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Chromosomal abnormalities in fetuses with brain anomalies on antenatal ultrasound scanning

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Objectives: Chromosome microarray (CMA) is indicated in the presence of fetal structural abnormalities. UK guidelines exist for the reporting of copy number variants (CNVs) in this setting. Structural brain anomalies may be isolated or associated with other abnormalities. We analysed data collected over 3 years to ascertain the prevalence of CNVs in fetuses with structural brain anomalies.

Methods: Retrospective analysis of genetic and clinical data was performed in cases of structural brain anomalies identified on ultrasound between January 2015 and January 2018. In cases where invasive testing was performed, QF-PCR and prenatal CMA data was collected for each type of structural anomaly. Cases with common aneuploidies (trisomy 13/18/21) detected on QF-PCR were excluded.

Results: 50 CMAs were performed in fetuses with structural brain anomalies (including both isolated and non-isolated cases); in 12 cases CMA abnormalities were reported. In 2 cases, ventriculomegaly was the only significant USS-detected abnormality. The USS findings and respective CMA results are outlined in Table 1.

Conclusions: In addition to common aneuploidies, there remains a high rate (24%) of chromosome abnormalities in fetuses with USS-detected brain anomalies, particularly when other structural abnormalities are evident. Careful counselling of parents regarding invasive testing and possibility of CNV is required even in cases of isolated brain anomalies. Future work to investigate the genomic outcomes in this cohort is proposed.
Table 1. Ultrasound abnormalities and reported chromosomal microarray findings

- Ventriculomegaly, small cerebellum, micrognathia: 18q22.3-18q23 deletion
- IUGR, ventriculomegaly: 11q13.4-q21 deletion
- Dandy-Walker malformation: Trisomy 9
- Agenesis of the corpus callosum: 17p12 deletion
- Dandy-Walker malformation: Mosaic trisomy 21
- Ventriculomegaly: 22q11.21 duplication
- Cardiac defect and posterior fossa cyst: 6p25.3p25.1 deletion and 14q24.3-q32.33 duplication - derivative chromosome 6 identified on subsequent G-banding
- Dandy-Walker malformation, abnormal kidneys, cystic placenta: Trisomy 8
- Short femur length, polyhydramnios, mild ventriculomegaly, oesophageal atresia: Mosaic trisomy 12
- Frontal cyst, absent nasal bone, echogenic focus in left ventricle: 22q11.21 deletion
- Agenesis of the corpus callosum, cardiac defect: 2q22.3-2q23.2 deletion
- IUGR, cystic hygroma, Dandy-Walker malformation, absent stomach: 4p15.2-4p16.3 deletion

Chromosomal microarray analysis on direct CVS can be complicated by confined placental mosaicism for aneuploidy and microdeletions

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Objectives: This study aims to establish the incidence and implications of confined placental mosaicism (CPM) in the context of prenatal chromosomal microarray analysis (CMA).

Methods: We retrospectively reviewed prenatal array data on 1382 consecutive CVS specimens spanning the past 6 years, focusing on those for which direct tissue (both cytotrophoblast and mesenchymal cells) was used for CMA and cultured cells (primarily mesenchyme) was also analyzed or amniotic fluid was used for confirmation, to determine the frequency of mosaic abnormal findings that were the result of CPM.

Results: Out of a total of 1382 consecutive CVS cases, we identified 42 (42/1382 = 3.0%) cases with abnormal array findings suggestive of mosaicism. Among them, 20 cases were unequivocally interpreted as CPM based on a normal amniotic fluid (AF) confirmatory result.
and/or normal results on cultured cells. Notably, 40% (8/20) of the cases revealed complex findings, including multiple mosaic aneuploidies, mosaic submicroscopic copy number variation (CNV) and mosaic aneuploidy plus mosaic CNV.

**Conclusions:** Abnormal CMA results from direct CVS specimens should be interpreted with caution when mosaicism is evident or suspected. Furthermore, confirmatory testing on amniotic fluid, which contains cells derived from the fetus, is recommended in these cases.

**P1-3 Table.**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Chromosome (culture)</th>
<th>CMA Result</th>
<th>Amnio follow-up Result</th>
<th>CMA mosaic fraction</th>
<th>CMA mean log2 ratio</th>
<th>CPM type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47,XY,+i(9)(p10)[2]/46,XY Y[70]</td>
<td>Mosaic +9p [direct]</td>
<td>Normal CMA</td>
<td>N/A †</td>
<td>0.66 †</td>
<td>II or III</td>
</tr>
<tr>
<td>2</td>
<td>46,XY</td>
<td>Mosaic +13 [direct]</td>
<td>N/A</td>
<td>0.2</td>
<td>0.14</td>
<td>I</td>
</tr>
<tr>
<td>3</td>
<td>46,XX</td>
<td>Mosaic –X [direct]</td>
<td>N/A</td>
<td>0.47</td>
<td>-0.39</td>
<td>I</td>
</tr>
<tr>
<td>4</td>
<td>+13</td>
<td>No growth</td>
<td>mosaic +13 (plus 22q11.21 loss) [direct]</td>
<td>CMA: 22q11.21 loss G-band: 46,XX</td>
<td>0.19</td>
<td>0.13</td>
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<tr>
<td>5</td>
<td>46,XY</td>
<td>Mosaic +X,+Y [direct]</td>
<td>N/A</td>
<td>N/A †</td>
<td>N/A †</td>
<td>I</td>
</tr>
<tr>
<td>6</td>
<td>46,XX</td>
<td>Mosaic –X [direct]</td>
<td>N/A</td>
<td>0.18</td>
<td>-0.14</td>
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<tr>
<td>7</td>
<td>46,XY</td>
<td>Mosaic +9, mosaic 0.098 Mb loss of Xp22.31 (STS gene) [direct]</td>
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<td>0.33</td>
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<tr>
<td>9</td>
<td>46,XX</td>
<td>Mosaic +13 [direct]</td>
<td>Normal CMA G-band: 46,XX</td>
<td>N/A ‡</td>
<td>N/A ‡</td>
<td>I</td>
</tr>
<tr>
<td>10</td>
<td>46,XX</td>
<td>mosaic +19, 14.595 Mb loss of 15q25.3q26.3 [direct]</td>
<td>N/A</td>
<td>0.08/0.72</td>
<td>0.06/-0.64</td>
<td>I</td>
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<tr>
<td>11</td>
<td>47,XX,+i(18)(q10)[9]/46,X X[11]</td>
<td>Mosaic +18q [direct]</td>
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<td>0.14</td>
<td>II or III</td>
</tr>
<tr>
<td>12</td>
<td>46,XX</td>
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<td>0.57/0.58/0.58</td>
<td>0.36/0.37</td>
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<tr>
<td>13</td>
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<td>Mosaic 0.182 Mb loss at Xq27.3 (FMRI gene) [direct]</td>
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<td>0.6</td>
<td>-0.51</td>
<td>II or III</td>
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<td>14</td>
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<td>Mosaic 1.333 Mb gain of 10p12.33p12.31 [direct]</td>
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<tr>
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<td>N/A †</td>
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<td>0.05</td>
<td>II or III</td>
</tr>
<tr>
<td>16</td>
<td>46,XX</td>
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<td>N/A</td>
<td>0.11</td>
<td>0.08</td>
<td>I</td>
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<tr>
<td>17</td>
<td>47,XY,+10[8]/46,XY[30]</td>
<td>Mosaic +10 and mosaic +13 ‡ [direct]</td>
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<td>0.23/0.71</td>
<td>0.16/0.4</td>
<td>I (mosaic +13)</td>
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</table>

*Unedited draft - unpublished*
<table>
<thead>
<tr>
<th>Case</th>
<th>karyotype</th>
<th>Diagnosis</th>
<th>Method</th>
<th>Normal [cultured cells]</th>
<th>Extra Chromosome [direct]</th>
<th>Log2 Ratio</th>
<th>Comparison</th>
<th>p-value</th>
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<tbody>
<tr>
<td>18</td>
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<td>N/A</td>
<td>Mosaic +3 [direct]</td>
<td>N/A</td>
<td>0.27</td>
<td>0.18</td>
<td>I</td>
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<tr>
<td>19</td>
<td>Normal</td>
<td>46,XX</td>
<td>Mosaic multiple CNVs on 8p [direct]</td>
<td>N/A</td>
<td>0.47/N/A‡</td>
<td>0.39/N/A</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal [cultured cells]</td>
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<td>0.46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>N/A</td>
<td>46,XY</td>
<td>Mosaic +5 [direct]</td>
<td>46,XY</td>
<td>0.11</td>
<td>0.08</td>
<td>I</td>
<td></td>
</tr>
</tbody>
</table>

CMA, chromosomal microarray analysis; CPM, confined placental mosaicism; N/A, not available.

† For non-mosaic 47,XY,+i(9)(p10) cells, the expected log2 ratio would be 1.32 (four copies of chromosome 9p in total); N/A, not applicable.

‡ Mosaic change not called by the in-house analysis program but noticed by visual inspection. See Figure 2 for CMA plots.

§ FISH analysis on cultured CVS cells of this sample showed no evidence of a copy number gain in chromosome 10.

¶ Normal chromosome analysis (46,XY) after this child was born.

†‡ Only mosaic trisomy 13 detected by CMA on direct CVS sample of this case was interpreted as CPM but not the mosaic trisomy 10.

**P1-4**

**Positive predictive value of fetal Nuchal Translucency (NT) for detection of genomic imbalances after rapid exclusion of “common” aneuploidies**

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²Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital in Olomouc, Olomouc, Czech Republic
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Objectives: In years 2014-2017 we performed the retrospective study focused on analysis of:

- positive predictive value of NT for chromosomal imbalances (different than common aneuploidies) using karyotype, Multiplex Ligation – dependent Probe Amplification (MLPA) (MRC,Holland) or chromosomal microarray (CMA): Cytoscan Optima - Affymetrix, Santa Clara, USA or CytoSNP-12-Illumina, San Diego, CA, USA.
- correlation between fetal gender and NT.

Methods: NT was measured in the group of 576 foetuses (297 males, 279 females). NT value was correlated to gender in four groups - Group A1: healthy children with NT< 95. centile, Group A2: healthy children with NT >2.1 mm, group B: normal karyotype and NT ≥3.0 mm, group C: normal karyotype and NT ≥3.5mm. In addition to QF PCR and karyotypig targeted MLPA test (with probemixes Telomeres 3 and 5, Microdeletions 1, DiGeorge syndrome, Congenital heart diseases) was performed in 105 samples stratified according to NT value and CMA was performed in addition to MLPA in 50 of these samples.

Results: Combination of karyotyping and MLPA revealed 17 chromosomal changes (16 pathogenic, 1 VOUS) including a mosaic trisomy 21, which was not detected by QF-PCR. CMA detected 3 additional CNVs to the 17 previously detected by MLPA and karyotyping. 3 (15 %) CNVs would be missed in the group of 50 samples tested only by karyotyping and MLPA, 7 (35 %) CNVs might be missed in the group of 105 samples tested only by karyotyping. The detection rate of MLPA and karyotyping was 11 (20.75 %), 3 (12 %) and 3 (11.1 %) in the group A, B, C respectively.

Conclusions: The higher rate of chromosomal imbalances in the group with NT below 3.0 mm (A) was influenced by an inclusion of pregnancies with detected US abnormalities in the second trimester of pregnancy into the investigated group. This shows that considerable amount of chromosomal imbalances is not associated with increased NT. The males/females ratio was 0.94, 1.58, 1.2 and 0.89 in the groups A1, A2, B and C respectively. The males/females ratio is higher with NT (2.1 – 3.4 mm) comparing to control group (A1) and group with NT≥3.5mm.

The whole study was supported by MH CZ – DRO (FNOI, 00098892).

P1-5

Four prenatal cases of unusual chromosomal rearrangements involving the chromosome 22q11.2 region

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Unedited draft - unpublished
Objectives: Chromosome 22q11.21 contains a cluster of low-copy repeats (LCRs), referred to as LCR22A–H, that mediate meiotic non-allelic homologous recombination, resulting in either deletion or duplication of various intervals in the region. The deletion of the DiGeorge/velocardiofacial syndrome interval LCR22A–D is the most common recurrent microdeletion in humans, with an estimated incidence of 1: 4,000 births. Although both deletion and duplication events should occur in equal proportions, microduplications of the 22q11.2 region are about half as frequent as microdeletions. Probably, microduplications are underdiagnosed by karyotype analysis and fluorescent in situ hybridization (FISH).

Methods: We present clinical, cytogenetic, molecular and genotype-phenotype analysis of four prenatal cases involving the 22q11.2 region. Microarray-based comparative genomic hybridization (array CGH; Agilent SurePrint G3 8x60K) was used for characterization of 22q11.2 abnormalities. All findings were confirmed by FISH and we also investigated parents in order to determine the parental origin of chromosomal rearrangements.

Results: In the first case, we report prenatal diagnosis of a 2,6 Mb deletion at 22q11.2 and a 1,7 Mb duplication at 16p13.11 in a fetus with congenital heart defect. In the second case, array CGH showed a 156 kb interstitial microdeletion of 22q11.21, including CRKL gene, in a pregnant woman with complex congenital heart disease. In the third case, we detected a 2,9 Mb 22q11.2 microduplication and fetal chromosome analysis also revealed a mosaic karyotype, 47,XX,+mar[15]/46,XX[41]. A fetus with normal ultrasound anatomy represents the last case. In this case, we identified a 846 kb 22q11.21 microduplication spanning LCR22B–D.

Conclusions: Our data exemplify the complexity of rearrangements in the 22q11.2 region and the clinical heterogeneity observed in cases with 22q11.2 microdeletion/microduplication syndrome. The array CGH is a suitable and reliable method for rapid and effective detection of atypical 22q11.2 abnormalities in prenatal cases.

P1-6

Pallister-Killian syndrome associated with Dandy-Walker malformation: A fetal case report

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Objectives: Pallister-Killian syndrome (PKS) is a sporadic genetic disorder. It is cytogenetically characterized by a tissue-limited mosaicism for a supernumerary isochromosome for the short arm of chromosome 12, which is present at high percentage in cultured fibroblasts. Prenatal diagnosis can be suspected on fetal images of characteristic craniofacial dysmorphism and a variety of malformations. We document a fetal case of PKS with detailed prenatal and autopsy findings.

Unedited draft - unpublished
Methods: We report on a case of PKS with prenatal diagnosis made by conventional karyotype analysis of cultured amniocytes.

Results: A 30-year-old pregnant woman underwent amniocentesis at 18 weeks' gestation. Prenatal ultrasound showed coarse face, micromelia and polyhydramnios. The karyotype, performed according to standard G-banding techniques, was: mos46,XX[13]/47,XX,+i(12p)[22] (Figure). Parental karyotypes made on peripheral blood samples were normal. A complete fetopathological examination was performed after pregnancy termination. The fetus had cranio-facial dysmorphism with hypertelorism, short nose, flat nasal bridge, long philtrum, labio-alveolar-palatal cleft, micrognathia and low-set dysplastic ears. The neck was short with excess of nuchal fold. Addition abnormalities were observed including rhizomelic micromelia, brachydactyly, arhinencephaly, vermian and corpus callosum agenesis, cystic dilatation of the fourth ventricle, and bilateral pyelocaliceal dilatation.

Conclusions: Our case report adds another types of central nervous system anomalies which may be associated with PKS, including Dandy-Walker malformation, arhinencephaly and agenesis of the corpus callosum. The short arm of chromosome 12 seems to be involved in the development of medline structures. Conventional karyotype in cultured fibroblasts is a pivotal tool to search for mosaicism of i(12p) and to determine its level.

P1-6 Image.
Utility of chromosomal microarray analysis in fetuses with major structural anomalies

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Objectives: To determine the association of copy number variants (CNV) with abnormal ultrasound scan in fetuses with normal karyotype. To evaluate the incremental yield over karyotyping in our cohort.

Methods: A total of 33 chromosomal microarrays (CMA) were performed from March 2016 to October 2017 on samples obtained from invasive testing using a custom array 60K from Agilent Technologies. The phenotype of the fetuses carriers of CNVs is described below. Case 1. Abnormality of ductus venosus blood flow (HP:0010947), increased nuchal translucency (HP:0010880) and bilateral pleural effusion (HP:0002202). Case 2. Increased nuchal translucency (HP:0010880), 5.7mm. Case 3. Talipes equinovarus (HP:0001762) and fetal akinesia sequence (HP:0001989).
Results: Two CMA have no result because of low quantity/quality of DNA and chromosomal imbalances were detected on 3 cases.
Case 1. arr[GRCh37]10q11.21q11.22(49004647_52372211)x1. CNV of Uncertain clinical significance (VUS), likely pathogenic, associated with facial dysmorphisms as hypertelorism (HP:0000316), micrognathia (HP:0000347) and prominent ears (HP:0000411).
Case 2. arr[GRCh37]15q11.2q13.1(22880274_28535051)x1. Pathogenic deletion associated with Angelman Syndrome (MIM#105830) or Prader-Willi Syndrome (MIM#176270).
Case 3. arr[GRCh37]1q21.1q21.2(145818502_147824348)x1. CNV related to 1q21.1 deletion syndrome (MIM # 612474). This syndrome has reduced penetrance and extremely variable phenotype. The most common clinical findings include developmental delay (HP:0001263), microcephaly (HP:0000252), hypotonia (HP:0001290) and variable dysmorphic features.

Conclusions: Chromosomal imbalances were therefore detected in 3 of 31 (9.7%) fetuses, indicating the additional value of routine array CGH in cases with abnormal ultrasound results and normal karyotype.

P1-8

Expanded QF-PCR analysis of 187 miscarriages samples with a homemade combination of STRs

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Objectives: To examine prevalence and types of chromosome abnormalities among samples of miscarriages received for prenatal diagnosis in the Hospital 12 de Octubre, Madrid from January 2016 to December 2017.

Methods: 187 samples from 137 pregnant women were received. Miscarriage samples consisted in trophoblast, chorionic villi, gestational sac, yolk sac, fetal skin or placenta obtained from abortions between 6th and 20th weeks of gestation. Multiplex and simple QF-PCR assays have been performed on miscarriage DNA samples, analysing specific short tandem repeat (STR) markers for chromosomes 13, 15, 16, 18, 21, 22, X and Y. Karyotyped have been performed after cultured cells with GTG-banded.

Results: 34 cases had QF-PCR results compatible with different chromosome numerical abnormalities: 4, 2, 7, 4, 3 and 5 cases of trisomy 13, 15, 16, 18, 21 and 22, respectively; one cases of Turner’s syndrome; 7 cases of triploidy and one case homozygous for all the short tandem repeat markers. The detection rate was 24.8% (34/137). A trisomy 2 confined to placenta was also detected with karyotyping analysis.

Unedited draft - unpublished
Conclusions: Identification of the cause of fetal loss reduces the feelings of anxiety, depression and self-blame in women with a miscarriage. Knowing the genetic cause of the fetal loss allows to predict the risk of recurrence in further pregnancies and to give an appropriate genetic counselling.

P1-9

47,XY,+del(X)(q21.31)/46,XY Mosaicism in prenatal diagnosis – case report of a rare event

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Objectives: Aneuploidies involving the sex chromosomes are the most common anomalies in humans. In many cases these anomalies are present in mosaic and may involve either the whole chromosome or just part of it. These anomalies constitute a challenge in prenatal diagnosis because it is generally very difficult to establish a reliable genotype-phenotype correlation. Here we report a rare event of a mosaic in which one cell line carries an additional abnormal X chromosome, with a terminal deletion at q21.31 region, and a normal XY constitution in the majority of the cells.

Methods: A healthy 36-year-old G1P1 woman was referred for prenatal diagnosis at 11+5 weeks of gestation for increased nuchal translucency. Chorionic villus biopsy was performed and molecular rapid aneuploidy result indicated an anomalous situation for the X chromosome in a male fetus. As the material was not sufficient to establish a culture an amniocentesis was performed at 17+3 weeks and karyotyping and microarray were performed in order to characterize the anomalous result.

Results: The results obtained indicated the presence of a mosaic involving an extra X chromosome with a terminal deletion, [47,XY,+del(Xq)/46,XY.arr[GRCh37] Xp22.33q21.31(169921_89283237)x1~2], which is compatible with a Klinefelter syndrome variant.

Conclusions: Pregnancies affected by X chromosome aneuploidies diagnosed prenatally are at an increased risk of adverse fetal and neonatal outcomes. High quality information is critical for informed decision-making in pregnancy following a prenatal diagnosis of sex chromosome aneuploidy.

P1-10

Mutation of IFNLR1, an interferon lambda receptor 1, is associated with autosomal dominant nonsyndromic hearing loss

Unedited draft - unpublished
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Objectives: Hereditary sensorineural hearing loss is a genetically heterogeneous disorder. Here, we show that mutations in the gene encoding interferon lambda receptor 1 (IFNLR1) – a protein that functions in the Jak/STAT pathway – are associated with autosomal dominant, nonsyndromic, progressive sensorineural hearing loss (ADNSHL) in a large Chinese family.

Methods: Using whole exome sequencing coupled with linkage analysis, we identified a cosegregating heterozygous missense mutation, c.296G>A (p.Arg99His) in IFNLR1. Morpholino knockdown of ifnlr1 leads to a significant decrease in hair cells, supporting cells and non-inflation of the swim bladder in late-stage zebrafish, which can be reversed by injection with normal Zebrafish ifnlr1 mRNA.

Results: These results suggest that ifnlr1 is essential for the normal development and function of hearing and swim bladder in zebrafish. Knockdown of ifnlr1 in zebrafish causes significant upregulation of cytokine receptor family member b4 (interleukin-10r2), jak1, tyrosine kinase 2, stat3, and stat5b in the Jak1/STAT3 pathway at the mRNA level.

Conclusions: In aggregate, these data suggest that IFNLR1 function is required in the auditory system and that IFNLR1 mutations are associated with ADNSHL. To the best of our knowledge, this is the first study implicating an interferon lambda receptor in auditory function.

P1-10 Image.
High resolution chromosomal microarrays in the general prenatal population: Pathogenic CNVs and VOUS in a 2016 state-wide cohort

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Objectives: Since the publication of a landmark study by Wapner et al\textsuperscript{1} showing the advantages of a targeted chromosomal microarray (CMA) over karyotype for prenatal diagnosis, CMA utilization has expanded rapidly. The yield of CMA and rates of variants of uncertain/unknown significance (VOUS) vary according to the CMA platform and indications for testing. This study aimed to analyse the diagnostic yield of whole genome high resolution SNP CMA in a state-wide cohort and compare this to the yield reported in the 2012 study.


Methods: Data on all amniocenteses and CVS done in 2016 in the state of Victoria, Australia were analysed. G-banded karyotype or CMA analysis with Affymetrix Cytoscan 750K (0.2Mb

Unedited draft - unpublished
resolution) was performed according to request by the clinical referrer. Results were analysed according to indication for testing and type of abnormality, specifically pathogenic copy number variant (pCNV), abnormality detectable by karyotyping, or VOUS. The major indications for prenatal diagnosis were ultrasound abnormalities, first trimester combined screening (FTC), non-invasive prenatal testing (NIPT), history of chromosome abnormality, single gene testing and advanced maternal age (AMA). Statistics were performed in STATAv14.

**Results:** Of the 1468 samples in 2016, 83.2% (n=1221) were analysed by CMA and 16.8% by karyotype. The results of the CMA group included: 29 (2.4%) pCNVs, 68 (5.6%) VOUS and 190 (15.6%) other major abnormalities detectable by karyotype (aneuploidies and large deletions). The highest rate of pCNVs occurred in those tested for ultrasound abnormality (3.2%), which was not significantly different to that reported by Wapner (2.8%). Rates of pCNVs by other indications were: 1.2%, 0.8%, and 1.9% for FTC, NIPT, and other combined indications respectively. The upward trend in annual total chromosome abnormalities in our population continued in 2016 (n=363).

**Conclusions:** Our overall rate of pCNVs was significantly higher than the 0.9% previously reported1, though the yield for ultrasound-indicated diagnosis was not significantly different. Likely factors contributing this difference include more specific indications for testing in our population, such as fewer for AMA alone and different CMA platforms. Also, as time progresses, a previously assigned VOUS may become a pCNV (or benign variant) when linked to a phenotype. The expansion of CMA to our general population has maintained high detection rates at a time of declining procedures. Our rates of VOUS are relatively high, posing challenges for the genetic counselling workforce.

P1-12

**ATAD3A deletions: A challenge in prenatal diagnosis**

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**Objectives:** The ATAD3 gene cluster is part of the ATPase family AAA-domain containing proteins consisting of three paralogs, ATAD3A, ATAD3B and ATAD3C located in tandem on chromosome 1p36.33. The ATAD3 genes encode mitochondrial membrane proteins that contribute to the stabilization of large-mitochondrial protein complexes. Recently, deletions in

*Unedited draft - unpublished*
the ATAD3 gene cluster were described in neonates with fatal congenital pontocerebellar hypoplasia.

Methods: A pregnant female, G3P2, was referred because of loss of a previous child due to severe pontocerebellar hypoplasia, intracranial calcifications of basal ganglia, corneal clouding, multiple contractures and underdeveloped lung alveoli. A second pregnancy resulted in a normal child. The present, third pregnancy presented at 30 weeks with pontocerebellar hypoplasia and progressive microcephaly, reminiscent of the clinical picture of their earlier child.

Results: SNP array analysis revealed no aberrant CNVs and therefore fast whole exome sequencing (WES) was performed for the foetus, the diseased sister and both parents. Analysis of the exome of the foetus revealed a homozygous variant in the ATAD3B gene for which only the mother was a heterozygous carrier, suggesting a deletion on the paternal allele. Further analysis of this gene cluster demonstrated biallelic deletions of the ATAD3B/ATAD3A genes in both children. Due to the highly homologous genes in this gene cluster it was not possible to map the exact breakpoints using the SNP array and WES results.

Conclusions: Two additional patients with biallelic ATAD3A deletions, that lead to infantile lethality, were detected indicating that it is essential to diagnose this abnormality prenatally, as it has a major impact on the obstetric and neonatal care. The results described above suggest that neither SNP array nor WES is sufficient for a precise reconstruction of the genetic defect, and that other laboratory screening methods needs to be developed and implemented.

P1-17

Prenatal diagnosis and long term follow up of a patient with mosaic variegated aneuploidy and its molecular analysis

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Objectives: Mosaic variegated aneuploidy (MVA) is a recessive condition characterized by mosaic aneuploidies, predominantly trisomies and monosomies, involving multiple chromosomes and tissues. Mutations in BUB1B, CEP57 and TRIP13 genes are found in a subset of patients with MVA. The phenotype of MVA syndrome includes severe microcephaly and growth deficiency, central nervous system anomalies, mental retardation, mild physical

Unedited draft - unpublished
anomalies, and predisposition to cancer. Several cases of MVA were diagnosed in prenatal period. We reported the cytogenetic and antenatal findings of a patient with MVA with long-term postnatal follow-up and molecular analysis result.

**Methods:** A 29-year-old woman seen in 1996 at 19 weeks gestation because of raised maternal serum alpha-fetoprotein level was found to have fetal growth restriction with pericardial effusion. Amniocentesis was performed. Baby was born at 36 weeks with birth weight of 1.55 kg. Cord blood and placental tissue karyotype was repeated. She had infantile neuroblastoma, failure to thrive, microcephaly, short stature, mild mental insufficiency, renal failure, and was seen by clinical geneticist. At age 21, she developed pleural effusion, chylothorax, and bilateral ovarian tumour (Meigs syndrome), confirmed to be Sertoli-Leydig cell tumour. Targeted gene panel analysis on extended hereditary cancer syndrome was performed.

**Results:** G-banded chromosome analysis on cultured amniocytes, placental tissue, and cord blood lymphocytes showed aneuploidies in multiple cell lines with composite karyotypes: 45~51,XX,+X[1],+2[3],+3[2],+5[6],-5[1],+6[4],-6[2],+7[6],+8[3],+10[3],+12[1],+14[1],+15[1],+16[1],+17[7],+18[1],+20[1],+21[2][cp150] in cultured amniocytes; 45~51,XX,-X[2],+1p[2],+1q[1],-1[2],+2[3],-2[3],+5[2],-5[3],+7[4],+8[9],+9[2],-10[2],+11[5],+12[4],+13[1],+14[1],-16[1],+17[3],+18[3],-18[1],+19[4],+20[5],+21[2],+fra[1][cp44] in cultured placental tissue, and 45~47,XX,-X[1],-13[1],+18[1],-21[1][cp8] in cord blood lymphocytes. Targeted gene panel analysis revealed biallelic pathogenic variants on $BUB1B$ gene c.1402-5A>G and c.2386-11A>G.

**Conclusions:** The case illustrated the clinical presentation of mosaic variegated aneuploidy syndrome from prenatal setting with intrauterine growth restriction, abnormal karyotypes on various tissues, to postnatal developmental problems including microcephaly, growth retardation and tumour formation. Pathogenic variants in $BUB1B$ gene were detected.

P1-19

**A novel method based on low-coverage whole genome sequencing for detection of chromosomal abnormalities associated with miscarriage**

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³Center for Medical Genetics, School of Life Sciences, Central South University, Changsha, China

**Objectives:** Chromosomal abnormalities contribute significantly to various congenital anomalies and pregnancy loss in humans. Cytogenetic karyotyping, in conjunction with array-based methods, has been the mainstream technology for genetic analysis for spontaneous miscarriage. More recently, next-generation sequencing (NGS) has represented a further evolution of molecular technologies for chromosomal analysis of clinical samples. In this study,
we compare the performance of traditional G-banding karyotyping and SNP array with that of copy number variation sequencing (CNV-Seq) for detection of chromosomal abnormalities associated with miscarriage.

**Methods:** Products of conception (POC) were collected from spontaneous miscarriages. Chromosomal abnormalities were detected using high-resolution G-banding karyotyping, SNP array and CNV sequencing. Quantitative fluorescent polymerase chain reaction analysis of maternal and POC DNA for short tandem repeat markers was used to both monitor maternal cell contamination and confirm the chromosomal status and sex of the miscarriage tissue.

**Results:** A total of 10ng of input DNA was sufficient for accurate CNV-seq diagnosis. In 48 samples with a normal karyotype, CNV-seq identified 10 small CNVs of <3Mb, representing a detection rate of 20.8%. And CNV-seq was assessed on 72 samples previously diagnosed by SNP array. In 43 of these samples (60%), CNVs were clearly associated with known chromosome syndromes. And CNV-seq detected five secondary CNVs of <1 Mb that were not detected by SNP array. Until now, a total of 10,000 POC samples were sequenced, of which 4,900 were found positive. 3,332 were with aneuploidy (68%) and 539 with CNVs (11%).

**Conclusions:** CNV-Seq is highly specific and reproducible for identifying chromosome copy number abnormalities associated with spontaneous miscarriage.

P1-21

**Novel heterozygous mutation in the RHO gene in a Chinese pedigree with retinitis pigmentosa**

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**Objectives:** Retinitis pigmentosa (RP) comprises a group of inherited disorders in which abnormalities affecting the photoreceptors (rods and cones) or pigment epithelium of the retina lead to progressive visual loss. Since many genetic alterations may influence RP, identification of gene rearrangements and mutations involved in each case is crucial to adequately classify, diagnose, and treat this disease.

**Methods:** Here, we used next generation sequencing (NGS) based on a custom AmpliSeq library and the Ion Torrent PGM system to detect the genetic alteration(s) underlying autosomal-dominant retinitis pigmentosa (adRP) in a four-generation Chinese pedigree. After NGS was
performed on the proband, a suspected variant was verified in the remaining family members through PCR amplification and Sanger sequencing.

**Results:** A heterozygous c.982delC (p.L328fs) mutation in the rhodopsin (RHO) gene was identified in the eight family members affected with adRP, but not in the nine unaffected relatives.

**Conclusions:** We conclude that the c.982delC (p.L328fs) mutation identified here is the causal factor of adRP in this pedigree. To the best of our knowledge, this RHO mutation had not been reported previously. These findings provide important new insight into the etiology of RP.

P1-22

**Genetic testing in stillbirth: Which is the best tissue to sample?**

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**Objectives:** To assess the effectiveness of genetic testing in stillbirths during a 5-year period, and to compare karyotyping success rate of according to the different tissue sampled.

**Methods:** From 2012 to 2017, 146 stillbirths (fetal losses above 22 weeks) were delivered in our hospital. Karyotype was performed in different tissues: amniotic fluid and fetal blood preferentially, and fetal skin or placenta if blood could not be sampled. In the last two years of the study microarray replaced karyotyping. Cases were divided into 5 groups according to genetic test types and results: a) testing not consented; b) testing failure; c) QF-PCR; d) Karyotyping; e) Array-CGH. Karyotype success rates were compared between fetal tissues with the use of 95% confidence intervals.

**Results:** Among 146 stillbirths, the genetic results were: group a) 9 cases, b) 21 cases, c) 12 cases, d) 60 cases, e) 43 cases. Four chromosomal abnormalities (trisomy 9, trisomy 22, tetrasomy 18p, and monosomy X) and 2 microdeletions (arr2p16.3(50790948-50936973)x1; arr11q13.2q13.4(67684435-73938053)x1) were found, resulting in a 3.5% (4/115) prevalence for chromosomal abnormalities and a 4.7% (2/43) for microdeletions. Karyotyping success rates according to the tissue sampled: Table 1.

**Conclusions:** Similar prevalences of chromosomal and submicroscopic anomalies were observed in our stillbirth series. A successful karyotype is more frequently achieved in amniotic fluid (83%) and placenta (78%), as compared to fetal skim (13%) or blood (6.3%).

Unedited draft - unpublished
Chromosomal abnormalities involved in pregnancy loss after assisted reproduction

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Objectives: Cytogenetic analysis of aborted tissue material in order to identify the type and frequency of chromosomal abnormalities occurring in first trimester pregnancy loss after assisted reproduction.

Methods: A total of 105 tissue samples, mostly from first trimester miscarriages were obtained. Mean maternal age was 38,6 years and mean gestation age was 9,3 weeks. Samples were collected in RPMI medium and sent to the laboratory within 48 hours. They were rinsed with antibiotics, dissected in order to remove maternal tissue and blood clots and the obtained chorionic villi were trypsinized. Long term cultures were set up and upon adequate growth, harvesting followed in order to obtain G-banded chromosome preparations. Maternal cell contamination was investigated by DNA genotyping assays when necessary. Cytogenetic results were described according to ISCN 2016.

Results: Cytogenetic analysis was obtained in a 100 out of 105 samples, thus a 95,2% success culture rate was achieved. An abnormal karyotype was observed in 58,2% of cases, a normal male karyotype in 13,4% and a normal female karyotype of fetal origin in 28,3% . Autosomal trisomy was the most frequent aberration, followed by X chromosome monosomy. Double autosomal trisomies were detected in 4,47% of cases, combined sex and autosome aneuploidy in 1,49% and mosaicism in 4,47%. A 48,XX,+15,+16[10]/48,XY,inv(9)(p12q13),+13,+15[2] karyotype, identified after analysis of a single sample, implied a dichorionic twin pregnancy. This was further confirmed by information provided by the referring physician.

Conclusions: Our findings provide further support to the fact that standard karyotyping of products of conception is essential in order to investigate the causes of pregnancy loss, estimate recurrence risks and offer proper genetic counseling to couples with reproductive

<table>
<thead>
<tr>
<th>Tissue sampled</th>
<th>Testing Success</th>
<th>Testing Failure</th>
<th>Total</th>
<th>Success rate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amniotic fluid</td>
<td>48</td>
<td>40</td>
<td>8</td>
<td>83% (82.8 – 83.9)</td>
</tr>
<tr>
<td>Fetal blood</td>
<td>16</td>
<td>1</td>
<td>15</td>
<td>6.3% (5.3 - 7.2)</td>
</tr>
<tr>
<td>Fetal skin</td>
<td>54</td>
<td>7</td>
<td>47</td>
<td>13% (12.7 - 13.7)</td>
</tr>
<tr>
<td>Placenta</td>
<td>27</td>
<td>21</td>
<td>6</td>
<td>78% (77 – 78.5)</td>
</tr>
</tbody>
</table>

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difficulties due to miscarriage. Since the cytogenetic aberrations identified in this study are in agreement with existing literature data, it seems that there is no particular difference between miscarriages occurring after assisted reproduction and those after natural conception with regards to the chromosome abnormalities involved.

P1-25

243 prenatal cases were recruited in this study with congenital morphological abnormalities detected by prenatal ultrasound scan as well as normal G-band including normal variants (November of 2013 to December of 2017)

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Objectives: The aim of this study is to investigate genetic causes of congenital multiple abnormalities detected by SNP microarray testing of DNA from chorionic villi or amniotic cells.

Methods: DNAs extracted from chorionic villi or amniotic cells in anomalous cases with normal karyotype were examined by SNP microarray. SNP microarray was performed by using CytoScan™ Assay with CytoScan™ 750K or HD Array (Thermo Fisher Scientific) and data analysis was done by Chromosome Analysis Suite (ChAS) (Thermo Fisher Scientific). The data are compared with 3 databases, DGV, ISCA and DECIPHER.

Results: Among 243 cases, abnormal results were revealed in 22 cases (9.05%). Ten pathogenic cases (4.12% of all recruited cases) were confirmed including one case of 1q21.1 duplication syndrome and other 9cases (0.4%). Five cases (2.06%) were confirmed as ‘likely pathogenic’. Two cases (0.82%) were ‘unknown significance’ and two cases (0.82%) ‘likely benign’. Two cases (0.82%) with trisomy 14 mosaicism and 4p16.3p15.31deletion mosaicism were considered as confined placental mosaicism.

Conclusions: SNP microarray is useful for the cases with ultrasound abnormalities with karyotypes accompanied with difficult break point. However we should be prudent in diagnoses and counseling patients, because there still be many of variants of uncertain clinical significance (VOUS) and it is essential to investigate familial variants as well as long-term follow up of infants with abnormal SNP. Of course need check Trio (Fetus, maternal and paternal) analysis. As a molecular genetic approach in prenatal diagnoses, whole exome sequencing or whole genome sequencing should be performed with combination of fetal detailed morphological ultrasound scan.

P1-26

Unedited draft - unpublished
A novel pathogenic NIPBL gene mutation in a Chinese patient with Cornelia de Lange syndrome

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Objectives: Cornelia de Lange syndrome (CdLS), a rare genetic and severe multisystemic development disorder which is a highly clinically and genetically heterogeneous congenital disease, is caused by mutations in various cohesion complex-related genes (\textit{NIPBL}, \textit{SMC1A}, \textit{SMC3}, \textit{HDAC8}, and \textit{RAD21}). To date, Sanger sequencing was employed to detect the pathogenic mutations in CdLS, but it is time-consuming and expensive. We aimed to provide a timely and advanced method for molecular diagnosis of CdLS by targeted next-generation sequencing (NGS).

Methods: Array-based Comparative Genomic Hybridization (aCGH) was used to detect the chromosomal alterations and targeted next-generation sequencing (NGS) was employed to screen five known genes involved in CdLS. Sanger sequencing was used to validate the results of NGS.

Results: In this study, we reported the clinical and molecular characterization of a patient with CdLS. We found a novel heterozygous nonsense mutation –c.3920C>A (p.S1307X)– in the \textit{NIPBL} gene, which has not been reported previously. The positive correlation of the mutation with CdLS was further validated via Sanger sequencing of DNAs from proband’s parents and fifty normal individuals, which indicated that the pathogenic mutation was not inherited from proband’s parents and the mutation was also not found in \textit{NIPBL} gene of fifty normal individuals.

Conclusions: Our finding expanded the spectrum of \textit{NIPBL} mutation and suggested that NGS was an accurate and cost-effective method for the genetic diagnosis of genetically heterogeneous disease.

P1-27

Fraser syndrome: A prenatal case possibly due to a multiallelic pattern of inheritance

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Unedited draft - unpublished
Objectives: We describe a male fetus of 20 weeks of gestation whose pregnancy has been terminated because of a multiple congenital anomalies. The ultrasound and morphological findings are strongly suggestive for Fraser syndrome. This recessive condition is due to those biallelic pathogenic variants in either GRIP1, FRAS1 or FREM2 genes impairing a specific multiprotein complex, called Fraser-complex. The genetic investigations performed so far did not show a clear molecular basis to support this clinical diagnosis. However, whole exome sequencing (WES) identified an excess of inherited variants in Fraser-related genes, possibly suggesting their functional involvement in the pathogenesis beyond a classic two-hit fashion of inheritance.

Methods: First-trimester screening was performed through biochemical tests in maternal blood (PAPP-A + free beta-HCG) and nuchal translucency measurement. After multidisciplinary counselling and accordingly to parents' will, invasive procedure was undertaken via chorionic villus sampling. The fetal sample was both prepared for conventional karyotyping and stored at -20°C for any further investigation. Second-trimester ultrasound investigation was carried out by skilled ecogaphist. After the termination of the pregnancy, fetal autopsy was performed and whole exome sequencing (WES) was undertaken on the DNA of the trio (foetus and parents) through next-generation sequencing. Array-CGH, still ongoing, is being realized at the highest resolution to exclude contributing copy number variants.

Results: First-trimester screening formulated an intermediate risk (1:608). Chromosomal analysis revealed a normal male karyotype. Second-trimester ultrasound described anidramnios, bilateral renal agenesis and right encephalocele. Fetal autopsy added facial dysmorphisms, bilateral cryptophthalmos, low-set ears, bilateral cutaneous syndactyly and talipes. WES found a monoallelic nonsense variant in FRAS1 (c.364delG) and a homozygous missense variant in FREM1 (c.4466G>A); frem1 protein interacts with the Fraser complex and its biallelic pathogenic variants cause a milder form of the Fraser phenotypic spectrum. All the variants were inherited from the healthy parents. No other possibly damaging variants in OMIM genes were found. The reads count seems to exclude a deletion of the second allele of FRAS1, however array-CGH is being perofomed to rule out this chance.

Conclusions: We present a prenatal case strongly suggestive for Fraser syndrome. However, WES did not identify biallelic pathogenic variants in any of the Fraser-related genes, found in about 50% of individuals with Fraser syndrome. Interestingly, WES revealed a nonsense variant in FRAS1 and a homozygous missense variant in FREM1. We speculate on the possible effects of this co-occurrence, which might impair the functioning of the Fraser-complex and thus produce the Fraser phenotype. Further investigations are needed to demonstrate this hypothesis. However, if this were the case, it would associate to Fraser syndrome a multiallelic pattern of inheritance, as already described for other conditions (ie ciliopathies).
Invasive diagnostic testing results in over 500 cases with ultrasound findings

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Objectives: This study aimed to characterize the number of abnormal prenatal diagnostic tests in patients with ultrasound findings who pursue chorionic villus sampling (CVS) or amniocentesis seen at a large and diverse academic medical center, and to differentiate between abnormalities seen on karyotype and chromosomal microarray (CMA). Additionally, we aimed to describe the types of ultrasound findings that are most likely to have a clinically relevant finding on CMA.

Methods: A retrospective review of diagnostic testing results from CVS and amniocentesis entered in the prenatal genetic counseling patient database from cases with ultrasound findings seen at the University of Texas Health Maternal-Fetal Medicine Clinics in greater Houston between 2013-2016 was performed (#HSC MS 16 0957). Descriptive statistics were used on the data set.

Results: Over four years, 517 procedures were performed on cases with ultrasound findings. Of 510 karyotypes ordered, 67% were normal, 30% were abnormal, and 3% had no growth. CMA was ordered on 211 cases, with 75% giving normal results, 24% abnormal, and 1% no result. Within abnormal karyotype results, 9% would not have been identified on CMA. In contrast, 77% of CMA findings would have been missed by karyotype alone. Cases most likely to yield abnormal findings on CMA were those with both structural and nonstructural abnormalities (34.4%) and those with only structural abnormalities (22.9%).

Conclusions: Clinically relevant CMA findings were seen in one quarter to one third of cases with structural abnormalities. Importantly, 77% of findings from CMA would have been missed
if only a karyotype had been ordered. Therefore, CMA should be routinely ordered as a first tier test for patients with structural abnormalities on ultrasound rather than as a reflex after karyotype. This may be particularly relevant in cases where access to pregnancy options is limited after 20 weeks gestation and timing of results is paramount.

P1-29

Cornelia de Lange syndrome caused by a novel heterozygous deletion mutation of the NIPBL gene

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Objectives: Cornelia de Lange syndrome (CdLS) is a congenital developmental disorder characterized by distinctive craniofacial features, growth retardation, cognitive impairment, limb defects, hirsutism, and multisystem involvement. To date, mutations have been identified in five genes responsible for CdLS: NIPBL, SMC1A, SMC3, RAD21, and HDAC8 and in about 60% of the patients, mutations in NIPBL could be identified. Here in this study we present a newborn female patient with classic CdLS and investigate genotype-phenotype correlation.

Methods: Venous blood samples were obtained from the family members and 100 unrelated healthy individuals. Genomic DNA was extracted following a standard protocol. The coding exons and flanking intronic sequences of NIPBL, SMC1A, SMC3, RAD21, and HDAC8 were amplified by PCR and bidirectional Sanger sequencing.

Results: Sequencing analysis of the proband revealed a 1395bp heterozygous deletion in exon 46 and part of exon 47 of NIPBL, which resulted in a frame-shift, leading to premature termination of Nipped-B-like protein. The variant was not detected in the parents and the controls, suggesting a de novo event. No mutations SMC1A, SMC3, RAD21, and HDAC8 were found.

Conclusions: In this study, we described the molecular genetic diagnosis of a Chinese newborn with classic CdLS, who carried a heterozygous 1395-based deletion in NIPBL. According to the information available in the Human Genome Mutation Database and to the best of our knowledge, this is a novel de novo mutation of NIPBL and has not been reported elsewhere.
Peptidomic analysis of fetal heart tissue for identification of endogenous peptides involved in tetralogy of fallot

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Objectives: Tetralogy of fallot (TOF) is one of the most prevalent types of congenital heart diseases. As a category of bioactive molecules, peptides have been proved to participate in various biological processes. However, the role of endogenous peptides in the pathogenesis of TOF has not been studied.

Methods: In this study, we performed a comparative peptidomic profile in the fetal heart of TOF and control group for the first time by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results: Our data demonstrated that a total of 201 peptides derived from 176 precursor proteins were differentially expressed in the heart tissues of TOF fetuses comparing with normal controls, including 41 up-regulated peptides and 160 down-regulated peptides. After analyzing the characteristics of these differentially expressed peptides and their precursor proteins, we found that these peptides were potentially involved in different biological processes, especially cardiogenesis and congenital anomaly of cardiovascular system. Interestingly, we detected several extracellular matrix (ECM)-derived peptides involved in our differentially expressed peptidomic profile.

Conclusions: In summary, our study constructed a comparative peptidomic profile from the heart tissues of TOF fetuses and normal controls and identified a series of peptides which could potentially participate in heart development and TOF formation. The emergence of our peptidomics study indicated a new perspective to explore the pathogenesis of abnormal heart morphology especially TOF.
Transgenerational effects of fetal growth restriction

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Objectives: To evaluate the transgenerational effects of fetal growth restriction.

Methods: A cross-sectional study examining parents-first-born offspring pairs. First, 623 neonates were identified from delivery books from 1975-1993. Current contact data was retrieved from the database of the Catalan health system. Perinatal data from an eventual first-born-offspring was obtained through telephone questionnaire from 152 adults. The relationships between the parents’ and the child's birth characteristics were estimated using multivariate quantile regression analysis.

Results: Perinatal characteristics from 152 offspring born 2005-2015 to 72 FGR and 80 AGA parents could be retrieved. Offspring from FGR adults presented a lower birthweight (median 2950g (interquartile range 2570-3288) vs 3200g (2830-3465), p=0.009) and birthweight centile (18 (5-50) vs 38 (19-65), p=0.004) as compared to those children born from AGA adults. A similar gestational age at delivery could be observed in both groups (40 weeks (38-40) vs 39.5 weeks (38-40), p=0.5). Interestingly, the prevalence of preeclampsia was higher in pregnancies from FGR individuals (8.3% vs 1.3%, p=0.05). Regression analysis showed that the best contributors for offspring’s birthweight were parental.

Conclusions: Our data provides evidence of transgenerational effects of FGR and reinforces the importance of preventive strategies for improving intrauterine growth.

Does placental size correlate with fetal weight and brain development in mouse models of Down Syndrome?

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2Medical Genetics Branch, National Human Genome Research Institute, Bethesda, MD, United States

Unedited draft - unpublished
**Objectives:** Prenatal growth restriction is associated with poor postnatal outcomes in humans with Down syndrome (DS). Previously, we observed a significant correlation between growth and behavioral delays in the Ts1Cje mouse model of DS. Here, we evaluated placental size, fetal growth and brain weight in the Dp(16)1/Yey, Ts65Dn and Ts1Cje mouse models of DS.

**Methods:** Dp(16)1Yey and Ts1Cje males were mated to C57BL/6 females and Ts65Dn females mated with C57BL/6XC3Sn males. Day 18.5 embryos were extracted from euthanized pregnant dams. Body weight, crown rump length (CRL), brain weight and placenta weight and diameter were measured. Genotyping and sex determination were performed by PCR. Means and standard deviations were calculated with statistical significance set at $p \leq 0.05$.

**Results:** Dp(16)1/Yey (n=39), Ts65Dn (n=33) and Ts1Cje (n=37) strains showed autosomal dominant Mendelian transmission. There was a linear relationship between body weight and brain weight in all mouse models; however, this relationship was stronger in euploid compared to trisomic embryos. Dp(16)1/Yey and Ts65Dn mice exhibited significant growth delays vs. euploids (Eup) ($p=0.03$ and $0.01$ respectively). Weight distribution in trisomic embryos followed a normal distribution that was shifted to the left (i.e. lower weights) compared to Eup. Brain weight was significantly reduced in Dp(16)1/Yey and TS65Dn embryos ($p<0.001$). The differences in placental weights and fetal: placental ratios did not reach statistical significance.

**Conclusions:** Although body and brain weights are correlated in three different mouse models of DS, they do not correlate with placental size. More detailed analyses of placental histology and gene expression are ongoing to determine if the placenta plays a role in decreased brain growth in Dp(16)1/Yey and TS65Dn embryos.
Etiology and outcome of non immune hydrops: A review of 47 cases

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Objectives: Non immune hydrops is the pathological accumulation of fluid in fetal serous cavities and soft tissues in the absence of red cell alloimmunization. It can be due to a variety of
causes and can recur in subsequent pregnancies. We tried to ascertain the etiology of hydrops in our patients and institute timely treatment where beneficial. Pregnancy outcome in non immune hydrops was studied.

**Methods:** A total of 47 cases of nonimmune hydrops who presented to us between January 2015 and December 2017 were reviewed. Patients with ultrasound suggestive of hydrops were evaluated using ICT to confirm nonimmune etiology. A detailed ultrasound assessment of fetus, placenta and amniotic fluid volume, MCA-PSV, fetal echocardiography and MRI where required, maternal blood studies for TORCH infections, MCV and DNA studies for alpha thalassemia where required and prenatal invasive testing for fetal karyotype and enzyme assays for lysosomal storage disorders was done. Appropriate therapy where available was provided. Regular monitoring was done and the outcome after delivery was determined.

**Results:** Ten patients had recurrent hydrops. Seven fetuses had chromosomal abnormalities. There were four cases of lysosomal storage disorders, three cases of TTTS, two cases each of heart block, CHAOS, chylothorax, bladder outlet obstruction and one case each of CCAM, fetal anemia, Ebstein anomaly, TRAP and placental chorioangioma. Nine patients opted for pregnancy termination while fifteen patients underwent intervention in the form of intrauterine transfusion, pleural or ascitic fluid tapping, pleuro-amniotic or vesico-amniotic shunt insertion, selective fetal reduction by RFA and bipolar fetoscopic coagulation. Nine babies were discharged in stable condition, three pregnancies are ongoing and remaining resulted in IUD or early neonatal demise.

**Conclusions:** An ultrasound finding of fetal hydrops requires a thorough investigation to look into the underlying cause. Prenatal testing can be offered in subsequent pregnancy in certain genetic conditions. Timely referral and intervention can improve fetal prognosis in selected cases.

P1-37

**Chromosomal disorders in congenital anomalies of the kidney and the urinary tract**

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Objectives: To assess the clinical implication of chromosomal microarray analysis (CMA) in prenatal diagnosis of Congenital Anomalies of the Kidney and the Urinary Tract (CAKUT).

Methods: We reviewed 107 cases of CAKUT detected by prenatal ultrasound in our Fetal Nephrourological Unit from 2015 to 2016. CAKUT cases were classified into 3 groups: 79 collecting system anomalies (including hydronephrosis, duplex collector system, megacystis and LUTO); 14 renal dysplasias (including echogenic, hypoplastic, polycystic and multicystic kidneys), and 14 number, fusion or location anomalies. Invasive test (amniocentesis or CVS) for CMA were always offered with the exception of isolated mild pelvic dilatation. CMA was performed by qGenomics (qChipCM, 8x60K).

Results: From 33 (31%) genetic studies performed, 5 (15%) anomalies were found: 3 of them in dysplastic and echogenic kidneys with normal amniotic fluid (two microdeletions 17q12 and one 47,XYY) and 2 in collecting system anomalies (T21 in a first trimester megacystis and untypical microdeletion 22q11.21 in one case of bilateral pelvic dilatation + ventricular septal defect, undetected by conventional karyotype). Of this 5 chromosomal anomalies, there was one termination of pregnancy (T21). The 3 newborns with echogenic kidneys present preserved renal function, but persistent anomalous appearance of kidneys. Untypical 22q11.21 microdeletion presents severe neurological delay and normal renal function.

Conclusions: Chromosomal microarray analysis could help in the diagnosis and prenatal counseling of CAKUT, particularly in those cases that represent a diagnostic dilemma such as echogenic/dysplastic kidneys with normal amniotic fluid.

P1-37 Image.
Fetal skeletal dysplasias: Fetopathological classification of 72 cases

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Objectives: Fetal skeletal dysplasias (FSD) are a large group of genetic disorders that may be detected by prenatal ultrasound or result in intrauterine or early neonatal death. Their diagnosis is described in the literature as one of the most difficult in the field of fetopathology. Genetic counseling is hampered by the phenotypic and genetic heterogeneity of these conditions. The aim of this study is to classify the FSD in a consecutive series of 72 affected fetuses on the basis of fetopathological criteria.

Methods: In this retrospective study, we gathered clinical, radiological and histopathological data of 72 cases of FSD which were diagnosed among 5995 autopsies performed over a 8-year period at the Embryo-Fetopathology Department of the Maternity and Neonatology Center of Tunis between January 2009 and December 2016. The classification was established according to the 2015 Nosology and Classification of Genetic Skeletal Disorders.

Results: The prevalence of FSD was 1.2:100 autopsies. The overall sex ratio (M:F) was 1.25. The mean gestational age at time of autopsy was 21.6 weeks gestation (range 12-41). Parental consanguinity and recurrence in siblings were reported in 39.3% and 11.5% of cases, respectively. The FSD were classified into 13 distinct pathological groups. Four major pathological groups were identified: (1) Osteogenesis imperfecta and decreased bone density group (21 cases, 29%); (2) FGFR3 chondrodysplasia group (18 cases, 25%); (3) Ciliopathies with major skeletal involvement (9 cases, 12%); and (4) Sulphation disorders group (7 cases, 10%). Thanatophoric dysplasia and osteogenesis imperfecta were the most frequently encountered skeletal dysplasias.

Conclusions: Our study demonstrates the usefulness of the fetopathological examination in the diagnosis and accurate classification of FSD, thus enabling better targeting of genetic counseling. The relatively high prevalence of the FSD in Tunisia implies establishment of a national registry and multidisciplinary collaboration for better management of these skeletal disorders. Besides, this study highlights the needs of future research to improve our current knowledge on FSD in Tunisian population regarding their molecular basis and pathogenetic mechanisms.
Value of placental examination in the diagnostic evaluation of stillbirth

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Objectives: Stillbirth is a tragic event for the parents and obstetrician. Fetoplacental examination (FPE) is important to identify the cause of stillbirth and to provide an appropriate genetic counseling. The aim of this study is to evaluate the contribution of placental examination in the identification of the causes of stillbirth.

Methods: In this retrospective and descriptive study, we gathered clinical and histopathological data of a cohort of 147/325 stillbirths which were selected from 757 autopsies performed at the Embryo-Fetopathology Department of the Maternity and Neonatology Center of Tunis between January and December 2013. Stillbirths before 14 weeks gestation, twin pregnancies and fetuses without placentas were excluded. According to the FPE results, the stillbirths were classified into homogeneous etiological groups.

Results: The incidence of stillbirth was 20/1000 births. The overall sex ratio was 1.45. The mean gestational age at time of autopsy was 21.5 weeks gestation (range 14-41). The stillbirths were classified into five distinct etiological groups: (1) placental causes (89 cases, 61%); (2) materno-fetal causes (23 cases, 16%); (3) fetal causes (14 cases, 9%); (4) multiple causes (13 cases, 9%); and (5) unexplained causes (8 cases, 5%). Placental examination provided essential or contributory informations in 88% and 12% of cases, respectively. The placental abnormalities were consistent with vascular, inflammatory and developmental lesions in 62%, 21% and 12% of cases, respectively.

Conclusions: Our study demonstrates the usefulness of the placental examination in the diagnostic evaluation of stillbirths and genetic counseling.

Fetal thrombotic vasculopathy associated with protein S deficiency: A case report

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Unedited draft - unpublished
Objectives: Fetal thrombotic vasculopathy (FTV) is a chronic disorder characterized by placental vascular obliteration and hypoperfusion. It is usually associated with adverse fetal outcomes, especially fetal growth restriction and stillbirth. The diagnosis is only made histologically. The objective of this study is to report a typical case of FTV due to protein S deficiency.

Methods: We report on a case of FTV with adverse perinatal outcome.

Results: A 20-year old pregnant woman, gravida 2 para 0, with previous miscarriage, was referred at 20 weeks gestation for severe oligohydramnios. She had positive family history with protein S deficiency. A complete feto-placental examination was performed after pregnancy termination. It revealed fetal growth restriction, Potter sequence, generalized visceral congestion and thymic lymphocyte depletion. The placenta was hypoplastic; it weighed 100 g (normal range: 135-150 g). Histological study of the placenta showed FTV with mural thrombosis of the funicular, chorionic and intraplacental arteries, and villous chronic hypoxic lesions. Testing for protein S deficiency was positive.

Conclusions: Our case report documents the major challenge raised by the FTV. The diagnosis is only based on the histopathological study of the placenta. There are no specific criteria allowing prenatal detection or suspicion of this condition. The early-onset fetal growth restriction without fetal structural abnormalities, as described in the present case, would prompt clinicians into an exhaustive search for thrombophilia.

Fetal eosinophilic insulitis: A pathognomonic histologic feature in infant of diabetic mother

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Objectives: The pancreas in an infant of a diabetic mother responds to maternal hyperglycemia by marked beta-cell hyperplasia and less frequently by eosinophilic insulitis. This rare entity should alert of the maternal diabetes or pre-diabetes. The present report describes the histological aspects of this uncommon entity.

Methods: We report on a case of eosinophilic insulitis in a premature male infant born to a diabetic mother.

Results: A male infant was born at the 38th week gestation to a 26-year-old mother, gravida 2 para 2, with a history of gestational diabetes. Antenatal ultrasound showed polyhydramnios and fetal macrosomia. At birth, the infant had immediate respiratory distress and died after 24 hours of resuscitation. At autopsy, he presented with macrosomia, facial dysmorphism, anterior
meningoencephalocele and talipes equinovarus. Microscopic examination revealed bilateral pulmonary atelectasis complicating hyaline membrane disease and severe disorganization of the pancreatic parenchyma with hyperplasia of islets, abundant fibrous connective tissue and diffuse inflammatory cell infiltration consisting of eosinophils, lymphocytes and plasma cells.

Conclusions: The case report documents marked hyperplasia of islets, increased peri-insular fibrosis and eosinophilic insulitis in a preterm macrosomic fetus. This constellation of pancreatic anomalies is pathognomonic of maternal diabetes. It alerts about patients who should be targeted for diabetes screening.

P1-43

Achondrogenesis type IB associated with renal cysts: A case report

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Objectives: Achondrogenesis type IB is a lethal skeletal dysplasia caused by mutations in the sulfate transporter gene, SLC26A2. It is characterized by extremely shortened limbs, poorly ossified skull, spine and pelvis, and absence of rib fractures. The key histologic change is the lack of development of normal hyaline cartilage which show homogeneous pale interterritorial matrix and round chondrocytes surrounded by a dense extracellular matrix forming rings. We aim to describe a case of achondrogenesis type IB with novel finding.

Methods: We report on a case of achodrogenesis type IB associated with renal cysts.

Results: A 27-year-old, G4P0 woman with history of previous two siblings affected by the same disorder, was referred at 15 weeks gestation for a prenatal ultrasound that demonstrated short limbs and cystic hygroma. The male karyotype was normal. Fetopathological examination showed marked edema of soft tissues, extreme micromelia, short chest with a protuberant abdomen, cleft palate, micrognathia, hypoplastic lungs and heart. Radiologic evaluation and histologic analysis of a femoral specimen were consistent with the diagnosis of achondrogenesis type IB. Histological study also revealed cystic dilatation of few distal tubules of renal cortex and medulla sparing the proximal tubules.

Conclusions: The present case report documents an unusual association of achondrogenesis type IB with renal cysts. The sulfate transporter, SLC26A2, shows some level of renal expression. Its role in mediating renal tubular sulfate transport and potential implication in
tubular dilatation are unknown. Further studies are needed to determine the exact physiological role of this sulfate transporter in the kidney.

P1-45

**Middle interhemispheric variant of holoprosencephaly: Case studies of 5 prenatal cases**

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**Objectives:** Middle interhemispheric variant (MIH) of holoprosencephaly (HPE) or syntelencephaly is a rare variant of HPE. It is characterized by an abnormal midline separation of the posterior frontal and parietal lobes with variable fusion of the thalami. We report on 5 cases prenatally diagnosed.

**Methods:** We conducted a retrospective study in our cases of MIH, prenatally discovered between January 2012 and September 2017. Array comparative genomic hybridization (CGH) was performed. All patients choose for a termination of pregnancy and virtual and/or conventional autopsy was offered to the parents.

**Results:** MIH is characterised by deficient interhemispheric separation of the posterior frontal and parietal lobes. The cavum septi pellucidi was absent in all cases and the corpus callosum is usually deficient in the region of interhemispheric non separation. Diagnosis was possible in the first and second trimester of pregnancy. In 4 patients antenatal or/and postmortem MRI was performed. In one patient fMRI was performed. In 4 patients conventional autopsy was performed and confirmed the antenatal diagnosis. In 1 patient a mutation of the ZIC2 gen was found and in another a deletion on the long arm of chromosome 10.

**Conclusions:** MIH variant of HPE should be considered in the differential diagnosis when the CSP is absent. MIH is detectable in early pregnancy. Prenatal and postmortem MRI as well as perinatal autopsy should increasingly be used to confirm US diagnosis and explore the extent of this rare and relatively new variant.

P1-46

**The investigation on the expression of HO-1 in the placenta shares of sIUGR pregnancies**

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*)Unedited draft - unpublished*
Objectives: To investigate HO-1 levels in placental shares of the twins in normal MCDA and sIUGR pregnancies, and to discuss the relationship of HO-1 expression and the pathogenesis of sIUGR.

Methods: Twenty nine pairs of placentas, from 19 sIUGR (enrolled in sIUGR group) and 10 normal MCDA (enrolled in normal group) twin pregnancies, were collected in this research. HO-1 mRNA and protein levels were detected in placentas of normal and sIUGR groups.

Results: Under the qRT-PCR and Western Blot, we found no significant difference of HO-1 mRNA and protein between the twins in the normal group (P > 0.05), while in sIUGR group, the levels of HO-1 mRNA and protein were higher in placental shares of the smaller fetus than larger fetus (P < 0.05).

Conclusions: HO-1 was up-regulated in placental shares of the smaller fetus in sIUGR pregnancies, suggesting the activation of antioxidant system in the smaller fetus, which may be involved with the pathogenesis of sIUGR.

P1-47

Regulation of NRF2 by hypoxia in trophoblastic cells

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Objectives: To investigate the relationship between hypoxia and NRF2 expression in trophoblast cells.

Methods: HTR-8/SVneo cells were were cultured with ASA for 24 hr. The proliferous abilities were detected by MTT assay; and the apoptosis were detected by Flow cytometry assay in the OS model.

Results: Compared to the normoxia group (21% O₂), NRF2 mRNA was significantly lower in the severe hypoxia group (3% O₂)( P < 0.001) and was significantly higher in the mild hypoxia group (10% O₂) ( P < 0.01).
Conclusions: NRF2 was significantly up-regulated under mild hypoxia but down-regulated under severe hypoxia, suggesting the up-regulation of NRF2 in placental shares of the smaller fetus may be related to hypoxia.

P1-48

The regulation role of NRF2 in trophoblastic proliferation and apoptosis

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Objectives: To investigate the regulation role of NRF2 in trophoblastic proliferation and apoptosis.

Methods: HTR-8/SVneo cells were transfected with NRF2 siRNA. NRF2 mRNA and protein levels were detected by qRT-PCR and Western Blot respectively; MTT assay was used to detect the cellular proliferation, and Flow cytometry assay was used to detect the apoptosis in the OS model.

Results: The viability was significantly decreased when HTR-8/SVneo cells transfected with NRF2 siRNA for 24 hr, compared to the NC group (NC group : siRNA group = 0.76±0.01 VS. 0.6±0.04), P < 0.05. Compared to the NC group, the apoptosis was significantly increased when HTR-8/SVneo cells transfected with NRF2 siRNA for 24 hr (NC group : siRNA group = 8.76±0.91 VS. 20.06±1.24), P < 0.001.

Conclusions: NRF2 siRNA could inhibit cellular proliferation and promote apoptosis in the OS model of HTR-8/SVneo cells, indicating the regulation of NRF2 in trophoblastic function.

P1-49

The effect of ASA on trophoblastic proliferation and apoptosis

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**Objectives:** To investigate the effect of ASA on trophoblastic proliferation and apoptosis, and to illustrate the possible mechanism of ASA in regulating the trophoblastic functions.

**Methods:** HTR-8/SVneo cells were cultured with ASA for 24 hr. HTR-8/SVneo cells were pre-treated with MK2206 2HCl (PI3K/Akt signaling pathway inhibitor) for 24 hr, and qRT-PCR was applied to detect the levels of *NRF2* and *HO-1* mRNA levels. The proliferous abilities and the apoptosis were detected in the OS model.

**Results:** HTR-8/SVneo cells were cultured with ASA (100 μmol/L) for 24 hr. MTT results showed that the proliferation was significantly increased in the ASA group, compared to the H2O2 group (*P* < 0.05). When transfected with NRF2 siRNA for 24 hr, the proliferation was significantly decreased (*P* < 0.05). Flow cytometry assay results showed that the cellular apoptosis was significantly decreased in the ASA group. Pre-treated with PI3K/Akt signal pathway inhibitor MK-2206 2HCl, HTR-8/SVneo cells showed a significant down-regulation of *NRF2* mRNA.

**Conclusions:** ASA induced NRF2 up-regulation through PI3K/Akt signaling pathway, promoted cellular proliferation and inhibited apoptosis in the OS model of HTR-8/SVneo cells. The antioxidant effect of ASA may involve in the molecular mechanism of NRF2 in regulating trophoblast functions.

P1-50

**PI3K/Akt signaling pathway was involved in the regulation of NRF2 by ASA in trophoblast cells**

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**Objectives:** Since we found to ASA can influence the biological behavior of trophoblast cells, then we want to illustrate the possible mechanism of ASA in regulating the trophoblastic functions.

**Methods:** HTR-8/SVneo cells were pre-treated with MK2206 2HCl (PI3K/Akt signaling pathway inhibitor) for 24 hr, and qRT-PCR was applied to detect the levels of *NRF2* and *HO-1* mRNA levels.

**Results:** Pre-treated with PI3K/Akt signal pathway inhibitor MK-2206 2HCl, HTR-8/SVneo cells showed a significant down-regulation of *NRF2* mRNA (ASA+H2O2 group : MK2206 group = 1.22±0.36 VS. 0.57±0.13), *P* < 0.05. *HO-1* mRNA was also significantly lower in the MK2206 group (ASA+H2O2 group : MK2206 group = 2.37±0.23 VS. 1.03±0.13), *P* < 0.001.
**Conclusions:** ASA induced NRF2 up-regulation through PI3K/Akt signaling pathway, promoted cellular proliferation and inhibited apoptosis in the OS model of HTR-8/SVneo cells. The antioxidant effect of ASA may involve in the molecular mechanism of NRF2 in regulating trophoblast functions.

P1-51

**In vivo investigation for the role of fibrinogen like protein 1 in preeclampsia**

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**Objectives:** Inhibition of nitric oxide synthase with $N^\omega$-nitro-l-arginine-methyl ester (L-NAME) has been employed as an experimental model of human preeclampsia (PE). Insulin resistance (IR) and liver injury are related to PE and recent literatures have demonstrated that fibrinogen like protein 1 (Fgl1) regulates energy metabolism and hepatocyte regeneration. However, the association between Fgl1 and PE is still unknown till now. Therefore, we used L-NAME-induced PE animal model to determine the role of Fgl1 in PE.

**Methods:** Pregnant mice were divided into two groups: (1) control group, (2) L-NAME group, receiving L-NAME (50 mg/kg/day) injected intraperitoneally, from day 7 to day 16 of gestation (n=6 in each group). The blood pressure and urine protein of mice was measured during this period of time. On day 17 of gestation, animals were fasted for 6 hours and then euthanized. Blood samples were collected for plasma level of Fgl1, glucose, insulin, and homeostasis model assessment-IR index (HOMA-IR) to define IR. Kidneys, livers, and placenta were removed for histological analysis. Western blotting analysis for Fgl1 expression in placenta were also performed.

**Results:** L-NAME treated group showed elevated blood pressure and exhibited more dominant proteinuria in late gestational stage compared to control group. Reduced litter sizes and smaller litter weight were demonstrated in L-NAME group. Glomerulonephritis and connective tissue of liver examined by collagen I expression were more severe in L-NAME-induced PE-like mice. Fasting plasma glucose and insulin increased in the L-NAME group and fasting HOMA-IR were elevated more significantly in this group. Plasma fgl1 level of L-NAME group was higher than control group, and significantly higher expression of fgl1 in the placenta of L-NAME-induced PE mice was also found.

**Conclusions:** Our preliminary data have demonstrated that administration of L-NAME in pregnant mice is a reliable method to develop a PE-like syndrome, including hypertension, proteinuria, and smaller live-born litters, which have been implicated in the pathophysiology of
PE. We also proved the association of Fgl1 and IR in PE-like animal model. Further clinical and in vitro studies are needed to investigate the relationship between Fgl1, IR, and PE, and the underlying mechanisms of Fgl1 in the pathogenesis of PE.

P1-53

Abnormal vertebral patterning in deceased fetuses and neonates: Evidence of selection against variation in the number of vertebrae and ribs

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Objectives: To assess the vertebral pattern in deceased fetuses and neonates and gain insight into the possible genetic etiology of abnormalities in vertebral patterning. The possible associations between an abnormal vertebral pattern and specific structural, genetic or chromosomal anomalies were examined.

Methods: Radiographs of 445 deceased fetuses and infants were assessed. Cases in which the pattern of the vertebral column could not be determined were excluded. SNP array results and copy number variants (CNVs) of 335 cases were analyzed.

Results: 274 of 374 included cases (73.3%) had an abnormal vertebral pattern. Cervical ribs were present in 187/374 (50.0%) and were significantly more common in stillbirths (68/126 (54.0%) and terminations of pregnancies (101/188 (53.7%), compared to live births (18/60, 30.0%, p 0.003). The prevalence of cervical ribs did not differ significantly between fetuses and neonates with and without structural anomalies (119/245 versus 38/71, p0.73). Significant associations between an abnormal vertebral pattern and specific structural, chromosomal or genetic abnormalities were not found. No recurrent or overlapping abnormal array results or rare CNVs involving candidate genes were found.

Conclusions: The presence of deviations from the regular vertebral pattern, particularly in the cervical region, could be regarded as a sign of disruption at critical and conserved stages of embryogenesis. Assessment of the vertebral pattern could provide valuable information regarding fetal and neonatal outcome. A common underlying genetic causal factor for the high occurrence of abnormalities of the vertebral pattern could not be identified.

Unedited draft - unpublished
Role of transabdominal cerclage in fetal membranes histology after term elective cesarean section

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**Objectives:** Transabdominal cerclage is considered as a surgical approach to cervical insufficiency in women with a failure of previous transvaginal cerclage and fetal loss. In fetal membranes overlying the cervix, a zone of altered morphology has been well described before labor at term. We aimed to examine the fetal membranes histology by comparing the

*Unedited draft - unpublished*
membrane thickness of normal pregnant women and pregnant women with transabdominal cerclage, at term, before labor, in the absence of premature rupture of membranes. Our hypothesis was that transabdominal cerclage can determine structural changes in the cervical area of membranes and can also lead to functional changes.

**Methods:** Chorioamniotic membrane samples were collected from women who underwent term elective cesarean section before the onset of labor at the same gestational age (≥ 37 weeks) without any maternal or fetal disorder. Women were divided into two groups: the abdominal cerclage group (n=5) and the control group (n=5). Membrane samples were collected in two different areas: membranes overlying the cervix and membranes located far from the cervix and the placenta.

**Results:** In the chorioamniotic membranes overlying the cervix: (1) the mean fetal membrane thickness was significantly decreased compared to the distal area. In the cervical area, the chorion is significantly thicker in membranes of women with transabdominal cerclage. (2) Hydroxyprostaglandin dehydrogenase (PGDH) and Toll-like receptor-2 (TLR-2) expressions are also significantly increased in fetal membranes in women with transabdominal cerclage. (3) Cellular senescence is significantly decreased in membranes of women with transabdominal cerclage.

**Conclusions:** Fetal membranes in presence of transabdominal cerclage exhibit structural and functional changes compared to controls at term before labor. In presence of transabdominal cerclage, the significant chorion thickening in the cervical area closely correlates with increased PGDH and TLR-2 expression and the reduction of cellular senescence. Our data suggest that these changes contribute to the creation of a specific microenvironment in membranes that prevent the triggering of parturition. Structural and functional changes in fetal membranes account for favorable outcomes and the high success rate of transabdominal cerclage.
Fetal growth restriction in pregnant cancer patients treated with chemotherapy: A search for biochemical markers for placental pathology – a pilot study

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Objectives: Fetal growth restriction (FGR) is more frequently seen in pregnant patients treated for cancer. The placenta releases multiple factors in the maternal blood, of which several have been proposed as predictors of FGR. This study was set up to evaluate the levels of these proposed biomarkers in the blood of pregnant cancer patients treated with chemotherapy during pregnancy.

Methods: A total of 17 samples of chemotherapy-exposed pregnant patients (FGR: n=7 no FGR: n-12) and 49 control samples (FGR: n=17, no FGR: n=32), recruited between January 2014 and September 2016, were eligible for inclusion. Maternal blood and umbilical cord blood samples were taken at delivery. The concentration in the maternal plasma of sFlt-1 and PIGF was determined by Elecsys immunoassays; the concentration of leptin by ELISA. RT-qPCR was used to examine the expression of Flt1, Ki67, Leptin-R, TNF, HSP70 and PCNA in the maternal and umbilical cord blood plasma.

Unedited draft - unpublished
Results: Lower sFlt1 expression in the maternal plasma after chemotherapy-exposure were observed as compared to the levels in non-exposed controls (median 4495 and 3086 vs 5043 and 4471 pg/mL). The PIGF expression, resulting sFlt1/PIGF ratio and leptin concentrations did not differ between all groups. In the umbilical cord blood the sFlt1, Ki67 and Leptin-R expression values were higher compared to the other groups. The expression of HSP-70 was higher in the maternal blood and lower in the umbilical cord blood after chemotherapy exposure as compared to the controls.

Conclusions: This first observational pilot study shows that levels of circulating factors secreted by the placenta may be imbalanced in chemotherapy-exposed patients compared to non-exposed controls. Performing large-scale studies on the circulating maternal levels of biomarkers during the course of pregnancy might be helpful to detect potential circulating biomarkers for FGR.

P1-55 Table.
**Congenital glioblastoma multiforme presented with intracranial bleeding: Case report**

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**Objectives:** Intracranial tumors are rarely seen in the neonatal period. The incidences of these tumors are 1.1-3.6 in every 100,000 live birth and they are constituted of teratomas, astrocytomas or glioblastomas. Infants might be stillborn or it may progress severely. The average lifespan is two months without treatment. It has a tendency to bleeding within the first week of life. GBM is located in the cerebral hemispheres, the cerebellopontine angle and the cerebellum. Patients exhibit macrocrania and hydrocephaly. The tumor is diagnosed by magnetic resonance imaging (MRI) or ultrasonography (USG). We aimed to report a congenital GBM with intracranial hemorrhage and tumor.

**Methods:** A 22-year-old gravida-2, parity-1 at 37 weeks patient was admitted to our clinic with the suspicion of fetal intracranial bleeding and tumor. There was no risk factor in her medical history. No pathological findings were recorded in previous ultrasound examinations. The 37th-week ultrasound revealed the findings; biparietal diameter was 107 mm, head circumference was 374 mm (>97% for gestational age), a 9x8 cm sized supratentorial tumor in the right cerebral hemisphere, causing shifting and herniation of the midline structures, and which illustrated internal blood supply in the Doppler USG. The neonatal intensive care unit and the neurosurgery department were informed.

**Results:** A male baby weighing 2295 gr was delivered by cesarean section at 37 weeks. The 1 and 5 minute APGAR scores were 6 and 8 respectively. The head circumference was 39 cm. Macrocephaly and bilateral setting-sun eye phenomenon were observed on physical examination. He was floppy, had no active movements and he exhibited spontaneous eye movements. The postnatal MRI identified a 9x8 cm tumor with hemorrhage, located in the right cerebral hemisphere with shifting and subfalcine herniation. The patient died during surgery because of bleeding. The pathology revealed grade IV, IDH-1 negative glioblastoma. His parents refused a postmortem autopsy.

**Conclusions:** This report illustrates congenital brain tumors should always be considered in the differential diagnosis of intracranial hemorrhage. Fetal MRI is required for advanced diagnosis and is helpful in treatment planning. The onset of most of the congenital brain tumors occurs in the 3rd trimester of pregnancy, and they cannot be diagnosed in earlier weeks.
MR spectroscopy of the placenta - a feasibility study

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Objectives: Placental ¹H MRS is a newly emerging technique with the apparent potential to non-invasively assess the function of the placenta in vivo. However, only a few previous studies have utilised this technique, all of which focus on a particular pathology. The influence of changing gestational age and varying placental maturity have also not been accounted for in these studies. Therefore, it remains unclear whether this tool could be feasible for routine clinical use.

Methods: The primary aim of this study was to assess the feasibility of using in vivo ¹H MRS to assess placental function, in terms of the quality of spectra it can produce. In utero placental spectra were obtained from women (n=43) referred for fetal MRI following abnormal ultrasound scans, presenting with a range of conditions. The quality of spectra obtained was assessed, along with the frequency of detected metabolites. The effect of gestational age and placental maturity on these spectra was also determined.

Results: Almost 75% of spectra obtained were considered good quality following visual assessment, with assessors in substantial agreement with each other. Neither gestational age nor placental maturity appeared to influence the quality of spectra or the spectral peaks observed. The metabolites detected in this study were generally concordant with those of previous studies, although not all metabolites were present in all placentae. Lipid and choline peaks were most prominent, present in all but four placentae. Some contamination of spectra was also observed.

Conclusions: Despite showing great promise, it would be inappropriate to use this technique to inform clinical decisions currently. However, in vivo placental ¹H MRS certainly warrants further investigation and could potentially be feasible for use in future clinical practice. Future studies establishing metabolite thresholds profiles for conditions and confirming our conclusions regarding the influence of potential confounding variables would be beneficial.

Detection of marginal placental cord insertion by prenatal ultrasound: Is it predictive of adverse perinatal outcomes?

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Unedited draft - unpublished
Objectives: Lack of placental tissue at the site of cord insertion (velamentous insertion) is associated with adverse perinatal outcomes, including IUGR. An association between more common marginal cord insertion (MCI) and poor perinatal outcomes remains controversial, yet many institutions perform heightened surveillance of pregnancies affected by MCI. Part one of our study examines an association between MCI and adverse perinatal outcomes. We then evaluate a correlation between ultrasound-measured distance from the site of placental cord insertion to the placental margin (PCI distance) and multiple perinatal outcomes, reasoning that this analysis might identify a subset of MCI pregnancies at increased risk.

Methods: Part one is a retrospective cohort study of MCI pregnancies presenting to a Stanford PDC 9.2014-9.2016. MCI was determined on routine ultrasound. Our primary outcome was fetal intolerance to labor (FITL). Our study was powered to validate the null hypothesis. Parameters were compared using Chi-squared test. In part two, PCI distance was determined by review of ultrasounds by a single individual. Association with continuous variables was evaluated using measures of correlation and linear regression models with log-transformed outcomes and/or PCI distance as needed. Association with binary variables was evaluated using Wilcoxon tests and logistic regression models with log-transformed PCI distance.

Results: Of 675 abnormal cord insertion cases, we identified 183 that met inclusion criteria. These patients were then matched 1:1 with controls for part one of our study. We found no statistically significant association between MCI and FITL or secondary outcomes, including preterm delivery and IUGR (Table 1). In part two of our study, we found no significant correlation between fetal weight percentile at last third trimester ultrasound before delivery (Figure 1), gestational age at delivery, maternal blood loss, or APGARS (data not shown) and PCI distance measured at either 18-22 weeks or during a third trimester follow-up scan.

Conclusions: Our study suggests that pregnancies with marginal cord insertion are not at increased risk of fetal intolerance to labor and/or numerous other adverse perinatal outcomes. We go on to show that among MCI pregnancies, the distance from cord insertion to the placental margin does not correlate with risk of numerous fetal and maternal outcome measures, including IUGR. Together, these findings challenge the notion that ultrasound detection of marginal cord insertion alone should be an indication for heightened antepartum surveillance measures, such as third trimester growth scans. We propose that further studies be conducted to validate our findings.
**P1-59** Table.

<table>
<thead>
<tr>
<th>Perinatal Outcomes</th>
<th># MCI Cases (%)</th>
<th># Controls (%)</th>
<th>p value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal Intolerance to Labor</td>
<td>17 (9.3)</td>
<td>14 (7.7)</td>
<td>0.71</td>
<td>1.24 (0.55-2.80)</td>
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<tr>
<td>Premature Delivery</td>
<td>15 (8.2)</td>
<td>13 (7.1)</td>
<td>0.84</td>
<td>1.17 (0.50-2.75)</td>
</tr>
<tr>
<td>Intrauterine Fetal Demise</td>
<td>1 (0.55)</td>
<td>0 (0)</td>
<td>0.50</td>
<td>3.0 (0.1 to 75)</td>
</tr>
<tr>
<td>Pre-Eclampsia (with and without severe features)</td>
<td>10 (5.5)</td>
<td>12 (6.6)</td>
<td>0.83</td>
<td>0.82 (0.31-2.14)</td>
</tr>
<tr>
<td>Intrauterine Growth Restriction</td>
<td>6 (3.3)</td>
<td>5 (2.7)</td>
<td>1.00</td>
<td>1.21 (0.3-5.09)</td>
</tr>
<tr>
<td>APGARS at 5 min &lt; 7</td>
<td>3 (1.6)</td>
<td>1 (0.55)</td>
<td>0.62</td>
<td>3.03 (0.25-160)</td>
</tr>
<tr>
<td>Cord gas pH &lt; 7.2</td>
<td>27 (14.8)</td>
<td>28 (15.3)</td>
<td>1.00</td>
<td>0.96 (0.52-1.77)</td>
</tr>
<tr>
<td>NICU admission</td>
<td>9 (4.9)</td>
<td>5 (2.7)</td>
<td>0.41</td>
<td>1.84 (0.54-7.13)</td>
</tr>
</tbody>
</table>

**Figure 1**

P1-60

**Correlation between prenatal ultrasound and fetopathological examination findings in a fetus with Jarcho-Levin syndrome**

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**Objectives:** Jarcho-Levin syndrome (JLS) is an autosomal recessive spondylothoracic dysostosis. It is characterized by multiple vertebral and rib defects, resulting in a short neck and small
constricted thorax. Thus, this condition is commonly complicated by respiratory failure and death in the first years of life. We aim to correlate fetal anomalies associated with this disorder and detected by ultrasound examination with those identified at autopsy following termination of pregnancy.

**Methods:** We report on a case of JLS, that was diagnosed by the second trimester routine ultrasound in a fetus of a consanguineous couple without a previous family history or teratogenic exposure during early pregnancy. Chromosome analysis of amniotic-fluid cells showed a normal karyotype.

**Results:** A 30-year-old woman, gravida 2, para 2, underwent a routine ultrasound at 21 weeks gestation. It revealed abnormal alignment of the vertebral bodies with abnormally spaced vertebrae and marked kyphoscoliosis. The parents opted for termination of pregnancy. Fetopathological examination showed a 22-week-old female fetus presenting with cranio-facial dysmorphism, short neck and trunk, severe thoracic kyphoscoliosis, camptodactyly and rock-bottom feet. Radiological evaluation confirmed disorganization and fusions of multiple hemi-vertebrae and rib synostoses resulting in a small thorax and showed a Sprengel deformity. Genetic counseling was offered to the couple and plan made to assess subsequent pregnancy with ultrasound.

**Conclusions:** The case report confirms the utility of the prenatal ultrasound diagnosis of JLS and suggests that when the prenatal diagnosis of JLS is made before viability, the choice of pregnancy termination should be offered to the parents.

P1-60 Image.
Jeune syndrome associated with diffuse chorangiomatosis related placentomegaly: A fetal case report

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Objectives: Placental diffuse chorangiomatosis (PDC) is characterized by multifocal stem villi enlargement containing increased number of small vessels. It can cause massive placentomegaly. The etiology of PDC is currently unknown. The final diagnosis is achieved by postpartum histological examination of the placenta. It may have major negative impact on both mother and fetus, especially growth restriction, pre-eclampsia and premature delivery. Here, we report on an unusual association of Jeune syndrome with PDC resembling placental mesenchymal dysplasia by ultrasonography.

Methods: We describe the case of a female fetus of a 25-year-old woman, gravida 2 with previous one miscarriage who presented at 18 weeks gestation for a routine ultrasound screening. Her past medical history was unremarkable. Fetal and placental structural abnormalities were detected. Amniocentesis revealed a diploid karyotype of the fetus.

Results: Ultrasound revealed placentomegaly with single live fetus presenting with very short long bones, bilateral pyelectasis and single umbilical artery. Placenta was 75 mm thick and showed multiple large anechoic areas suggestive of partial mole (Figure). In view of high incidence of polymalformative fetal syndrome, the parents opted for termination of pregnancy. The placenta was received incomplete and had multiple grape-like vesicular structures. Placental histopathology showed diffuse vascular proliferation involving the stem villi and intermediate villi, which was confirmative of PDC. Fetopathological examination findings were consistent with Jeune syndrome and confirmed the bilateral pyelectasis without renal dysplasia and the single umbilical artery.

Conclusions: PDC should be considered in the differential diagnosis when the ultrasound shows a cystic placentomegaly. Careful attention should be paid in detection of fetal malformations that may justify the termination of pregnancy.

Unedited draft - unpublished
Antenatal diagnosis of alobar holoprosencephaly

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**Objectives:** Holoprosencephaly is a spectrum of cerebrofacial anomalies resulting from the complete or partial failure of the prosencephalon cleavage. The prosencephalon forms the cerebral hemispheres, the thalami and the basal ganglia. Thus, abnormal development of the prosencephalon results in variable fusion of these structures. The alobar variety is the most severe and lethal form of holoprosencephaly. Early diagnosis by fetal ultrasound allows early termination of pregnancy. The purpose of this case report is to describe the characteristic ultrasonographic features of the alobar holoprosencephaly and to stress the importance of its early detection in order to allow legal medical termination.

**Methods:** We report on a case of alobar holoprosencephaly with hypothalamic hamartoma due to inherited translocation trisomy 13. The mother was a carrier of the Robertsonian translocation.
Results: A 26-year-old second gravida was referred for routine obstetric ultrasonography at the 22th week of gestation. Her first child was normal. Ultrasonography revealed hypotrophic male fetus. The supratentorial brain was replaced by a large central monoventricle with thin cerebral parenchyma and fused thalami (Figure). The posterior fossa structures were normal. The facial features were dysmorphic with median proboscis and cyclopia. A complete fetopathological examination was performed after pregnancy termination. It confirmed the ultrasound findings. In addition, it showed hypothalamic hamartoma, accessory spleen with intrapancreatic splenic tissue, elongated and lobulated kidneys, single umbilical artery and polydactyly of the right foot.

Conclusions: We described a typical case of alobar holoprosencephaly with characteristic facial dysmorphism. The ultrasound diagnosis was easy allowing early medical termination of pregnancy. Furthermore, genetic counseling could be provided as the translocation trisomy 13 was inherited.

P1-62 Image.

P1-63

Prenatal sonographic findings in fetal hemochromatosis

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Objectives: Neonatal hemochromatosis is a congenital lethal alloimmune disease. It is an uncommon cause of hydrops fetalis. Prenatal ultrasound may detect features strongly suggestive of this disease, which we detail in the present case report.

Methods: We describe the prenatal ultrasound features of neonatal hemochromatosis complicated with hydrops fetalis and in-utero fetal death. The diagnosis was confirmed by a complete fetopathological examination.

Results: A 37-year-old gravida 3 para 2 woman underwent routine obstetric ultrasonography at 37 weeks gestation. Ultrasound demonstrated severe fetal hydrops, cardiomegaly without structural anomaly, distinct inhomogeneity of liver structure and heterogeneous content of the gall-bladder (Figure). On macroscopic examination, the fetus had cardiomegaly with marked ventricular hypertrophy, splenomegaly, cirrhotic hepatomegaly and very distended gall-bladder filled with stones. Microscopically, the liver displayed extensive portal fibrosis and marked siderosis of hepatocytes. Hemosiderin deposits were also demonstrated to a lesser extent in renal tubules, pancreatic acini and zona glomerulosa of the adrenal cortex. Fibrous thickening of the splenic parenchyma was noted.

Conclusions: Fetal hydrops must alert the obstetrician to evaluate the hepatic structure and the content of the gall-bladder as part of the etiological evaluation. Prenatal recognition of the previously described sonographic features associated with neonatal hemochromatosis is of great help in appropriate diagnosis and genetic counseling.
A fetal case of Greenberg dysplasia associated with massive intracranial hemorrhage: Correlation of prenatal imaging features with autopsy findings

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Objectives: Greenberg dysplasia is an autosomal recessive lethal chondrodysplasia. It is related to a defect in cholesterol biosynthesis due to mutations in the gene encoding the lamin B receptor, LBR. The most prominent radiological sign is the moth-eaten appearance of the markedly shortened long bones with unusual ossification centers. We describe a case of Greenberg dysplasia associated with massive intracranial hemorrhage.

Methods: A fetal case of Greenberg dysplasia is presented including prenatal, radiographic, macroscopic and histopathologic findings.

Results: A 28-year-old woman, G4P3, was referred to our institution at the 20th week gestation because of the third recurrence of a lethal skeletal dysplasia, previously diagnosed as achondrogenesis. Ultrasound showed oligohydramnius, hydrops fetalis, macrocrania with suture disjunction, platyspondyly, extreme tetramicromelia, narrow thorax, protruding abdomen and massive hemorrhage in cerebral parenchyma, ventricles and subarachnoid space. The MRI confirmed the sonographic abnormalities and revealed brain midline shift with subfalcorial herniation (Figure). The parents decided to terminate the pregnancy. The fetopathological examination including macroscopic, radiological and histological analysis of a bone specimen, confirmed the diagnosis of Greenberg dysplasia.

Conclusions: Our case report documents a massive intracranial hemorrhage in a fetus presenting with Greenberg dysplasia. Cholesterol deficiency may be involved in this complication.
Objectives: Factors predisposing to in utero intracranial hemorrhage (ICH) include a wide variety of conditions, mostly maternal trauma and fetal coagulation disorders. In most cases, however, the etiology remains unrevealed. The incidence is estimated at 1 in 10,000 pregnancies. Aim: To determine sonographic features of fetal ICH in our series of 36 fetuses. Furthermore, we reviewed the role of magnetic resonance imaging (MRI), genetic investigation and the outcome of this condition in order to optimize counseling.

Methods: Retrospective review of all cases of ICH antenatally diagnosed from 2005 to 2017. Prenatal and postnatal medical records were revised. The cases were divided into extra-axial (subdural and subarachnoid) or intra-axial (intraparenchymal with or without intraventricular extension). ICH was categorized according to the classification commonly used in neonates.

Results: 36 cases of fetal ICH included 3 subdural hematomas, 1 subarachnoid bleeding and 32 intra-axial hemorrhages (5 cases of Grade I, two cases of Grade II, 6 cases of Grade III and 17 cases of Grade IV hemorrhage and 2 cases had a bleeding in the posterior fossa). In 26/36 cases ventriculomegaly was the earliest ultrasound finding. In 31/36 cases MRI was performed,
confirming in 27/31 cases the ultrasound findings. No functional loss was reported in ICH grade I or II. Grade III hemorrhage had neurological impairment. All grade IV had unfavourable outcome. Subdural hematomas in our series were due to coagulation disorders.

**Conclusions:** Diagnosis of ICH is usually made late in second/third trimester of pregnancy with ventriculomegaly being the most common ultrasound finding in our series.

MRI allows evaluation of the entire cerebral parenchyma although it adds little information to expert prenatal neurosonography.

Genetic exploration with a thrombogenetic platform may reveal additional counseling information in some of the unexplained cases.

P1-66

**Real-time virtual sonography using MRI and ultrasound fusion imaging in the evaluation of CNS anomalies**

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**Objectives:** Recently, prenatal ultrasound has experienced the benefits of high-resolution probes and the use of transvaginal US to diagnose central nervous system anomalies. In addition, MRI has become a complementary tool. Both techniques deliver their maximal potential when performed by clinical experts, unfortunately this is not the same individual most of the time. MRI and US fusion has been successfully introduced postnatally. The technology provides synchronization of MRI and US images that could provide the benefits of both techniques. To evaluate the additional value of real-time virtual sonography using fusion imaging in CNS anomalies and to look for influence of the gestational age.

**Methods:** In our Fetal Medicine Unit, we performed 14 MRI-US fusion imaging examinations during a 4-month period to evaluate CNS anomalies. MRI was performed on a 1.5 Tesla system (Siemens Area Erlangen, Germany) with maternal sedation and included T2-weighted images in 3 planes and 2 3D T2-weighted series with different resolution. After acquisition, the fetal MRI dataset was loaded in the fusion system (GE Logic E9, Ziph, Austria) and scanning was started within 60 minutes after MRI. 3 in-plane anatomical reference points were used for synchronisation. Feasibility was evaluated compared to B-Mode US and MRI, separately.

**Results:** We included 5 patients with CMV seroconversion, 3 patients with ventricular abnormalities, 3 patients with spina bifida, a patient with a subdural bleeding, a patient with IVH grade 1 and another with a subependymal cyst. Data registration, matching and fusion
were performed within 60 minutes after the MRI and from 26 weeks onwards. Fusion imaging did not find additional information in the assessment of CMV anomalies. Fetal MRI depicted an abnormality in 2 cases, polymicrogyria and a posterior horn cyst, not seen on US. Fusion was inconclusive for the polymicrogyria and confirmed the cyst. US demonstrated the septa in the subependymal cyst.

**Conclusions:** Fusion imaging is feasible in the assessment of fetuses with CNS abnormalities from 26 weeks onwards. The technique does not show anatomical details that would have been missed by MRI or US alone. However, data integration of both modalities improves the multidisciplinary prognostic appraisal as well as the prenatal counseling.

**P1-68**

**Subependymal cysts in the fetal brain: Prenatal diagnosis of 41 cases**

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5*Department of Fetal Medicine, Leuven, Belgium*

**Objectives:** Subependymal cysts (SEC) are usually located in the wall of the caudate nucleus or in the caudothalamic groove. They are found in up to 1% of all neonates. Due to improved ultrasound detection, the prenatal incidence increases. AIM: To determine the sonographic appearance, the role of MRI in the work-up, the clinical implications and the outcome in 41 fetuses diagnosed with subependymal cyst(s).

**Methods:** Retrospective review of all our cases of antenatally diagnosed subependymal cysts from 2007 to 2017. We distinguished 5 groups of SEC: (1) the isolated SEC, (2) the SEC with an associated non progressive lesion, (3) SEC associated with a progressive CNS lesion, (4) SEC associated with ventriculomegaly and (5) CMV related SEC.

**Results:** In group 1 (n=3) perinatal outcome was normal. In group 2 (n= 4) club feet, a Blake pouch cyst, an isolated rhabdomyoma and a duplex kidney were found. These children had normal follow-up. In group 3 (n=3) a molybdenum cofactor deficiency, a PROSC mutation and delayed cerebral gyration were diagnosed. They all opted for TOP. In group 4 (n=3) Pyruvate carboxylase deficiency, respiratory long chain deficiency and congenital toxoplasmosis were diagnosed. Outcome was unfavourable. In group 5, 20/28 MRI confirmed the US CMV- findings; 3 patients declined MRI and 5 had no MRI. Eight patients opted for TOP, 14 had normal outcome and 6 were lost for follow-up.

*Unedited draft - unpublished*
Conclusions: Subependymal cysts develop mainly in the second and third trimester. Fetal MRI adds to the detection of associated brain abnormalities. Isolated supependymal cysts seem to have better prognosis. Small cysts are often CMV related. Subsequential ultrasound follow-up is mandatory to evaluate the number and the growth of the cyst and associated brain lesions. Large and multiple cysts show more adverse outcome.

P1-69

The ultrasound elastography in placenta might predict high risk pregnancies

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Objectives: Ultrasound elastography (UE) is used as a new technology that allows the objective evaluation of tissue hardness, such as breast, liver and placenta. Placenta has an important role in fetal development and maintenance of pregnancy, and its pathological change was correlated with major obstetrical disease, such as hypertensive disorders of pregnancy or small for gestational age. The pathological finding of placenta in these patient showed an acute sclerosis of the blood vessel and fibrosis of the villus. In this study, we prospectively evaluated an UE as a new assessment tool for placenta.

Methods: Elastographic evaluation was performed using the ratio of the elasticity of the target tissues (placenta) to subcutaneous fat (reference). Secondly, we made a benchmark to easily determine the hardness of the placenta, and the color mapping patterns of certain ROIs of the placenta were evaluated by an observer. The pattern was categorized into 3-step scores according to the frequency of the blue area (Figure 1). After delivery, pathological examinations were performed in placentas. This study was approved by the Institutional Review Board of our University (No. 2949).

Results: A total of 111 pregnant women received examinations including elastography. A significant positive correlation was observed between the ratio of the elasticity of the abdominal wall fat tissue and a z score of birth weight (correlation coefficient, \( r = -0.39; \ p < 0.01 \)). For the groups with scores of 1, 2, and 3, the mean birth weights were -0.11 SD, -0.09 SD, and -0.93 SD, respectively. The differences between scores 2 and 3 was \( p = 0.054 \), respectively. Furthermore, in the hard placenta, we can find the deposition of fibrin and chorangiosis which is thought to be an adaptation for hypo-oxygenation during the gestational period.

Conclusions: These results suggested that placental hardness according to elastography correlated with lower fetal birth weight and higher risk of pathologic abnormality of the placenta. Ultrasound elastography might offer a predictor of high risk pregnancies.
P1-69 Image.
Population-based trends in ultrasound-indicated prenatal diagnosis from 1994 to 2016: Two decades of change

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Objectives: To assess population trends in ultrasound-indicated prenatal diagnostic testing over the past two decades, in the context of rapidly-changing practices in aneuploidy screening and chromosome analysis.

Methods: Retrospective analysis of ultrasound-indicated amniocenteses and chorionic villus sampling from the Australian state of Victoria from 1994-2016. Ultrasound-indicated prenatal diagnostic testing included those performed for: fetal structural abnormality, fetal death, fetal growth restriction, abnormal amniotic fluid volume, “soft marker”, or unspecified ultrasound abnormality. Maternal age, indication for testing, type of procedure, gestational age, type of chromosome analysis (G-banded karyotype or chromosomal microarray, CMA), and test results were obtained. Diagnostic yield (% tests yielding a major abnormality) was analysed by year, maternal age, and gestational age. Statistical analysis was performed using the $\chi^2$ tests for trend or proportions as appropriate.

Results: During the 23-year study period there were 1,533,317 births and 16,152 diagnostic procedures performed for the primary indication of ultrasound abnormality. In recent years, ultrasound abnormality became the most common indication for prenatal diagnosis (29.4% of tests in 2013-2016) due to steep declines in testing for combined first trimester screening or maternal age alone. In 2016, > 95% of ultrasound-indicated procedures were performed with CMA. and pathogenic copy number variants (CNVs) were the most common abnormal findings (3.5%), followed by trisomy 21 (2.8%) and trisomy 18 (2.6%). The diagnostic yield for ultrasound-indicated tests < 16w was significantly higher than > 20w (31.5% vs 9.0%).

Conclusions: Ultrasound-indicated procedures are contributing to prenatal diagnosis in new ways in the genomic era. A pathogenic CNV is now the most likely diagnosis after ultrasound-indicated testing, rather than trisomy 21 or other whole chromosome aneuploidy. Despite steady improvements in first trimester screening for aneuploidy, the diagnostic yield of ultrasound-indicated tests > 20 weeks has remained stable due to increased utilization of CMAs. However, procedures performed for structural abnormalities < 16w continue to have the highest diagnostic yield, supporting the benefits of early fetal structural assessment at 11-13 weeks.
The normal cavum septum pellucidum during foetal development

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Objectives: The cavum septum pellucidum (CSP) is a fluid filled cavity found in the centre of the foetal brain. It is often used as a marker in ultrasound (US) and magnetic resonance imaging (MRI) scans to establish the neurodevelopmental progress of the foetus and highlight abnormality. There have been previous, older US studies into the size of the CSP, as well as MRI studies on the abnormal CSP however in our literature search we have not yet found a study which looks at the normal size of the CSP in foetal MRI, this study aims to address that gap.

Methods: This retrospective study uses data collected in routine clinical practice. In the coronal plane the slice must contain: the sylvian fissures, the 3rd ventricle inferiorly, the arms of the lateral ventricles laterally with the corpus callosum bordering superiorly. The width of the CSP is then taken at the widest point. In the axial plain, the sylvian fissures must again be present in the slice, or consecutive slices, and the anatomical boundaries used to measure the length are the genu of the corpus callosum anteriorly and the splenium of the corpus callosum posteriorly. The width is also measured again in this plane.

Results: Initial data using over 200 measurements is promising. The current average gestational age measured is 25.3 weeks. The mean coronal width is 5.3+-1.1mm and the mean axial width is 5.5+-1.2mm. When plotted against gestational age, both graphs show a weak trend of increasing as gestational age increases. The axial length when plotted against the gestational age shows a very clear trend of increasing as gestational age increases until it begins to plateau and fall at 32 weeks.

Conclusions: The coronal and axial CSP widths are highly comparable to existing ultrasound studies (Mott et al. 1992 - 5.7 ± 1.9mm, Jou et al. 1998 - 5.5 ± 1.48mm, Falco et al. 2000 - 5.3 ± 1.7mm). As they are so similar it also indicates their interchangeability in clinical practice depending on the available foetal views. The clear trend in axial length with gestational age indicates the strong possibility of creating a normal range growth curve with the data.

Prenatal sonographic features and parental origin in triploid pregnancies

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Unedited draft - unpublished
Objectives: To analyze prenatal sonographic features in triploid pregnancies and evaluate the association between sonographic features and parental origin. This distinction is essential because of the maternal complications that may occur in triploid pregnancies from paternal origin.

Methods: We performed a retrospective, multicenter cohort study that included all triploid pregnancies diagnosed between 2001-2016 in the Academic Medical Centre and VU University Medical Centre in Amsterdam. Prenatal, perinatal and postnatal parameters were collected from medical records. Fetal anomalies and extra-fetal anomalies on ultrasound were systematically reviewed. Prenatal sonographic features (phenotypes) were compared to parental origin.

Results: 109 triploid pregnancies were identified. In 49.5% of cases anomalies were detected before 14'weeks gestation. Main indication for referral was intra-uterine growth restriction in combination with anomalies detected on ultrasound (27%). Detected sonographic features included early intra uterine growth restriction (67%), brain anomalies (41%), limb defects (38%) and cardiac anomalies (28%). In 17% ultrasound showed placental molar changes. Parental origin was confirmed in 32% (n=35) by DNA-analysis: 34% derived from paternal origin and 66% from maternal origin. Triploidy from paternal origin was associated with a high free β-hCG (median 4,70 MoM), an increased nuchal translucency and placental

Conclusions: Suspicion of a triploidy should rise in case of: an abnormal first trimester combined test, intra-uterine growth restriction, HC/ AC > 95th percentile, or placental molar changes in combination with the presence of fetal anomalies. Paternal and maternal triploid pregnancies show distinctive differences on ultrasound. The present data indicate that it is possible to make a proper assessment of the parental origin of triploidy based on prenatal sonographic features. Molecular genotyping allows a definitive diagnosis but is more expensive and inessential.

P1-73

First trimester ultrasound detection of trisomy 18

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Objectives: Anomalies that were previously detected at 20 weeks’ gestation can nowadays be identified during the first trimester scan. Therefore knowledge and awareness of the appearance of trisomy 18 at different stages of pregnancy is an important prerequisite for
improving the detection in first trimester. The aim of this study was to examine which ultrasound features are noted in trisomy 18 pregnancies during routine first trimester ultrasound exams in the Netherlands. Based on the results an assessment protocol is proposed with the aim to minimize late diagnosis.

**Methods:** In a retrospective study prenatal ultrasound and outcome data were collected for pre- or postnatally diagnosed trisomy 18. Cases were included if one or more ultrasound examinations were performed in the region for which the Academic Medical Centre in Amsterdam provides tertiary care. First, second and third trimester ultrasound findings were retrieved from the regional screening database. The estimated date of delivery was between 01-01-2012 and 31-12-2017.

**Results:** Results on first trimester ultrasound (FTU) findings in 243 fetuses with trisomy 18 examined between 9 and 14 weeks of gestation are presented. Additional details on structural abnormalities will be presented.

<table>
<thead>
<tr>
<th>Ultrasound findings</th>
<th>normal</th>
<th>abnormal</th>
<th>not assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuchal translucency</td>
<td>70</td>
<td>131</td>
<td>42</td>
</tr>
<tr>
<td>Nasal bone</td>
<td>23</td>
<td>44</td>
<td>176</td>
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</tbody>
</table>

**Conclusions:** At present, the first trimester scan is not conducted according to a formal protocol. Therefore information on nuchal translucency, nasal bone and items such as shape of the fetal head, intactness of the abdominal wall are not standardly examined. Routine assessment of these items according to a structured protocol can considerably improve timely diagnosis of trisomy 18.

**P1-74**

**Is the cisterna magna width a useful first trimester marker of aneuploidy?**

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**Objectives:** To assess whether the cisterna magna width (CM) measured in first trimester fetuses was a useful marker for aneuploidy detection.
Methods: This was a prospective study using 2 different cohorts in a tertiary referral center. The first cohort was used to establish the CM reference ranges and the second included high risk fetuses undergoing chorionic villus sampling (CVS) during the period 2012-2016. Women were enrolled in the study after giving informed consent for the ultrasound scan, and also for invasive procedures in the high risk group. Normal CM ranges were constructed using the Lambda-Mu-Sigma (LMS) method. Mean and detection rates using the 95th percentile for CM observed in chromosomal anomaly groups were compared with those obtained in chromosomally normal fetuses.

Results: The 50th percentile for CM ranged from 1.66 mm to 2.70 mm when CRL increased from 45 mm to 84 mm. Among the 714 high risk fetuses, genetic analysis revealed anomalies in 125 (17%) fetuses: trisomy 21 (n=63), trisomy 18 or 13 (n=20), monosomy X (n=9), submicroscopic anomalies (n=11), and other (n=22). The Mean CM for euploid fetuses was 2.4 mm (95%CI: 2.3-2.5 mm). While a significant increased CM was observed in trisomy 21; (mean: 2.72 mm; 95%CI: 2.5-2.9 mm) (p>0.05), no differences were found in the other groups. Among the 63 trisomy 21 fetuses, CM> 99th percentile was observed in 36.5%.

Conclusions: The CM in first trimester fetuses appears to be increased in trisomy 21 although its value as an ultrasound marker is limited, because of its 37% detection rate.

P1-75

Measurement of pulmonary vascular volume using virtual reality three-dimensional ultrasound in foetuses with congenital diaphragmatic hernia

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Objectives: To study the pulmonary vascular volume (PVV) using three-dimensional (3D) virtual reality (VR) ultrasound in foetuses with a left sided congenital diaphragmatic hernia (CDH).

Methods: 3D power Doppler volumes of the pulmonary vasculature were obtained in foetuses with left sided CDH between April 2012 and June 2017. Of these foetuses the observed-to-expected lung-to-head ratio (O/E LHR) was retrieved. The PVV was measured selecting and saving the 3D ultrasound volume of the vasculature of the right lung in 4DView. This volume was then analysed in the BARCO I-Space Virtual Reality (VR) system using the V-Scope software (figure). The PVV measurements are correlated with survival and presence of chronic lung disease (CLD) in surviving foetuses.

Results: Forty-nine foetuses were included, of which 109 volumes. Fifty-nine percent of the volumes was of good quality. The survival rate was 80%, of which 46% developed CLD. The
median [interquartile range] of the first measured O/E LHR was 42.4% [31.3-51.5], indicating a moderate/mild lung hypoplasia group. The median [interquartile range] PVV in mm3 at 20 (n=44), 26 (n=39) and 30 (n=25) weeks gestational age (GA) for survivors versus non-survivors 69.9 [37.9-190.0] vs 3.2 [1.9-91.0] (p0.04), 180.7 [48.0-485.1] vs 100.5 [58.3-384.1] (p0.46) and 251.3 [73.0-580.2] vs 96.2 [2.7-452.9] (p0.31), respectively. The PVV was not statistically significantly different between foetuses with and without CLD.

Conclusions: The PVV measured before 24 weeks GA is significantly larger in surviving foetuses with left sided CDH compared to non-surviving foetuses in a group of foetuses with moderate/mild lung hypoplasia.

P1-75 Image.

The validity of the viscero-abdominal disproportion ratio for type of surgical closure in all omphalocele patients throughout pregnancy

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Unedited draft - unpublished
Objectives: To assess the predictive value of the omphalocele circumference/abdominal circumference (OC/AC) ratio for type of postnatal surgical closure and survival in all foetuses with a non-lethal omphalocele, and to evaluate the trend of the OC/AC-ratio during gestation.

Methods: This retrospective cohort study was performed in all prenatally diagnosed liveborn cases with an omphalocele between 2000-2015. The OC/AC-ratio and liver position were determined using 2D-ultrasound at three time points during gestation from 12 weeks onwards. Primary outcome was type of surgical closure. In a secondary analysis we examined the predictive value of the OC/AC-ratio for survival and the trend of the OC/AC-ratio for type of closure.

Results: In 27/45 patients the defect was closed primarily and 38/45 survived. We found a significant association between the OC/AC-ratio and 1) primary closure (optimal cut-off: 0.61), and 2) survival (optimal cut-off: 0.75). The cut-off values for predicting closure decline with increasing gestation from 0.71 in first trimester to 0.63 in third trimester. Multiple OC/AC-ratios were measured in 33/45 cases. We found no association between the trend of the OC/AC-ratio and type of closure. In all cases without liver herniated in the cele (n=23), the defect was closed primarily.

Conclusions: Type of closure and survival in omphalocele patients can be predicted prenatally using the OC/AC-ratio. The cut-off values decline with increasing gestational age.

P1-77

Prenatal diagnosis of corpus callosum anomalies

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Objectives: The diagnosis of Corpus callosum (CC) anomalies by prenatal ultrasound (pUS) has improved over the last decade due to enhanced diagnostic tools and transvaginal sonographic approach. Our aim was to investigate the presence of associated anomalies or isolated forms. We differentiated between complete agenesis (cCCA) and dysgenesis of the CC (CCD)
(either partial agenesis or abnormally shaped CC), and investigated short term outcome, being termination of pregnancy (TOP) or live birth, and neurological outcome.

**Methods:** We performed a retrospective study from January 2007 to December 2017. CC anomalies were diagnosed by prenatal ultrasound in 143 cases. We compared the outcome of pUS and prenatal MRI (pMRI). Associated malformations (structural and chromosomal) in both groups were compared as well as the population characteristics, gestational age at diagnosis and the obstetric outcome. Standardized questionnaires (PARCA-R questionnaire) were send to collect the neurologic outcome of the infants.

**Results:** Of 143 cases, 57 showed cCCA (40%) and 86 CCD (60%). Outcome was unavailable in 26 cases (7 cCCA and 19 CCD) and were therefore excluded. Of the remaining 50 cCCA, 22 were isolated on pUS or genetic analysis. pMRI in 17/22 revealed anomalies in 5. In 33 cCCA with additional anomalies, 25 opted for TOP. Of the remaining 67 CCD, 12 had no associated malformations on pUS or karyotyping. MRI revealed 2 cases with additional anomalies. In 57 CCD with additional malformations, 38 opted for TOP. Upon completion of the data, additional obstetric and neurological outcome will be presented.

**Conclusions:** Compared to the c CCA group, the CCD group revealed additional malformations more frequently. In case of associated anomalies TOP was more frequently performed in the cCCA group. TOP was uncommon if an isolated dysgenesis of the CC was present on ultrasound. More often, additional malformations were found on MRI in cCCA than in the partial agenesis group. These additional findings didn’t lead more frequently to TOP.

P1-78

**Chronology of lesions on ultrasound in CMV-infected fetusses and comparison with MRI findings**

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**Objectives:** Objectives: Primary CMV seroconversion during the first trimester of pregnancy, may result in a serious CMV related fetopathy in 2% of neonates. PCR examination on the amniotic fluid increases the sensitivity/specificity of prenatal imaging by detailed neurosonogram. Very often a fetal MRI scan is added to the investigation.

The main goal of this study is to investigate the chronology in the development of lesions. Witch lesion appears first in CMV infected fetuses. The added value of a MRI scan in a CMV PCR positive baby after a detailed neurosonogram is a secondary goal.
Methods: We conducted a retrospective study including all fetuses with a positive amniotic fluid (AF) PCR for CMV (from 2007 to 2015). At least one fetal ultrasound and fetal MRI scan had to be performed in our center. AF PCR was expressed as copies/ml.

Results of ultrasonography were compared over time. The duration of pregnancy when the first lesion(s) appeared was noted and the lesions were compared with the final abnormalities found on neurosonogram and MRI.

Results: A total of 150 amniotic fluid samples showed a positive CMV PCR. In 90 of these patients a prenatal ultrasound and prenatal MRI was performed. First lesions that appeared included periventricular halo and echogenic bowels. These findings showed around the 22nd week of pregnancy. Later in pregnancy (around week 30) calcifications and lenticulostriate vasculopathy are visible. Subependymal and periventricular cysts are usually visible later in the third trimester. All fetuses with severe lesions on ultrasound had serious anomalies on MRI scan.

Conclusions: Conclusion: Chronologically first CMV-related lesions seem to appear in the second trimester and are usually subtle findings like echogenic bowels and periventricular halo. Later in pregnancy calcifications, cysts and lenticulostriate vasculopathy become more apparent. Additional fetal anomalies picked up with an MRI scan after a detailed neurosonogram do not seem to change the further treatment of the pregnancy in CMV-PCR positive patients.

P1-79

The added value of the first-trimester scan in the prenatal detection of isolated severe congenital heart defects

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³Leiden University Medical Center, Department of Obstetrics and Fetal Medicine, Leiden, Netherlands
⁴VU University Medical Center, Amsterdam, Netherlands

Objectives: To assess the added value of the first-trimester scan after implementation of a national screening program in the Netherlands on prenatal detection rate, time of diagnosis and outcome of fetuses diagnosed with an isolated severe congenital heart defect (CHD).

Methods: Retrospective case series of 255 pre- and postnatally diagnosed cases of isolated severe CHD born between 01-01-2007 and 31-12-2015 in the Academic Medical Center

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(Amsterdam) and its affiliated centers. Severe CHD was defined as being potentially life threatening and requiring surgery or intervention within the first year of life.

We compared cases with prenatally both a first and second-trimester scan to those with only a second trimester structural anomaly scan. A first-trimester scan was classified as an additional examination of the fetus between the 11th and 14th week of pregnancy.

Results: The total prenatal detection rate was 68.5% during the whole study period. A first-trimester scan was performed in 55.3% of the women and in these cases 23.4% of the CHDs were found before 18 weeks gestational age. The group with first-trimester scan showed a significantly higher and earlier prenatal detection rate compared to cases with only a second trimester structural anomaly scan, 75.9% versus 59.6% and 19+1 versus 20+4 weeks, respectively. Prenatal detection of CHD in the first trimester showed a strong correlation with severity, which was reflected in a significantly higher rate of pregnancy termination of 33.3% versus 15.8%.

Conclusions: The first-trimester scan has an added value in the detection of congenital heart defects. It has a significant impact on the prenatal detection rate, time of diagnosis and outcome. Introducing a protocol for first-trimester screening would significantly improve the overall detection rate of congenital heart defects.

P1-80

MR spectroscopy of amniotic fluid for lactate and lecithin - a feasibility study

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Objectives: To assess the feasibility, quality and value of MR spectroscopy of amniotic fluid in a clinical setting to detect lecithin as a marker of fetal lung maturity and lactate as an indicator of fetal distress.

Methods: 31 patients were prospectively recruited from patients attending for fetal MRI in a tertiary referral setting. MR spectroscopy was performed in the largest pool of amniotic fluid available. T2 Weighted imaging was done pre and post spectroscopy to assess fetal position and likely hood of contamination. Spectra were graded for quality and analysed in terms of the distinct peaks seen. Single speactr were obtained and no repeats were performed. Phantoms of lactate and lecithin at known concentrations were also used and scanned at 1.5T, 3T and 9.4T.

Results: 19 spectra were high quality. In 27 a peak was seen at 1.3ppm consistent with lactate on the spectra obtained both from 1.5T and 3T scanners and was not dependent on Gestational age. No other peaks were seen. Phantom studies showed that lactate was detectable by MRS at
1.5T and 3T even at levels lower than seen physiologically. Phantom studies using lecithin did not show a peak on MRS at 1.5T or 3T. A peak was seen on 9.4T at 10mM.

**Conclusions:** The defined lactate peak in the 0.5mM solution suggests clinical scanners are able to identify lactate in concentrations much smaller than those seen physiologically. The level of lecithin, even at term, is too low to be detected by MR spectroscopy at 1.5 or 3T. Detection at 9.4T at 10mM (over 100X higher than seen physiologically) suggests that MR spectroscopy is not clinically useful for assessing lung maturity. To our knowledge, this is the first study to analyse both the feasibility of using MRS to analyse amniotic fluid in a clinical setting, and the metabolites present in a relatively large cohort.

P1-81

**Differentiation of fetal abdominal cystic mass between fetal intestinal obstruction and meconium peritonitis**

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**Objectives:** To explore the difference of abdominal cystic mass between fetal intestinal obstruction and meconium peritonitis (MP) in prenatal.

**Methods:** We reviewed a fetus who diagnosed MP by ultrasound in prenatal and finally confirmed intestinal obstruction in postnatal surgery. A 30-year old pregnant woman was referred to our center for amniotic fluid reduction, cystic mass aspiration and invasive diagnosis because her ultrasound at 29 weeks of gestation found a dilated bowel loop, abdominal cystic mass, echogenic bowel and polyhydramnios.

**Results:** Result of infection testing and chromosomal microarray analysis (CMA) was normal. At 30+2 and 36+4 weeks' gestation, ultrasonography in our hospital indicated a large abdominal cystic mass with patchy high echo, bowel dilation along with polyhydramnios and the fetus was diagnosed MP. Prenatal ultrasound never detected other distinctive features of MP particularly ascites and abdominal calcifications. The infant was spontaneously delivered at 36+5 weeks' gestation without abdominal distension. In addition, radiography revealed dilated bowel and MP. Laparotomy was performed at 1 day after birth and the diagnosis of intestinal obstruction was confirmed. The infant was subsequently discharged in good health.

**Conclusions:** Our case illustrated that careful examination of the fetal abdominal cystic mass along with other ultrasound finding, especially the fetal ascites and abdominal calcifications, during fetal life might be helpful for distinguishing meconium peritonitis and intestinal obstruction in prenatal, and it also is vital to postnatal management.
Preparing to evaluate pre- and postnatal mesenchymal stem cell therapy to improve prognosis in osteogenesis imperfecta – the Boost Brittle Bones Before Birth study (BOOSTB4)

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Objectives: Osteogenesis imperfecta (OI) is a heterogeneous inherited condition. Severe forms present prenatally and carry significant long-term morbidity. Preliminary clinical experience indicates that transplantation of fetal mesenchymal stem cells (MSC) before and after birth may ameliorate symptoms. To inform the development of the BOOSTB4 trial that will evaluate the safety and efficacy of pre- and/or postnatal MSC transplantation in OI types III and severe type IV we aim to: (1) explore stakeholder views to understand perceived benefits or concerns, identify ethical issues and parental support needs, and (2) to establish rapid molecular diagnosis using exome sequencing to enable accurate case selection.

Methods:

1. Semi-structured qualitative interviews were conducted with four groups: parents/carers of children affected with OI, adults with OI, health professionals and patient advocates. Interviews were digitally recorded, transcribed verbatim and analysed using thematic analysis.

2. Women undergoing invasive testing following ultrasound detection of abnormalities suggestive of OI following multidisciplinary team (MDT) review, were consented for parental and fetal (excess amniocytes) DNA sequencing. Agilent SureSelectXT and a clinical exome enriched for a 20Mb region focused on 240 genes causative of skeletal dysplasias was used for variant identification. Potential pathogenic variants were reviewed at MDT, confirmed and a diagnostic report produced.
Results:

1. To date the 49 interviews held were generally positive. Early treatment was considered advantageous for reducing severity by avoiding fractures during rapid bone development and could bring psychological benefits for parents. Common concerns were procedure safety, short/long-term side effects, effectiveness of infusions and difficulties in decision-making with unknown treatment efficacy. Support needs differed in families with a history of OI versus an unexpected sonographic diagnosis.

2. OI, including types III/IV and VIII, was diagnosed in 6/9 (67%) cases referred so far. Two cases, both with good outcomes, remain undiagnosed, and one had hypophosphatasia. Time to diagnosis was reduced to 12 days.

Conclusions: Stakeholders have mixed views regarding stem-cell therapy for OI. Good communication, significant support and time for reflection during decision-making will be crucial for parents to make informed decisions about this approach to management. Definitive diagnosis of OI can be made in less than two weeks using clinical exome sequencing with variant analysis targeted to skeletal genes. A multidisciplinary approach to case selection and variant interpretation with access to good ultrasound images and expert genetic, scientist and fetal medicine input results in high diagnostic yield.

These data are informing development of the BOOSTB4 pan-European trial that will commence in 2018.

P1-84

Fetomaternal hemorrhage and the association with persistent pulmonary hypertension of the newborn

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Objectives: Fetomaternal hemorrhage (FMH) and persistent pulmonary hypertension of the newborn (PPHN) are both significant causes of neonatal morbidity and mortality. Although there is an increased risk of PPHN in anemic newborns, reports on the association between FMH and PPHN are rare. Unknown is whether treatment of fetal anemia by intravascular intrauterine transfusions (IUTs) in case of FMH affects the risk of PPHN. In this present study, we reviewed all consecutive cases with FMH at our center, evaluated the incidence of PPHN and speculate on a possible effect of IUTs on the risk of PPHN.
Methods: We extracted all cases of severe FMH from our prospective NICU database of neonates admitted between January 2006 and January 2018. We evaluated the incidence of PPHN and described the prenatal and postnatal features of FMH cases, including any treatment by IUT and presence of severe hypoxic brain damage on imaging.

Results: PPHN occurred in 11 of 29 (37.9%) newborns with severe FMH. Six (21%) FMH cases were treated by IUT, of whom one developed PPHN (17%). 43% (10/23) of neonates without IUTs developed PPHN. Eight cases had (proven or suspected) early onset sepsis, four of these cases developed PPHN. PPHN occurred in five out of nine cases with perinatal asphyxia, of whom two were treated with hypothermia. 31% (9/29) of newborns had severe hypoxic brain damage.

Conclusions: This case series demonstrates that anemia as a result of FMH should be recognized as a cause or at least a significant contributing factor for PPHN. As the development of severe PPHN is difficult to predict and FMH may cause serious harm to the infants, we advise that all patients with suspected FMH should be examined for fetal anemia, and if possible treated by an IUT, and deliver in a tertiary care center with iNO treatment options.

P1-85

Translating human cognitive tests to evaluate communication and hippocampal learning in mouse models of Down Syndrome

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Objectives: Children with DS utter fewer vocalizations than typically developing children. Adolescents and adults with DS exhibit hippocampal learning deficits in the Cambridge Neuropsychological Test Automated Battery (CANTAB). Here we used ultrasonic vocalization (USV) and the CANTAB visual discrimination (VD) task to analyze communication and hippocampal learning in the Dp16, Ts65Dn and Ts1Cje mouse models of DS. Our goal was to establish baseline data in untreated mice be able to compare the effects of therapy.

Methods: For USV, postnatal days (PND) 3 to 10 trisomic and euploid (Eup) littermates were separated from their mother for 20 min. The number of USVs was recorded for 10 min. For CANTAB studies, adult mice (Dp16=14, Ts65Dn=12, Ts1Cje=12, and Eup mice) were trained to press a “Flower” image on a touchscreen in exchange for a milkshake reward during pre-training (stages 1-5). During VD the mice had to choose between two images (“Airplane,” associated with a reward, and “Spider,” with no reward). Percent of correct responses and the number of days to reach criteria (70% correct responses) were analyzed.

Unedited draft - unpublished
Results: For USV experiments, Ts65Dn pups showed delayed vocalization patterns between PND3 and 7 (p<0.001). Analyses of USV profiles in Dp16 and Ts1Cje pups are ongoing. For CANTAB experiments, all Ts1Cje, Dp16 and Eup mice reached Stage 5 in less than 50 days of training. Five Ts65Dn and 1 Eup animals reached Stage 5 after 63 days. Ts1Cje mice took longer to move to VD vs. Eup (p=0.09). There were no differences between Dp16 and Eup mice. At VD, the average percent of correct answers was significantly lower in Dp16 (23.53±3.39%) and Ts1Cje (22.70±1.93%) versus Eup (35.51±2.45% and 32.18±1.49%, respectively, p<0.05).

Conclusions: We successfully applied human cognitive tests to demonstrate communication and learning deficits in three mouse models of DS. Future experiments will evaluate if pre- and/or postnatal therapies decrease the time intervals to achieve training milestones.

P1-86

Role of amniocentesis in the management of cervical insufficiency with bulging membranes in the second trimester

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Objectives: Describe perinatal outcome of pregnancies affected by cervical insufficiency and bulging fetal membranes in the second trimester of gestation, and to compare admission-to-delivery interval and pregnancy outcomes in this patient, according to amniotic fluid analysis at admission and cervical cerclage placement.

Methods: This study is a retrospective cohort study including 77 cases of singleton second trimester pregnancies between 150/7 and 266/7 with diagnosis of cervical insufficiency with exposed fetal membranes at physical examination in two tertiary health centers between 2009 – 2017. We compared the pregnancy outcomes in three different groups: Group 1: amniocentesis negative with placement of cervical cerclage. Group 2: amniocentesis not performed with placement of cervical cerclage. Group 3: positive amniocentesis with or without cervical cerclage.

Results: 70 patients were evaluated at admission with diagnostic amniocentesis to detect intraamniotic infection /inflammation. In 7 patient’s amniocenteses was not performed. The prevalence of intraamniotic infection / inflammation observed in this group was 18.9%. The outcomes of patients with positive amniocentesis (group 3) was remarkably poor: Median latency to delivery in this group was 1.0 day (interquartile range: 1.0 – 10.0) and median
gestational age at delivery was 22.2 weeks (interquartile range: 20.7 – 23.4). Neonatal survival in this group was only 7.7%. Group 1 was the group in which cerclage was most beneficial.

Conclusions: In patients with cervical insufficiency and bulging membranes during the second trimester of pregnancy, the prevalence of IAI/I is high. The outcome of affected pregnancies is poor. Performance of amniocentesis before placing a cervical cerclage is an easy and safe procedure that permits selection of the best candidates for cervical cerclage and exclude those who will get little or no benefit from the therapy.

P1-87

In utero treatment of a large symptomatic rhabdomyoma with sirolimus

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Objectives: Rhabdomyomas are the most common fetal cardiac tumors detected prenatally and can rarely cause complications such as arrhythmias, hydrops, vascular obstruction and spontaneous fetal demise. Although most rhabdomyomas seen in fetal tuberous sclerosis decrease in size after birth, very large lesions can cause ventricular dysfunction or obstruct the ventricular outflow tracts, thereby compromising neonatal outcomes. Inhibitors of the mammalian target of rapamycin (mTOR) pathway have been used with success for the treatment of symptomatic rhabdomyomas in infancy. We describe the use of sirolimus for the treatment of a large, rapidly growing, fetal rhabdomyoma causing ventricular dysfunction at 31 weeks gestation.

Methods: A healthy 27-year-old nulliparous woman was found to have multiple cardiac rhabdomyomas in her fetus at 21 weeks gestation, with the largest lesion in the left ventricle measuring 10x5mm. Neurosonography and fetal MRI demonstrated the presence of cerebral tubers, suggesting a diagnosis of fetal tuberous sclerosis. Progressive growth of the cardiac rhabdomyomas was noted on serial echocardiography, the largest measuring 47x39 mm at 32 weeks gestation with concomitant deterioration of cardiac function. Given the poor neonatal prognosis, the option of experimental prenatal treatment with sirolimus, a mTOR inhibitor, was offered following multidisciplinary counselling and written informed consent.
**Results:** Transplacental treatment with sirolimus was initiated at 31.6 weeks gestation via maternal administration, aiming for maternal serum trough levels between 10-15ng/mL. Weekly surveillance was performed with fetal ultrasound and maternal bloodwork, to assess immune, renal and hepatic function. Within 4 weeks of treatment, the mass decreased significantly in size with improvement in ventricular function and resolution of tricuspid regurgitation. Sirolimus was discontinued at 36.1 weeks gestation to allow recovery of maternal immune system prior to delivery. A male neonate weighing 4300g was delivered at 39 weeks. The first postnatal echocardiogram demonstrated only mildly reduced biventricular systolic function and trivial pericardial effusion.

**Conclusions:** This case suggests sirolimus is a therapeutic option for the treatment of large cardiac rhabdomyomas in utero. Nonetheless, given the limited safety data, this option should only be reserved for cases with a poor prognosis, after extensive counselling.

P1-87 Table.

**Rhabdomyoma diameter and volume**

- Largest diameter (cm)
- Volume (cm³)

![Graph showing rhabdomyoma diameter and volume over weeks of gestation.](image)

Sirolimus started
Sirolimus stopped

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P1-90

**Daily gentamicin using ideal body weight demonstrates lower risk of postpartum endometritis and increased chance of successful outcome compared with traditional 8-hour dosing for the treatment of intrapartum chorioamnionitis**

Daniel Martingano¹, Audrey Renson², Sharon Rogoff³, Shailini Singh⁴, Meera Kesavan², Juliette Kim², Jeanne Carey²

*Unedited draft - unpublished*
Objectives: This study seeks to determine whether daily dosing of gentamicin using ideal body weight for the treatment of intrapartum chorioamnionitis is more or equivalently efficacious when compared to traditional 8-hour dosing regimens.

Methods: We conducted a 7-year retrospective cohort study and reviewed charts on all women receiving treatment for intrapartum chorioamnionitis which included intravenous gentamicin daily dosing calculated using 5 mg/kg ideal body weight (IDW) or receiving traditional every 8 hour dosing of gentamicin. Our primary outcomes were resolution of infection following delivery without the development of maternal endometritis and/or neonatal sepsis. We calculated the risk ratios of each outcome in the ideal v traditional dosing groups using modified Poisson regression both crude and adjusted. Adjusted models were controlled for variables determined to be potential confounders.

Results: The study included 500 patients with 255 patients receiving daily dosing of gentamicin and 245 receiving traditional dosing of gentamicin. In crude analysis, IDW daily dosing was associated with a lower risk of postpartum endometritis (RR 0.42, P=0.032). After adjusting for BMI, diabetes mellitus, gestational blood pressure >140/90, group β-Streptococcus status, race, advanced maternal age (>34 y), and parity, the IDW daily dosing group had a 5% greater chance of successful outcome (RR 1.05, P=0.046) and a 64% lower risk of endometritis (RR 0.35, P=0.017).

Conclusions: Daily dosing of gentamicin using ideal body weight is associated with a lower risk of postpartum endometritis and higher chance of a successful outcome in the treatment of intrapartum chorioamnionitis compared with traditional 8-hour dosing in our ethnically diverse, urban population and thus may be considered a superior option to every 8 hour dosing regimens.

P1-91

The first results after continuous amnioinfusion via a subcutaneously implanted port system with PPROM and anhydramnios <28 + 0 weeks of gestation

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2 Voronezh State University, Belgorod, Russian Federation

Unedited draft - unpublished
Objectives: Preterm premature rupture of membranes (PPROM) represents one of the main causes of high neonatal mortality and morbidity. Amniotic fluid loss with oligo/anhydramnios is associated with extreme preterm birth, pulmonary hypoplasia and the “fetal inflammatory response syndrome” (FIRS). Preterm birth, for those babies who remain alive, is in higher risk of disability, which surely burdensome for families and health care’s system. The aim of the study was to prolong the PPROM-delivery interval without to increase the risk of FIRS using continuous amnioinfusion with artificial amniotic fluid “flush out” therapy, through a subcutaneously implanted port-system.

Methods: The five patients were carefully selected according to the developed protocol which includes entry and exclusion criteria. Continuous amnioinfusion (100 ml/h, 2,4 L/24h, SDP (4±2 cm) via a subcutaneously implanted port system (Tchirikov Perinatal Port System, PakuMed GmbH, Germany) in all patients with PPROM and oligohydramnios on 25/0-27/0 weeks’ gestational using hypoosmotic amniotic fluid like solution. The treatment was conducted according with developed protocol including verification of classical PPROM (PAMG-1, SDP, amnio-dye test), antibiotic therapy with Amoxicillin. The newborns study included leukocytosis, CRP and procalcitonin control.

Results: Prolongation of pregnancy by amniotic lavage after a PPROM for 10 days or more. The level of CRP in the blood decreased 2.3 times to 0.47 ± 0.13 ng / ml (Z-2.66, p = 0.007), interleukin-6 1.75 times to 1.63 ± 0 , 33 pg / ml (T-9.5, p = 0.02), procalcitonin 1.9 times to 0.34 ± 0.08 ng / ml (Z-3.05, p = 0.002). The “flush-out” was the obstruction a FIRS. The level of leukocytosis was -8.46 ± 0.7 * 109 / l, C-reactive protein -0.29 ± 0.05 mg / dl, procalcitonin-0.41 ± 0.08 ng / ml).

Conclusions: The «flush-out» method decreased the PPROM-delivery interval for 10 days. The new method could be certainly use for the treatment of PPROM with oligo/anhydramnion < 28/0 weeks’ gestation. The prospective randomized international trial has been started 2016. Sponsoring: Center of Fetal Surgery, University Hospital Halle (Saale) and Russian Science Foundation, grant no. 15-15-00137.

Fetal interventions for left atrium decompression

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Unedited draft - unpublished
Objectives: Certain severe left-heart obstructive lesions are associated to a highly restrictive or intact atrial septum (RAS) that significantly increases perinatal mortality. In these patients, fetal left atrial hypertension results in abnormal lung development with lymphangiectasia and pulmonary vein muscularization. Fetal therapy has been proposed in order to decompress the left atrium and prevent the disease’s progression. Our aim is to report our experience with percutaneous ultrasound-guided atrial septum puncture and/or stenting of the fetal atrial septum to decompress the left atrium.

Methods: Retrospective review of clinical records of patients who underwent a fetal cardiac left atrium decompression procedure at the Fetal Medicine Unit of the Hospital Italiano de Buenos Aires between 2015-2018. The inclusion criteria for fetal intervention were severe left heart obstructive lesions and RAS, maternal age >18 years, no significant maternal morbidity, and a singleton pregnancy with no fetal extra cardiac malformations nor genetic abnormalities.

Results: We included 5 patients, 2 (40%) with intrauterine death during the procedure and 3 (60%) with technical success, including 1 dilatation of the foramen ovale in a fetus with hypoplastic left heart syndrome (HLHS) and RAS, and 2 stent placements after 2-3 weeks of a previous aortic valvuloplasty in fetuses with critical aortic stenosis and RAS. One of the cases is still ongoing and the other 2 were stable at birth, not requiring neonatal Rashkind procedure for hypoxemia nor presenting pulmonary hypertension. There were no maternal complications.

Conclusions: Fetal left atrial decompression is a feasible procedure that might confer postnatal benefit in a selected group of patients with very high perinatal mortality. As all fetal cardiac interventions it is a safe procedure for the mother but it is associated with a high risk of complications or death for the fetuses, and more studies are needed to determine its potential benefits in clinical practice.

P1-93

The TAPS trial: fetoscopic laser surgery vs. standard care for twin anemia-polycythemia sequence - an open label randomized controlled trial

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¹Leiden University Medical Center, Leiden, Netherlands
²Leiden University Medical Center, Department of Obstetrics and Fetal Medicine, Leiden, NH, Netherlands

Objectives: The aim of this trial is to investigate whether fetoscopic laser surgery improves the outcome for monochorionic twins diagnosed with twin anemia polycythemia sequence (TAPS) as compared to the control group (standard care consisting of expectant management, IUT, preterm delivery). The hypothesis is that fetoscopic laser therapy will improve neonatal outcome by prolonging pregnancy.

Unedited draft - unpublished
Methods: We propose to conduct a multi-center open-label randomized controlled trial to assess if fetoscopic laser surgery improves the outcome of TAPS compared to standard care. We will randomly assign 140 monochorionic twin pregnancies diagnosed with TAPS between 20-28 weeks of gestation to the fetoscopic laser surgery group or the standard treatment group, using a web-based application with a computer-generated list with random permuted blocks, stratified by gestational age at inclusion (20-24 weeks vs. 25-28 weeks) and TAPS type (spontaneous vs. post-laser TAPS). The inclusion period will be 2.5-3 years.

Results: Primary outcome will be a gestational age at birth. Secondary outcomes include a composite of perinatal mortality and severe neonatal morbidity, hematological complications, procedure-related complications and long-term neurodevelopmental outcome at a corrected age of 2 years.

Conclusions: We will present the design of the first randomized controlled trial investigating the best therapy option for TAPS twins. We aim to start recruiting patients around June 2018.

P1-94

Eligibility for and uptake of open fetal surgery for fetal myelomeningocele

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2Prenatal Diagnosis and Medical Genetics, Mount Sinai Hospital, Toronto, ON, Canada
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4Dept of Medical Imaging, Mount Sinai Hospital, Toronto, ON, Canada
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6Dept of Neurosurgery, Hospital for Sick Children Hospital, Toronto, ON, Canada
7Holland Bloorview Kids Rehabilitation Hospital, Toronto, ON, Canada
8PD fellow, Toronto, ON, Canada

Objectives: Fetal surgery for myelomeningocele (fMMC) halves the incidence of both hindbrain herniation and the need for ventriculo-peritoneal shunting for ventriculomegaly and improves motor outcomes in children when compared to those undergoing postnatal surgery. Our aim was to assess the need for a fetal therapy program for fMMC repair in Canada.

Methods: We reviewed all cases of fMMC that were referred to Mount Sinai Hospital from 2008-2017 and assessed their potential eligibility for fetal surgery using the inclusion and exclusion criteria used in the MOMS Trial (NEJM 2011).

Results: We identified 130 cases of fMMC. Fifty-two cases(40%) were ineligible for in-utero repair. Reasons for exclusion were maternal age <18 years(n=2), BMI ≥35 kg/m2(n=9),
associated fetal anomalies (n=24), uterine anomalies (n=2), diagnosis after 26 weeks (n=7),
previous history of preterm delivery (n=4), absence of hind-brain herniation (n=16) or multifetal
pregnancies (n=12). Six fetuses (4.6%) had chromosomal anomalies, all of which had other
anomalies. Twenty-four patients had a combination of maternal and fetal contra-indications. Of
the remaining 78 eligible patients, 42 (54%) terminated their pregnancy, leaving only 36 of
130 (28%) as potential candidates for fMMC repair. Five of 24 (20%) patients referred since
publication of the MOMS trial have undergone fMMC repair, 3 of which were done in Toronto.

Conclusions: Twenty-eight percent of fetuses with MMC are potential candidates for prenatal
repair and 20% of these actually underwent surgical intervention. With an estimated number of
150 cases of spina bifida/year in Canada, this would mean 42 potential surgical candidates, of
whom, at least 8 would undergo fMMC repair. These numbers support the need for a
national fMMC repair program in Canada.

P1-95

The role of antepartum amnioinfusion in the management of severe mid-trimester
oligohydramnios: A case series

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²Prenatal Diagnosis Center & Fetal Medicine Unit, Department of Obstetrics, International Peace
Maternity & Child Health Hospital, Affiliated to Shanghai Jiao Tong University School of
Medicine, Shanghai, China

Objectives:

1. The aim of this study was to assess the benefit of amnioinfusion in patients with
oligohydramnios.

2. To confirm the findings of previous researches that antepartum diagnosis amnioinfusion
followed by cordocentesis in cases of mid-trimester with severe oligohydramnios is feasible and
credible.

Methods:

1. Thirty-seven women with the diagnosis of oligohydramnios between June 2014 and
November 2017 at International Peace Maternity&Child Health Hospital were evaluated.

2. Amnioinfusion and chromosomal studies were recommended and preformed under
informed written consent.
3. Data collect included fetal and maternal clinical characteristics, ultrasound findings and prenatal outcomes.

4. SPSS was used for descriptive statistics.

**Results:** Our work provides reliable data for the management of oligohydramnios by diagnostic amnioinfusion and no major adverse effects happened during this procedure. 86.5%(32/37) of cases received artificial abortion for most reason of fetal abnormalities after AI, 5 cases among them were confirmed the diagnosis of occult PROM, 2 were chromosomal anomalies by cordocentesis. 13.5%(5/37) women gave birth alive, there were 2 preterm infants and one of which delivered before 34 gestational week, another 3 cases had a full-term delivery, all the babies were taken care of and discharged in good condition from NICU.

**Conclusions:** Antepartum amnioinfusion followed by cordocentesis is a valuable technique in cases of mid-trimester with severe oligohydramnios in prenatal diagnosis.

P1-95 Table.

**Table 1. The pregnant outcomes of antepartum amnioinfusion (n=37)**

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The evolving of prenatal screening and diagnosis in Taiwan in 2006 to 2015

Ching Hua Hsiao¹, Ran Chou Chen²

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²Health Promotion Administration, Ministry of Health and Welfare, Taipei, Taiwan

Objectives: To study the evolving of prenatal screening and prenatal diagnosis in Taiwan in 2006 to 2015.

Methods: This was a national registry-based cohort study of 2,017,519 baby births since January 2006 to December 2015. Over the ten year period, the evolving of prenatal screening methods from second trimester double serum test progressed to the first trimester combined test conducting with secondary trimester quadruple, preceding towards the advancement of non-invasive cell free DNA prenatal screening used today. Personal registration numbers of women having had an amniocentesis or a CVS were retrieved from the Taiwan Central Cytogenetic Registry, and cross-linked with the National Registry of Patients to determine the outcome of each pregnancy.

Results: This work was funded by the Health Promotion Administration, Ministry of Health and Welfare. We estimated the total invasive prenatal diagnosis was 440,182 and the overall invasive procedure rate was (440,182/2,017,519) 21.82%. The population prevalence in trisomy 21, 18, and 13 is one in 750, 2967, and 10731, respectively. The proportion AMA of trisomy 21 at 16 weeks’ gestation in women 35–51 years old increased from 11.8% in 2006 to 24.9% in 2015 (Fig. 1). The prenatal detection rate trisomy 21 and 18 increased from 74.1% and 83.3% in 2006 to 97.2% and 99% in 2015, respectively.
Conclusions: The amount of advanced maternal age pregnant women increased year by year and the invasive procedure along with increased by age. The total invasive prenatal diagnosis increased concurrently with the significant increase of maternal age pregnant women in the years 2006 - 2015.

P1-96 Table.

Figure 1. The amount of total, AMA, and younger than 35 years old performance invasive procedure from 2006 to 2015.

P1-97

Prenatal diagnosis of fetal congenital heart disease in Beijing, China

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Objectives: To understand the prenatal diagnosis of fetal congenital heart disease in Beijing, China.

Methods: Descriptive statistics were used to analyze the incidence of fetal congenital heart disease, prenatal ultrasound screening and diagnosis in Beijing during 2014-2016.

Results: From 2014 to 2016, Congenital heart disease incidence in all births of any gestational week was 4.90 %, 6.21 % and 5.38 %. Congenital heart disease incidence in birth after 28 gestational week was 4.90 %, 6.21 % and 5.38 %, in which the proportion of critical

Unedited draft - unpublished
congenital heart disease is 5.48% in 2015, 4.88% in 2016. Prenatal ultrasound diagnosis rate of complex congenital heart disease was 75.20% in 2014, 76.21% in 2015 and 79.91% in 2016.

**Conclusions:** Prenatal ultrasound screening in Beijing is an essential secondary prevention measure of fetal congenital heart disease. The rates of screening and diagnosis have been increasing year by year. At the same time, further efforts on screening and diagnosis of abnormalities other than cardiovascular system should be made.

P1-98

**Hepatic artery flow in first trimester aneuploid fetuses**

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7Department of Maternal-Fetal Medicine, Institute Gynecology, Obstetrics and Neonatology, Hospital Clinic of Barcelona, Barcelona, Spain

**Objectives:** To assess the hepatic artery (HA) flow changes with the use of color and pulsed Doppler in aneuploid fetuses at 10-14 weeks.

**Methods:** HA flow was assessed with the use of color Doppler at a 2.2 – 3.2 Hz PRF range in high risk fetuses undergoing chorionic villi sampling (CVS) at 11-14 weeks due to: a) positive first trimester screening; b) previous aneuploidy; c) increased nuchal translucency >p99; d) risk of genetic syndrome or e) ultrasound anomalies. The HA pulsatility index (PI) was measured when flow was noted. Differences were assessed with the use of $\chi^2$-test.

**Results:** HA flow was assessed in 539 fetuses. Mean maternal age was 36.2 years, and mean CRL was 65 mm. CVS revealed a chromosomal anomaly in 16% (84/539) of the fetuses by means of karyotyping or chromosomal microarray analysis. HA flow was observed in 119 fetuses (22%) with a 1.58 mean PI. In trisomy 21, the proportion of fetuses with present AH flow was 43% (20/47), significantly higher than 20% in euploid fetuses (93/455) ($p = 0.016$). No differences were noted in other anomalies. The mean PI in trisomy 21 was 1.35, similar than 1.54 in euploid fetuses.

**Conclusions:** When HA flow is observed at the first trimester scan there is an increased risk of trisomy 21.
Wolf Hirschhorn syndrome (monosomy 4): Early prenatal diagnosis of a rare genetic condition

Coralia Stefanescu, Eduart Balasa, Elena Cupsa
Euromaterna Hospital, Constanta, Romania

Objectives: The authors analyze a case of IVF-ICSI obtained gestation associated with a particular rare genetic disease.

Methods: During the first trimester scan a particular facial feature was detected and an amniocentesis was performed. The result was monosomy 4.

Results: Wolf-Hirschhorn syndrome (WHS) is an extremely rare chromosomal disorder caused by a missing piece (partial deletion or monosomy) of the short arm of chromosome 4. It occurs in approximately 1 in about 50,000 live births with a female to male ratio of 2:1. More recent studies suggest that the frequency of the disorder is underestimated because of misdiagnosis. The clinical phenotype includes typical facial features, microcephaly, growth retardation, developmental delay and major congenital malformations.

Conclusions: The clinician’s ability to identify and early diagnose WHS is essential due to the severe impairment and high mortality associated to this condition. Genetic counseling is recommended for families of children with Wolf-Hirschhorn syndrome.

Maternal age plus fasting plasma glucose at the first prenatal visit to screen gestational diabetes

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¹NTUH, Taipei City, Taiwan
²Taipei City, Taiwan

Objectives: Identifying first-trimester biomarkers could serve both diminish the need for provocative testing in all pregnant women and allow early intervention to improve outcomes or prevent gestational diabetes mellitus (GDM). In this study, we aim to reveal the first-trimester screening potential of these variables for predicting subsequent GDM and to reduce the need of oral glucose tolerance tests (OGTT) by using a simple model as a specific cutoff.

Methods: A prospective cohort study was conducted among patients who were admitted to our obstetric clinic between January 2013 and December 2017. Participants who provided blood samples in the first trimester, completed prenatal care, and delivered a live, term infant
at our institution were included in the study. Maternal blood samples were obtained during the first trimester examination to determine the HbA1c, fasting plasma glucose (FPG), total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides (TG) and homeostatic model assessment for insulin resistance (HOMA-IR) levels. Furthermore, screening algorithm using FPG in the first trimester (1st FPG) with maternal age was developed.

**Results:** A total of 413 women met all inclusion criteria, 65(15.7%) were in the group of GDM. The age and 1st FPG were significant independent predictors for GDM [adjusted odds ratio (OR= 1.18 (95% CI, 1.1-1.27) and OR = 1.28(95% CI, 1.2–1.36), respectively]. Use of the algorithm with “1st FPG plus maternal age” cutoff of could reduce the need of OGTT (OGTT%) from 62% to 53%, while maintaining good sensitivity (from 92.3% to 90.8%) and specificity (from 100% to 100%). The screening algorithm with a “1st FPG plus maternal age” cutoff could further reduce OGTT% by 27%-38%.

**Conclusions:** 1st FPG and maternal age should be considered in the risk assessment of GDM for they were both independent risk factors associated with the presence of GDM. A screening algorithm for GDM that takes 1st FPG with maternal age into consideration can successfully reduce the need of OGTT when women become pregnant at older ages.

P1-100 Table.

Table 1. Clinical characteristics and laboratory test results at 6–13 weeks of gestation in pregnancy women with and without gestational diabetes mellitus (GDM)

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>All</th>
<th>Non-GDM</th>
<th>GDM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>413</td>
<td>348</td>
<td>65</td>
<td>NA</td>
</tr>
<tr>
<td>Age(years)</td>
<td>33.9±4.3</td>
<td>33.5±4.2</td>
<td>36.2±3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age≥35(N, %)</td>
<td>198(47.9%)</td>
<td>152 (41.4%)</td>
<td>46(100%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nulliparous(N, %)</td>
<td>139(33.7%)</td>
<td>107 (30.7%)</td>
<td>32(49.2%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Family history of DM</td>
<td>247(59.8%)</td>
<td>199(54.2%)</td>
<td>48(73.8%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Past history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GDM</td>
<td>21(5.1%)</td>
<td>8(5.1%)</td>
<td>13(2.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PIH</td>
<td>3(1.6%)</td>
<td>2(1.6%)</td>
<td>1(1.2%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>7(1.7%)</td>
<td>3(0.8%)</td>
<td>4(8.7%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Preterm delivery</td>
<td>11(2.7%)</td>
<td>7(1.9%)</td>
<td>4(8.7%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Macrosomia</td>
<td>4(1%)</td>
<td>2(0.5%)</td>
<td>2(4.3%)</td>
<td>0.06</td>
</tr>
<tr>
<td>PCOS</td>
<td>18(4.4%)</td>
<td>16(4.4%)</td>
<td>2(4.3%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Pre-pregnancy BW(Kg)</td>
<td>56±9</td>
<td>55.7±9</td>
<td>57.6±9.3</td>
<td>0.11</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
<td>22.2±3.6</td>
<td>22.1±3.5</td>
<td>22.8±3.6</td>
<td>0.11</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>113.4±12.6</td>
<td>112.4±12.2</td>
<td>118.7±13.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>67.6±9.2</td>
<td>66.9±9</td>
<td>71.4±9.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Laboratory test results at 6–13 weeks of gestation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>177.1±32.5</td>
<td>177.5±32.2</td>
<td>175.5±34.5</td>
<td>0.66</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>93.4±26.6</td>
<td>93.2±26.5</td>
<td>94.6±27.5</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Unedited draft - unpublished
Integration of rapid molecular techniques including chromosome microarray and short tandem repeat markers in selective fetal reduction of the fetus carrying genetic anomaly in twin when sonographic differentiation between the cotwins is difficult

Wan-Ju Wu¹, Ming Chen²

¹Department of Genomic Medicine and Center for Medical Genetics, Changhua Christian Hospital; and Department of Genomic Science and Technology, Changhua Christian Hospital Healthcare System, Taiwan, Changhua, Taiwan

²Changhua Christian Hospital, Changhua, Taiwan

Objectives: Three women pregnant with dizygotic twins were referred to our center and requested selective fetal reduction because one of the fetuses carried genetic anomalies, including Down syndrome, a pathologial microduplication about 14.50 Mb in size at chromosome 8q24.21q24.3, and one pathological microdeletion about 11.08MB at chromosome 24.2p23. However, ultrasound all revealed two concordant male fetuses without any anomalous features to identify the affected fetus. To avoid iatrogenic errors from label switching of twins, we integrated molecular techniques, intra-amniotic dye and conventional ultrasound to identify the at-risk fetus and finally allocated the procedure correctly during the second trimester.

Methods: In addition to conventional ultrasound makers (placental location, cord insertion and fetal location), we labeled the twin by karyotyping, comparative genomic hybridization (aCGH) and short tandem repeat (STR) maker analysis. Two days before performing selective fetal reduction, we repeated amniocentesis on each fetus for confirmation of the prior results by a 2-day rapid protocol including interphase fluorescence in situ hybridization (FISH), chromosome microarray and STR makers. Simultaneously, we injected 20 mL of maternal peripheral blood into the sacs which were suspected the at-risk fetues.
**Results:** By using the aforementioned measures including genetic, sonographic and amniotic staining techniques, we performed intra-cardiac injection with 20 mL 2% lidocaine on the affected fetues. In addition, a blood sample from fetal heart was preserved for confirmation. The results of FISH, aCGH and STR study were concordant with the prior survey, which demonstrated that our procedure was accurate.

**Conclusions:** Accurate selective fetal reduction in twins is not always an easy task when one fetus carries genetic disorder without prenatal phenotype and no other reliable features (e.g., gender) as a proxy. Although standardized and reliable labeling is mandatory in antenatal ultrasound screening for twin pregnancy, there remains a risk in differentiating fetuses by single sonographic markers beyond gender or anomalous features. In addition to conventional ultrasound examination, we claimed that integrating various techniques (Table 1) for mapping as well as minimizing the interval between diagnostic and interventional procedure are acceptable approaches.

**P1-102 Table.**

<table>
<thead>
<tr>
<th>Method for mapping twins</th>
<th>Proxies</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound</td>
<td>Most reliable: gender, structural anomalies, fetal discordance, etc.</td>
<td>1. In utero switching.</td>
</tr>
<tr>
<td></td>
<td>Others: Placental and cord insertion site, Upper-lower pairs, the sac near cervix</td>
<td>2. Maximization the interval of ultrasound survey and interventional procedure.</td>
</tr>
<tr>
<td>Molecular techniques</td>
<td>FISH, MLPA, STR markers, etc.</td>
<td>1. Depending on clinical scenario and laboratory capability.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Time-saving consideration</td>
</tr>
<tr>
<td>Intra-amniotic dye</td>
<td>Indigo carmine, sodium fluorescein and phenolsulfonphthalein, maternal blood</td>
<td>1. Dye may not remain long enough.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Application on the abnormal fetus for neonatal morbidity consideration.</td>
</tr>
</tbody>
</table>
Ultrasound structural anomalies in patients with trisomy 18

María de Jesús Zavaleta, Monica Aguinaga, Sandra Acevedo

National Institute of Perinatology, Mexico City, Mexico

**Objectives:** The second more common aneuploidy is trisomy 18. It is known as Edward’s Syndrome and is due to the presence of an extra chromosome 18, either full, mosaic trisomy or due to a complex rearrangement. The prevalence of trisomy 18 rises with increasing maternal age. Based on maternal first trimester screening and ultrasound, the majority of patients with trisomy 18 are detected prenatally (Cereda and Carey, 2012). The objective of this study is to present the ultrasound structural anomalies found in the first, second and third trimesters of pregnancy in 12 fetuses with a prenatal cytogenetic diagnosis of trisomy 18.

**Methods:** We performed a retrospective analysis of all fetuses with a prenatal diagnosis of trisomy 18 during 18 months study period. Patients were referred to the National Institute of Perinatology, Mexico City and the ultrasound was performed by a certified fetal maternal medicine medical doctor. We diagnosed twelve patients with trisomy 18. The cytogenetic study was made in fetal cells obtained by a chorionic villus biopsy or amniocentesis. All cases were processed by in situ harvest and GTG technique. We analyzed 20 clones of at least four cultures. Ultrasound reports, biochemical markers, maternal and gestational age were obtained from medical records.

**Results:** Mean maternal age was 35.5 years-old (19 to 45). Mean gestational age by USG was 19.2 weeks with a range between 13 to 26.4 weeks of gestation. All cases showed a full trisomy 18. Five cases were detected in the first trimester, results are shown in the Table. We found six cases in the second trimester of pregnancy and one case in the third trimester. All fetuses showed structural multiple anomalies: Cardiac defects (n=8); hand abnormalities (n=7); Central Nervous System (SNC)(n=6), diaphragmatic hernia (n=3), facial cleft (n=1), clubfeet (n=1) and radial agenesis (n=1). Minor anomalies observed were: retrognatia (n=2); hypothelorism (n=2) and single umbilical artery (n=2).

**Conclusions:** Two patients in the first trimester of pregnancy were detected by abnormal serum screening. Most patients are referred late for study, multiple anomalies are detected by a second trimester ultrasound.
P1-103

Table. First trimester ultrasound anomalies in patients with trisomy 18

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-HCG</td>
<td>0.337 MoM</td>
<td>ND</td>
<td>0.055 MoM</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>0.060 MoM</td>
<td>ND</td>
<td>0.081 MoM</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Nuchal Translucency</td>
<td>1.95</td>
<td>ND</td>
<td>2.04</td>
<td>3.08</td>
<td>ND</td>
</tr>
<tr>
<td>Nasal Bone</td>
<td>Present</td>
<td>ND</td>
<td>Present</td>
<td>Absent</td>
<td>ND</td>
</tr>
<tr>
<td>Maternal Age</td>
<td>40</td>
<td>45</td>
<td>43</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>Echogenic bowel</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Single umbilical artery</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cystic hygroma</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Omphalocele</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anomaly of Ductus venosus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac defect</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Risk for Trisomy 18</td>
<td>1:12</td>
<td>1:5</td>
<td>1:5</td>
<td>1:5</td>
<td></td>
</tr>
</tbody>
</table>

ND: Not Done; PAPP-A: Pregnancy Associated Plasma Protein A; β-HCG: Beta subunit of the chorionic gonadotrophin hormone.

P1-104

Prenatal screening for trisomy 21 using enhanced first trimester screening: Experience and performance

Tianhua Huang, Melanie Bedford, Shamim Rashid, Alan Dennis, Karen Boucher, Ellen Mak-Tam, Wendy Meschino

North York General Hospital, Genetics Program, Toronto, ON, Canada

Objectives: To summarize our experience in the implementation of enhanced first trimester screening (eFTS) in a routine prenatal screening population and to estimate its screening performance for trisomy 21.

Methods: Enhanced FTS was introduced at North York General Hospital in April 2016 to replace FTS. Since November 2017, the 2-sample Integrated Prenatal Screen (IPS) was replaced with eFTS. Women who had a nuchal translucency (NT) measurement and a blood sample taken between 11+0 and 13+6 weeks gestation received an eFTS result. The blood sample was measured for serum pregnancy-associated plasma protein A (PAPP-A), free-β human chorionic gonadotrophin (hCG), placental growth factor (PIGF) and α-fetoprotein (AFP). The risk cut-off
for eFTS is 1 in 350 at term. The study summarized our experience with eFTS and its performance for trisomy 21.

**Results:** Between April 2016 and January 2018, 32,186 women were screened using eFTS. Requests for eFTS surpassed IPS and became the most common screening test at NYGH before IPS was discontinued. Racial specific median and weight equations were introduced for free-β hCG, PlGF, and AFP to adjust ethnic differences in marker measurements. During the study period, the detection rate (DR) of eFTS was 92.7%. The positive rates (PR) were 9.1% and 6.7% respectively before and after IPS was discontinued. The drop in the PR suggested an impact of population bias on screening performance.

**Conclusions:** EFTS was well accepted by pregnant women and health-care providers. By replacing IPS with eFTS, thereby eliminating the need for the second specimen (IPS2), has resulted in laboratory workflow efficiencies and savings. The observed DR was close to its theoretical value. The PR was higher initially but notably lower after the discontinuation of IPS. The higher PR was likely due to population bias as women dropping out of IPS, due to miscarriage or other abnormalities, received an eFTS result. EFTS performance is continually monitored in our screening program.

P1-105

**Fewer women age 35 and older chose Down syndrome serum screening: Impact and implications**

Glenn Palomaki, Geralyn Lambert-Messerlian

*Women & Infants Hospital, Providence, RI, United States*

**Objectives:** Over the last five years, our prenatal screening program has seen changes in the numbers of women screened, the screening test chosen, the distribution of maternal ages in the tested population and the accompanying false positive rate (FPR). Our aim was to quantify these changes and determine whether it might be appropriate to consider modifying the Down syndrome risk cut-off levels to provide more reliable serum screening. This information might also allow for more appropriate educational content and improved screening performance.

**Methods:** Tests available are the Integrated (with a sequential option), Combined and Quadruple tests for Down syndrome. We extracted over 54,000 records in six month intervals from 2013 through 2017 that included, maternal age, test chosen, gestational age, AFP MoM and Down syndrome risk. The number of tests, FPRs and proportion of women age 35 and older were computed for each of the 10 time intervals. Modeling was based on the 2015 US birth records and age-specific DRs / FPRs for each test to determine the overall DRs/FPRs for theoretical distributions of ages with 100% to 0% women age 35 and older.
Results: Over five years, the proportion of older women choosing Integrated or Combined screening declined by 1.1% and 2.0% every six months, respectively. The corresponding FPRs also declined. For Integrated screening, the observed FR declined from 1.9% in 2013 to 1.3% in 2017 (32%) while the proportion of older women declined from 14.9% to 8.5%. Based on modeling, this latter reduction is consistent with an expected FPR reduction of 43%. This indicates the observed FPR changes were driven by fewer older women participating. Modeling also showed the detection rates were reduced by 6 to 10%. Interestingly, the positive predictive values did not change materially.

Conclusions: For the first time, we have documented the declining proportion of women age 35 and older choosing serum screening for Down syndrome and its subsequent impact on the screening performance. ACOG recommends cfDNA screening be offered to such ‘high risk’ pregnancies and insurance tends to cover most costs for these women. One option that screening programs might consider is to lower their screening risk cut-off such that their 2017 FPR would be similar to that in 2012 or 2013 resulting in regaining most of the lost detection.

P1-106

The system of prenatal genetic screening in Kazakhstan: 10 years results and perspectives

Gulnara Svyatova, Damilya Salimbayeva, Meruert Kirikbayeva

RGP on PHV, Scientific Center of Obstetrics, Gynecology and Perinatology, Ministry of Health of Republic of Kazakhstan, Almaty City, Kazakhstan

Objectives: Prenatal screening (PS) in Kazakhstan was established in 2007. It covers all regions with annual level of 400,000 births and fully financed by the government. There are 21 regional departments of Medical Genetics, this is a 1st level of PS, 2nd level of PS is Republican medical genetics consultation in SCOGP. A centralized screening system with regular monitoring and evaluation of the efficiency indicators of PS was created. We use uniform ISPD algorithm, have a bioethics legislation policy and introduced annual PS specialist’s education and trainings.

Methods: PS in Kazakhstan consists of combined test of the first trimester screening for aneuploidies, ultrasound (US) screening in 11-13 weeks 6 days, 18-22 and 30-32 weeks of pregnancy, invasive prenatal procedures for group of high risk. Biochemical screening is carried out on dry blood spots by using the Perkin Elmer uniform certified equipment and reagents with obligatory external QC control (RIQAS, NEQAS UK). The information system “National Genetic Register” of malformations was established in 1998 year for control of PS efficiency.

Results: Since 2007 2.3 mln pregnant women were screened, PS coverage has increased from 10.5% in 2007 to 70.6% in 2017. The effectiveness of invasive procedures has increased from 2.0% to 10.7%. The frequency of congenital malformations has a tendency to decrease from 12.2 in 2007 to 10.5 per 1000 newborns in 2017. The frequency of “indicative congenital
malformations” decreased from 4.5 in 2007 to 3.3 per 1000 newborns in 2017. The most effective result of PS is the significant decrease of defects neural tubes frequency from 0.54 to 0.18 per 1000 newborns.

Conclusions: PS national system was created that is comprehensive, integrated and sustainable. Perspectives of PS in Kazakhstan include the improvement in regulatory framework, increasing of PS coverage and economical effectiveness, the introduction of new nosology (preeclampsia) and new methods (noninvasive prenatal test).

P1-107

Evaluation of PAXgene ccfDNA tube performance versus streck on the VeriSeq NIPT solution NGS platform

Lindsay van Limpt, Jens Philtjens, Brenda Gabriëls, Mario Berth, Davy Vanden Broeck

Algemeen Medisch Laboratorium, Antwerp, Belgium


Methods: Samples were collected in pair in Streck and PAXgene tubes from 49 pregnant women. Streck tubes were treated according to IFU and centrifuged 10 minutes at 1600g at 4°C, without brake, processed within 1 day and analysed within 6 hours. PAXgene tubes were centrifuged 15 minutes at 1900g at room temperature with medium brake, were kept at room temperature for median 6 days before centrifugation or frozen as an aliquot for median 3 days at -20°C before analysis. Plasma was analysed on the Illumina platform (ccfDNA yield, trisomies 13, 18, 21 and sex chromosomes).

Results: Plasma preparation of PAXgene was 50% shorter because of the long swing-out time without break. Both tubes have different caps; an in-depth interview with end-users (n=2) showed a preference towards the cap of PAXgene as this reduced risk of blood exposure. Prolonged storage time of PAXgene did not influence final result: ccfDNA yields were comparable between Streck and PAXgene (10.1 % and 9.8 % respectively and non-significant with paired T-test) with a Pearson correlation of 0.904. Results for trisomies 13, 18, 21 and sex chromosomes were 100% concordant between both tubes and reported back 1 positive screen for trisomy 13.

Conclusions: Performance of PAXgene tubes on the Illumina platform was non-inferior to the validated Streck tubes. Despite challenging storage conditions of PAXgene tubes, ccfDNA yield was similar. PAXgene tubes improved laboratory efficiency by reducing plasma preparation phase with 50%. End-users showed a slight preference for the cap of the PAXgene tube because of ease-of-use and safety.
The revelation of complex chromosomal rearrangements through genome-wide cfDNA testing

Theresa Boomer¹, Erica Soster², Samantha Caldwell¹, Eyad Almasri¹, Jenna Wardrop¹, Sidra Boshes¹, Michelle Hackbardt¹, Phillip Cacheris¹, Vanessa Nitibhon¹, Ron McCullough¹

¹Sequenom Laboratories, San Diego, CA, United States
²Sequenom/Integrated Genetics, San Diego, CA, United States

Objectives: Recent adoption of genome-wide cfDNA prenatal screening provides unique insight into placental findings not previously recognized. Here we present data from our first 28K clinical samples for expanded cfDNA screening, including genome-wide aneuploidy detection and subchromosomal copy number variants (CNVs) ≥ 7Mb, with specific attention to complex chromosomal rearrangements.
Methods: Maternal blood samples submitted for genome-wide cfDNA testing were subjected to DNA extraction, library preparation, and whole-genome massively parallel sequencing as described by Jensen et al. Sequencing data were analyzed using a novel algorithm as described by Lefkowitz et al.

Results: A total of 28,760 samples were submitted to the clinical laboratory, resulting in 1392 positives (4.8%) reported. Complex CNV samples showed an enrichment of ultrasound findings (61%), high risk personal and/or family histories (34%), and multiple high risk indications (20%) when compared to our testing population as a whole (20%, 6%, and 12% respectively). Of the 64 complex CVNs reported, 49 were interpreted as possible translocation events between two chromosomes and 15 as possible recombinant events, isolated to one chromosome.

Subsequent fetal confirmation was reported in the majority (56%), with 27% pending, 11% lost to follow-up, and 5% discordant.

Conclusions: Identification of complex chromosomal rearrangements via cfDNA prenatal screening marks a new era in prenatal testing. These findings tend to segregate with significant high risk prenatal indications. Many known familial rearrangements not previously amenable to cfDNA screening may now benefit from early identification or added reassurance. New discovery of families at risk of carrying a recombinant event often helps to explain past pregnancy complications, as well as clarify future reproductive risk.
Genome-wide cfDNA screening: Trends and lessons from > 40,000 samples

Theresa Boomer\textsuperscript{1}, Erica Soster\textsuperscript{2}, Samantha Caldwell\textsuperscript{1}, Eyad Almasri\textsuperscript{1}, Jenna Wardrop\textsuperscript{1}, Sidra Boshes\textsuperscript{1}, Michelle Hackbardt\textsuperscript{1}, Phillip Cacheris\textsuperscript{1}, Jason Chibuk\textsuperscript{2}, Ron McCullough\textsuperscript{1}

\textsuperscript{1}Sequenom Laboratories, San Diego, CA, United States
\textsuperscript{2}Sequenom/Integrated Genetics, San Diego, CA, United States

Objectives: Genome-wide cell-free DNA prenatal screening continues to increase our insight into placental findings not previously recognized. Here we present data from the first two years of clinical testing for expanded cfDNA screening, including genome wide aneuploidy detection and subchromosomal copy number variants (CNVs) larger \(\geq 7\text{Mb}\).

Methods: Maternal blood samples submitted to Sequenom Laboratories\textsuperscript{\textregistered} for MaterniT\textsuperscript{\textregistered} GENOME testing were subjected to DNA extraction, library preparation, and whole-genome massively parallel sequencing as described by Jensen et al. Sequencing data were analyzed using a novel algorithm as described by Lefkowitz et al.

Results: 41,634 samples were submitted to the clinical laboratory, with 1979 positive results. Similar to prior reported trends, 49\% of all positives showed USFs (either in isolation or combined with another high risk indication), yielding an increased 11\% positivity rate among this cohort. Likewise, 21.2\% of all positives report multiple high risk indications, yielding an increased 13\% positivity rate among this cohort. Late GA testers (\(\geq 20\) weeks) continue to account for a disproportionate amount of positive results (24\%), with the vast majority (81\%) reporting USFs. CNV size range holds steady at \(<10\text{Mb}\) to \(\sim 100\text{Mb}\), with the majority 10-20Mb.

Conclusions: Previously reported trends in genome wide cfDNA prenatal screening results remain consistent, including a higher positivity rate among pregnancies with USFs and multiple high-risk indications, as well as a higher proportion of late GA screening compared to targeted screening. The overall positivity rate as well as positive result distribution among the various result categories remains constant. However, the growing emergence of an ‘average risk’ screening cohort is noted, with a statistically significant increase (p-value <0.001) in size since our last report \(~ 6\) months ago. This may indicate a growing acceptance for genome-wide cfDNA screening among average risk.
Non-invasive prenatal testing and diagnosis on fetal cells from endocervical samples

Laura Bourlard¹, Julie Désir², Bruno Pichon²

¹Erasme Hospital U.L.B, Anderlecht, Belgium
²Center of Human Genetics, Université Libre de Bruxelles, Bruxelles, Belgium

Objectives: The risk for every couple to have a child born with a genetic abnormality is 2-5%. Currently this risk is evaluated during pregnancy with screening tests such as ultrasound and Non Invasive Prenatal Testing (NIPT) which is based on cell-free DNA sequencing from maternal blood sample. Fetal DNA represents ≤ 10% of the total cell-free DNA in maternal blood, so positive results of NIPT have to be confirmed by invasive testing such as amniocentesis or chorionicentesis. Aim of our project is to realize a Non Invasive Prenatal Diagnosis (NIPD) on fetal cells present in cervical samples from 5 WA.

Methods: The first step of this project is detection and isolation of fetal cells from standard cervical samples realized with cytobrush. Samples are washed to eliminate cervical mucus. Then cells are labelled with different fluorescent antibodies which seem to be potentially effective for their detection and isolation by Fluorescent Activated Cell Sorting (FACS). The second step is realization of genetic analyzes either directly or after amplification of these cells. Indeed, there are only few cells isolated from cervical samples (1/1,000-1/2,000) but they are more frequent than in blood (1/100,000-1/200,000) and so their amplification can be necessary to realize genetic analyses.
Results: First results for detection and isolation of fetal cells with FACS are hopeful. It will be necessary to improve removal of cervical mucus to not distort detection and to be sure that isolated cells have a fetal origin and that no cells will be recovered when test is used on not pregnant women. First results for detection of fetal genetic abnormalities are achievable either directly on few cells or after Whole Genome Amplification and show that it will be possible to detect genetic anomalies by genetic molecular techniques (including array-CGH) from few fetal cells retrieved from cervical samples.

Conclusions: It seems feasible to detect fetal genetic abnormalities completely non-invasively much earlier in pregnancy (5 WA) than with current techniques (from 11 WA) In concrete terms, this test will enable pregnant women, whose fetuses are affected by a genetic disease which can be responsible for serious consequences on their health or even their survival to interrupt their pregnancy earlier than with current invasive tests. It seems both medically and psychologically less difficult and traumatic. In addition, it will enable fertile women to avoid heavy and long in vitro fecundation treatments in a context of preimplantation genetic diagnosis (PGD).

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Over a half million noninvasive prenatal tests: A clinical laboratory experience

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Objectives: The adoption of noninvasive prenatal testing (NIPT) for screening has been rapid, resulting in a paradigm shift in patient management. Here, we describe the laboratory experience and clinical performance of the MaterniT®21 PLUS test, including for select subchromosomal deletions.

Methods: Over 600,000 maternal blood samples submitted to Sequenom Laboratories for MaterniT®21 PLUS testing were subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing as described by Jensen et al.1 Testing includes aneuploidies of chromosomes 13, 18, 21, X, Y and selected subchromosomal events. Statistical analysis of this large patient cohort was undertaken.

Results: The predominant indication for testing in this large cohort was advanced maternal age (56.7%), followed by abnormal ultrasound findings (9.5%) and positive serum screening (6.1%). Compared with 2015, when only 3.7% of samples were from average risk pregnancies, in 2016 20.2% of samples were from average risk pregnancies. Overall, the positivity rate in singletons for trisomy 21 was 1.23%, 0.39% for trisomy 18, and 0.19% for trisomy 13. More than 26,000
cases (~4.05%) were reportedly multifetal. Estimated performance based on ad hoc clinical outcome shows that sensitivity and specificity meet or exceed the original clinical validation studies.

**Conclusions:** MaterniT®21 PLUS offers pregnant patients accurate and reliable screening for fetal aneuploidy. This laboratory developed test has demonstrated positivity rates for trisomy 21, 18 and 13 that mirror those found in large studies on high-risk populations that underwent invasive testing. The addition of subchromosomal events was shown to perform well, with good clinical correlation. Operational performance demonstrated a robust and efficient process that has met or exceeded performance from the original clinical validation studies.

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**Clinical outcomes of genome-wide cfDNA for cases screening positive for trisomies 7, 16, 22, 3, and 15**

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**Objectives:** Cell-free DNA (cfDNA) testing for common aneuploidies has been integrated into prenatal care for both high-risk and average-risk pregnancies. Expansion of cfDNA technology includes select microdeletions, large copy number variants, and esoteric aneuploidies. Initial data regarding outcomes from a commercial genome-wide cfDNA test has been previously described.¹²³ When positive results for esoteric aneuploidies are reported, residual risk for fetal mosaicism/aneuploidy, confined placental mosaicism, uniparental disomy, and adverse pregnancy outcome may exist. The outcomes of the five most common esoteric aneuploidies seen with a commercial genome-wide cfDNA test are described here and are consistent with recent literature⁴⁵ from other groups.

**Methods:** A retrospective analysis was performed on over 28,000 maternal blood samples submitted for genome-wide cfDNA analysis. Samples were subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing.⁶ Sequencing data were analyzed using a novel algorithm to detect trisomies and subchromosomal, genome-wide copy number variant (CNVs) 7Mb and larger.¹ The results that screened positive for an esoteric aneuploidy (excluding 21, 18, 13, X and Y) were reviewed. Clinical outcomes were requested from ordering providers as part of routine follow-up of all positive samples. Adverse pregnancy outcomes were defined as growth restriction, preterm labor, miscarriage/fetal demise, or structural ultrasound anomalies.

**Results:** Approximately 1400 of over 28,000 samples (~5%) returned a positive result for any aneuploidy, CNV or microdeletion included on the test. Among these, approximately 200 cases
(~14%) were positive for an esoteric aneuploidy. In order of frequency, the most common positive result for an esoteric aneuploidy involved chromosomes 7, 16, 22, 3, and 15. Cases that screened positive for trisomy 16, 22, or 15 were more likely to have an adverse outcome (>60%) while trisomy 7 was less likely to have an adverse outcome (<30%). Cases with reported trisomy 3 risk appear to fall in between the two groups.

Conclusions: The differences between outcomes can likely be attributed to the origin of the trisomic event. Literature on placental mosaicism describes meiotic events (as is usually seen with trisomies 15, 16 and 22) to be at increased risk for fetal trisomy, uniparental disomy, or pregnancy complications. Conversely, mitotic events (as is usually seen with trisomies 3 and 7) are more likely to be benign. Although sample sizes are limited, preliminary conclusions can be drawn from this cohort. Missing or incomplete follow-up for clinical cases is a limitation of this cohort, but the available data provides a valuable tool for clinical counseling.

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Development of VeriSeq NIPT Solution, a PCR-free, whole-genome paired-end sequencing-based methodology for noninvasive prenatal screening of fetal chromosomal aneuploidies

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Objectives: Several different methodologies have been used for cell-free DNA (cfDNA)-based prenatal testing for fetal chromosomal aneuploidies, commonly referred to as noninvasive prenatal testing (NIPT). These include array comparative genomic hybridization, single nucleotide polymorphism-based targeted sequencing, and whole-genome sequencing (WGS; single-end or paired-end sequencing). The objective of this study was to develop a paired-end WGS-based test that used fragment size information to estimate fetal fraction. In addition, we aimed to develop an analysis method that combined a fetal fraction (FF) estimate with other quality control metrics to generate a robust measure of sample quality.

Methods: We developed a PCR-free, paired-end sequencing-based NIPT, the VeriSeq™ NIPT Solution, for detection of fetal chromosomal aneuploidies in maternal plasma samples. We developed a new analysis method incorporating fragment size and FF information in aneuploidy calling. To evaluate performance across different FFs, a dilution series was prepared by mixing extracted cfDNA from a trisomy 21 maternal plasma pool with cfDNA from a nonpregnant-female plasma pool, and we developed an in-silico titration series through controlled mixing of sequencing data. We determined the limit of detection (LOD₉₅) and compared performance against a validated single-end sequencing-based NIPT (Verifi™ Prenatal Test, Illumina, Inc.).
Results: The combination of data from the dilution series and the in-silico titration series demonstrated that this paired-end sequencing based methodology can accurately estimate FF. The LOD was determined for each chromosome-of-interest, all of which were under 3% FF at a sequencing depth of 8M reads. We developed a QC metric, ‘iFACT’ (Individualized Fetal Aneuploidy Confidence Test) that enables sample-specific assessment of whether sufficient reads are present to detect aneuploidy given the estimated FF. The dynamic threshold employed by the assay for sample coverage allowed for accurate classification of most samples, including samples with FFs below the LOD.

Conclusions: Use of a PCR-free methodology results in a simpler workflow, shorter turnaround time, and elimination of biases inherent with PCR methods. Incorporation of iFACT, a dynamic LOD threshold, allowed confident calls on samples with low estimated FF, provided the coverage in those samples was sufficient. This prevents a no-call result based solely on a FF estimate. The VeriSeq NIPT Solution, based on paired-end sequencing, showed high test performance for the detection of fetal chromosomal aneuploidies, even at low fetal fractions.

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Performance of a novel PCR-free, paired-end sequencing-based noninvasive prenatal screening test, VeriSeq NIPT Solution, in the detection of fetal chromosomal aneuploidies

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Objectives: To evaluate performance of a highly automated PCR-free solution for cell-free DNA (cfDNA)-based noninvasive prenatal testing (NIPT) in the detection of fetal chromosomal aneuploidies on chromosomes 21, 18, 13, X, and Y.

Methods: Frozen plasma samples from pregnant women with a gestational age of at least 10 weeks were tested using a PCR-free, paired-end sequencing-based NIPT assay, the VeriSeq™ NIPT Solution. Samples had previously been tested with a single-end sequencing-based NIPT (Verifi™ Prenatal Test, Illumina Inc.). Clinical outcomes were available for all cases based on cytogenetic results (amniocentesis, CVS, products of conception, and/or testing at birth) or newborn physical exam. Laboratory staff performing analysis with VeriSeq NIPT Solution were blinded to the original NIPT results and the clinical outcomes. Sample reporting rate as well as assay sensitivity and specificity were determined.

Results: Test performance was determined using 3107 samples, of which 21 (0.7%) were not reported because of QC failure on the only plasma aliquot available. Sensitivities were 98.9% (90/91), 90.0% (18/20), and 100% (8/8) for trisomy 21, trisomy 18, and trisomy 13, respectively.

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Specificities were ≥ 99.9% for all three trisomies; there were 8 false-positive results (one trisomy 21, three trisomy 18, and four trisomy 13). A total of 3082 samples received a sex chromosome classification; concordance for sex chromosome aneuploidies ranged from 80.0–100%. A biological explanation (eg, confined placental mosaicism) for false-positive and false-negative results cannot be ruled out.

Conclusions: This paired-end sequencing-based VeriSeq NIPT Solution has both high detection rates and specificities as well as a low assay failure rate. Because this paired-end sequencing-based assay incorporates a PCR-free approach, the workflow is simpler and the turnaround time reduced compared with single-end sequencing-based NIPT methods.

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Performance of Semiconductor sequencing platform for non invasive prenatal genetic screening for fetal aneuploidies: Results from a multicenter prospective study in clinical setting

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Objectives: To validate and evaluate an integrated protocol for non invasive prenatal genetic screening (NIPGS) for common fetal aneuploidies in a clinical setting, using the semiconductor sequencing, Ion Proton, technology.

Methods: This prospective study included 2505 pregnant women from seven academic genetic laboratories (695 in a validation study and 1810 in a real NIPGS clinical setting). Cell free DNA from plasma samples was sequenced using Ion Proton sequencer, and sequencing data were analyzed using the open-access software WISECONDOR. Performance metrics for detection of trisomies 21, 18 and 13, were calculated based on either fetal karyotype result or clinical data collected at birth. We also evaluated the failure rate and compared three methods of fetal fraction quantification (RASSF1A assay, and the software DEFRAG and SANEFALCON).

Results: Sensitivities and specificities were: 98.3% (95%CI: 93.5 - 99.7) and 99.9% (95%CI: 99.4 - 100) for T21, 96.7% (95%CI: 80.9 - 99.8) and 100% (95%CI: 99.6 - 100) for T18, 94.1% (95%CI: 69.2 - 99.7) and 100% (95%CI: 99.6 - 100) for T13. Our failure rate was initially 1.2%, and decreased to 0.6% after re-testing of some of the failed samples. Fetal fraction estimation by RASSF1A assay was consistent with DEFRAG results, both of which are adequate for routine diagnosis.

Conclusions: We report here one of the largest studies evaluating Ion Proton based NIPGS, and the first clinical study with a follow-up of pregnancies outcome up to birth. We demonstrate that this platform is highly efficient in detecting the three most common trisomies. The described protocol is robust and can be easily implemented in any genetic laboratory.
Objectives: Non-invasive prenatal screening (NIPS or NIPT) is available using a variety of technologies. Whole Genome Sequencing technology (Illumina) allows for analysis of chromosomes other than the NIPS validated chromosomes (13, 18, 21, X and Y). It remains uncertain if reporting these incidental findings is beneficial. We prospectively analysed the data on all pregnancies where an incidental finding (also known as Rare Autosomal Trisomies: RATS) was reported from a single Laboratory from March 2015 to August 2017 to determine if these findings added clinical benefit to the patient and the management of her pregnancy.

Methods: 28 incidental findings (increased signals in chromosomes other than 13, 18, 21, X and Y) were reported to Clinicians from 23,388 consecutive samples having NIPS analysed on the Illumina platform in the Genea Laboratory from March 2015 to August 2017. Eligibility was based on spontaneous singleton pregnancy with no obvious abnormality on ultrasound in women attending routine prenatal management through both private and public clinics in NSW/ACT Australia. All outcomes were prospectively obtained from the patients' Obstetrician and recorded including invasive testing result, structural anomalies, pregnancy outcome, birth data, timing of birth and Paediatric follow up where necessary.

Results: Abnormal outcomes occurred in 16 of 28 RATS including miscarriage (n=6), fetal structural anomaly on ultrasound (n=6), true fetal mosaicism (TFM n=4) and premature delivery (n=4). Significant growth restriction occurred in 8 cases. It is noteworthy that invasive testing only yielded abnormal results in the six miscarriage and four TFM cases. The other cases were all normal on amniocytes. Three of the 17 (25%) liveborn babies also had a structural anomaly despite normal microarray on amniocytes. One livebirth has a mosaic Trisomy 16 phenotype despite normal prenatal microarray result and normal cord blood analysis. Skin and muscle biopsy have been declined.

Conclusions: Our findings support recent published literature that RATS are not rare and are often associated with poor obstetric outcomes. Only 7 of 28 cases resulted in appropriately grown, liveborn and phenotypically normal neonates. RATS should be reported to guide pregnancy management from the first to the third trimester of pregnancy. Prognostic value varied by chromosome being generally more favourable for some (eg. trisomy 7 as long as Uniparental Disomy is negative) and less favourable for others (eg. trisomy 22) with variability in the implications for Clinical management. Our results provide early data to help guide Genetic Counselling of these patients.

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Evaluation of a cost-effective, fully automated, imaging based NIPT assay for T21, T18 and T13 screening

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Unedited draft - unpublished
Objectives: The Vanadis assay was developed and automated to make cfDNA based aneuploidy screening more accessible for laboratories and patients by eliminating cost and complexity. The objective of the current study is to investigate the feasibility of the Vanadis assay for aneuploidy screening. One prospective and one case control study were performed to analyze chromosome 21 followed by one prospective and one case control study performed to assess analysis of chromosome 13 and 18 in combination with chromosome 21. An automated workflow without the use of sequencing, microarrays or PCR was used.

Methods: A molecular analysis test was developed that use probes to capture thousands of loci across target chromosomes. Upon recognizing the correct loci probes are circularized using ligase and the corresponding circles are replicated by rolling circle replication to generate one sub-micrometer size rolling circle replication products (RCP) from each target. RCPs were labelled with a complementary fluoroescently labeled oligonucleotide, captured using a nanofilter microplate and then imaged using automated microscopy. Images were automatically analyzed using dedicated software to quantify molecular counts, asses normalized chromosomal ratios across samples and perform quality controls. A fully automated liquid handling workflow was developed.

Results: The feasibility to analyze trisomy 21 was tested by analyzing a total of 286 plasma samples of which 30 were affected and all cases were classified correctly. To investigate the feasibility of combining T21 with T13 and T18 analysis 507 samples were analyzed, 11/11 cases of T13, 16/17 cases of T18 and 16/16 cases of T21 were correctly classified. Out of the 507 samples 427 were analyzed using the automated workflow. In total 794 samples were analyzed and 46/46 T21, 16/17 T18 and 11/11 T13 cases were classified correctly with four false-positives and six single-pass analysis failures.

Conclusions: We report feasibility results from four studies performed during the development of a new T13, T18 and T21 aneuploidy screening technology that eliminate sequencing, microarrays and PCR. Results show that high detection rate was achieved in combination low false positive rate and assay failure rate. Additional studies are ongoing to further evaluate the Vanadis assay for implementation in clinical routine. An automated workflow that can process up to 84 samples per day with a total of 2,5 h hands on time from blood to result was implemented that makes cfDNA screening dramatically more accessible and cost effective for clinical laboratories.
Clinical validation study of noninvasive prenatal testing (NIPT) for detection of common aneuploidies

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Objectives: Non-invasive prenatal testing (NIPT) of circulating cell-free DNA (cfDNA) has become part of the standard of care in the screening for fetal aneuploidies. As a part of our initial validation study, we present a prospective cohort of 240 samples obtained from July 2016 to December 2017.

Methods: Cell-free DNA was isolated from maternal plasma collected from 10th to 16th weeks of gestation. Targeted amplification was performed in order to prepare the libraries and subsequent sequencing by synthesis was done. The fetal fraction was calculated using informative SNP loci. Our study includes a validation set of 84 samples without aneuploidy and a prospective cohort of 156 samples from pregnant women with high risk of aneuploidy but without major sonographic markers. All results were confirmed by invasive testing.

Results: The mean number of reads per sample was 2,37 million. The lower cut-off value for fetal fraction was 3%, with an average of 7.05%. All the 84 validation samples have negative results for NIPT, as expected. In the high-risk subset, 142 samples were correctly sequenced: 117 as no risk of aneuploidy, 8 were as risk of trisomy 21, 4 as risk of trisomy 18 and 1 as risk of trisomy 13. 5 samples have a non-informative result for trisomy 18 (3.2%) and 7 for 13 (4.49%). 14 samples have no result because of bad quality or low fetal fraction (8.97%).

Conclusions: In our cohort the use of this NIPT would avoid 117 invasive tests (80.75%). NIPT is an advanced screening test, so high risk of aneuploidy results must be confirmed with an invasive test, preferably amniocentesis. In the case of non-informative results, it is necessary to perform an invasive test to discard the presence of aneuploidy, due to the increased rate of trisomies 18 and 13 described in the bibliography.
Foetal copy-number variants (CNVs) detected by genome-wide non-invasive prenatal testing: The UZ Brussel-ULB experience

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Objectives: Non-invasive prenatal testing (NIPT) is a screening method for the early detection of foetal aneuploidies in pregnant women. While mainly developed for the detection of trisomy 13, 18 and 21, it is becoming clear that in rare cases genome-wide NIPT can identify foetal copy-number variants (CNVs) as well. Here we describe a limited number of foetal CNVs detected via NIPT in UZ Brussel-ULB, evaluate the circumstances that led to the detection of these foetal CNVs and investigate the clinical relevance of the detected foetal CNVs.

Methods: NIPT was performed using an in-house shotgun Massive Parallel Sequencing (sMPS) protocol on over 10 000 cfDNA samples extracted from maternal plasma, collected at >11 weeks of gestation. A minimum of 7x10E6 reads per sample were generated on the HiSeq 1500 (single read, 1x50bp). Data analysis was performed with a KU Leuven developed and a further in-house (BRIGHTcore) optimized and validated NIPT pipeline (freeze v1.4). After the detection of a potentially foetal CNV, microarray analysis was performed on both maternal and foetal derived tissues. Clinical follow-up was done in collaboration with the corresponding clinicians.

Results: In rare cases foetal CNVs can be detected via NIPT, although the distinction between maternal, placental and foetal aberrations can only be made by prenatal invasive testing (amniocentesis). Generally many factors can influence whether or not a foetal CNV can be
detected, including the size of the foetal CNV, foetal fraction, standard deviation, quality of the blood sample, number of reads. Here, we describe a limited number of prenatal clinically relevant foetal CNVs detected by NIPT, which have been confirmed via prenatal invasive testing. In some cases, specific echographic anomalies were observed that might be explained by the detected CNV.

Conclusions: These results show the added value of a genome-wide NIPT, since professional follow-up and counseling can improve pregnancy management for these patients. However, the detection of foetal CNVs via NIPT remains a rare event given the fact that test conditions must be optimal. Therefore, at this stage, NIPT should not be used specifically as a routine clinical screening method for these foetal CNVs. Also, care must be taken in the detection and reporting of these foetal CNVs, so that this does not lead to a needless increase in invasive testing.

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Positive predictive value (PPV) estimates for cell-free DNA based screening and choice of confirmatory invasive procedure: Experience of a large Italian referral prenatal diagnostic laboratory

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Objectives: With any new test, it is critical to determine performance characteristics in the ‘real’ world clinical setting and impacts care. However, studies confirming the clinical validity and utility of cfDNA-test, particularly for sex-chromosomal aneuploidies (SCAs), microdeletions and genome-wide imbalances, are still ongoing. The aim of this study is to contribute to that data by determining the PPV for common trisomies and SCAs using cfDNA screening, based on karyotype results obtained following a high risk cfDNA testing result.

Methods: Initial CfDNA screening was ordered by the referring physicians and performed by a variety of laboratories using MPSS based technology, SNP-based or DANSR technologies. The confirmatory samples were sent to our lab following a high risk cfDNA test result. Karyotype analysis was conducted after a high risk cfDNA testing result for Trisomy 13 (T13, n=13), T18 (n=9), T21 (n=62), 45,X (n=13), 47,XXX (n=6), 47,XXY (n=13) and 47,XYY (n=2). PPV stratified by maternal age was calculated for maternal age-dependent aneuploidies.

Results: PPV for T13, T18, T21 was 23.1%, 66.7%, 91.9%, respectively. PPV for SCAs was 7.7% for 45,X, 50% for 47,XXX, 30.8% for 47,XXY and 50% for 47,XYY. In women <35y and ≥35y the
PPV for T21, T18 and T13 was, 92%(81,97)*, 57%(25,84)*, 33.3%(12,65)*, and 90(60,98)*, 100%(34,100)*, 0%(0,49)*, respectively. Most of prenatal confirmations were performed on amniocytes (81/116; 69.8%); this trend was more evident when the high-risk result involved a SCA (28/33; 84.8%) versus a trisomy (54/84; 64.3%) (OR: 3.1111; 95%CI: 1.1-8.9). However, CVS shows a higher PPV (28/30, 93.3%) for non-mosaic trisomies than AF (37/54, 68.5%) (OR: 6.43; 95%CI: 1.4-30.2).

**Conclusions:** The higher rate of confirmatory AF after a high-risk result for SCA probably indicates that the referring clinicians are aware of the higher risk for sex chromosomes to generate mosaicism confined to placenta, therefore causing a decreased PPV when using CVS for SCAs. Although detailed information on ultrasound results at the time of invasive procedure are not available to the laboratory, a possible hypothesis for the higher PPV of CVS for non-mosaic trisomies might be the performance of an ultrasound-scan before the choice of confirmatory invasive procedure, whereby amniocentesis is preferred when no ultrasound anomalies are identified.

* 95th% CI

**P1-121**

**Internal verification and ongoing clinical prospective validation after tech-transfer process of a targeted cfDNA testing with microarray quantitation in an European laboratory**

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**Objectives:** Decentralized laboratories have been performing cfDNA testing on-site, either by tech-transfer from the source company laboratory or by setting-up their own home-brew test.

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To ensure quality and clinical accuracy, several measures are being undertaken. Quality assessment providers developed schemes for cfDNA laboratories. Furthermore, laboratories are required to verify or validate their assays, even if kit/software are CE-IVD marked, and to use internal quality control (IQC) monitoring processes. This study presents the results of the internal verification and ongoing validation of a targeted cfDNA analysis with microarray quantitation in an independent European laboratory after the tech-transfer from the source laboratory.

**Methods:** A standardized tech-transfer process was performed in the local laboratory. Thereafter, in agreement with European Guidelines for the validation and verification of molecular genetic tests for diagnostic use (Mattocks et al, EJHG,2010,18, 1276–1288) an internal verification on 410 retrospective unique plasma sample tubes with a known euploid or aneuploid result (from karyotype or matched sample result analysis by the source laboratory) was performed. Verification was performed in blinded fashion for operators. After clinical implementation, test performances (specificity, no result rate and PPV) were subjected to an ongoing audit to compare with performance specification of the source laboratory.

**Results:** Analytical validation: no false positives (FP), no sex discordances, 14/14 T21, 8/8 T18, 2/2 MX, 3/3 other SCAs and 2/3 T13 were detected. Fetal fraction after thawing overlapped with previous measurement on the fresh first tube in 92% of the samples. Clinical validation: 6701 cases were audited. No-result rate after 2\textsuperscript{nd} draw is 0.65%; assuming all screen-positive cases lost to follow-up were FP and FN cases would have been reported by clinicians, then sensitivity and specificity for T21,18,13 are: 100.00%(95%CI:87.66-100.00), 100.00%(95%CI:39.76-100.00), 100.00%(95%CI:29.24-100.00) and 99.95%(95%CI:99.87-99.99), 99.98%(95%CI:99.92-100.00), 99.98(95%CI:99.92-100.00), respectively. PPV for T21,18,13 were 90.32%(95%CI:86.58-93.11), 80.00%(95%CI:60.03-91.42) and 75.00%(95%CI:49.18-90.29), respectively.

**Conclusions:** With the multiplication of available cfDNA tests in the market, transparency around validation testing and ongoing performances, including reasons and percentage of no-result rate is becoming increasingly important. In this study, we have demonstrated that a standardized tech-transfer process of the targeted cfDNA analysis monitoring 172 IQC metrics is reliable, and provides an assurance that clinical performances in the decentralized laboratory matches that of the source laboratory.

P1-122

**Internal analytical verification of a targeted microarray-based cell-free DNA test for 22q11.2 deletion**

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*Unedited draft - unpublished*
Objectives: Laboratories are required to verify their assays determining that the test is being performed correctly, even if kit/software are CE-IVD marked, when a new test is implemented using a technology that is already well established in a laboratory, or using a test for which a suitable performance specification is available from a source laboratory where the test has already been validated. All these conditions apply to the targeted microarray-based cell-free DNA (cfDNA) test for 22q11.2 deletion syndrome (22q11.2DS). This study presents the results of the internal analytical verification after the implementation of 22q11.2DS cfDNA-test in an independent decentralized European laboratory.

Methods: Analytical sensitivity: 30 samples with 22q11.2DS were tested (2 maternal plasma and 28 simulated pregnancy samples). Deletions spanned through the A-D 22q11.2 LCRs, sizes ranged from 2.37 and 2.89Mb and simulated fetal fractions (FF) ranged from 7 to 39%. Analytical specificity: 423 prospectively ascertained maternal plasma samples with no known diagnosis of fetal/maternal 22q11.2DS were tested. These samples received a fetal trisomy risk assessment using targeted microarray-based cell-free DNA test, were singleton pregnancies without fetal malformations and/or increased risk after combined serum screening. This study was approved by the laboratory IRB. All samples were de-identified before study.

Results: Analytical sensitivity was 93.33%(95%CI:78.68-98.15) (28/30). Deletions were detected across the entire range of FF. Analytical specificity was 99.76%(95%CI:98.67-99.96) (422/423). The specificity must be considered a lower-bound estimate as the sample classified as “false positives” may be a true positive from an undiagnosed mother or affected fetus. Analytical specificity is not statistically different from that reported in a previous clinical validation/verification (OR 0.54;95%CI:0.07-4.31). Analytical sensitivity is statistically different from previous validation/verification of the source laboratory (OR 4.62; 95%CI:1.04-20.48).

Conclusions: Possible reasons for the statistically different sensitivity are the use of genomic DNAs instead of cfDNA from plasma samples from affected individuals to simulate affected pregnancies and the range of tested FF≥7% in the present validation. In this study, we have verified the analytical performances of a targeted microarray-based cfDNA test for the assessment of fetal 22q11.2 deletions inside the typical 3Mb region in the decentralized laboratory match the performance specification of the source laboratory. The low false positive rate, below 0.5%, for this cfDNA test expansion is critical when testing low-risk population as it highly impacts positive predictive value.

P1-123

NIPS for patients with high BMI: Evaluating the impact of whole genome sequencing

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Unedited draft - unpublished
Objectives: Fetal fraction (FF) is one of the many factors that influences the performance of noninvasive prenatal screening (NIPS). Low FF is associated with early gestational age, a compromised placenta (e.g., from triploidy and certain aneuploidies), and high Body-Mass Index (BMI). By far, the most common of these is high BMI: patients with high BMI constitute ~25% of US pregnancies. The most recent ACMG statement recommends “offering aneuploidy screening other than NIPS in cases of significant obesity.” We sought to examine whether high-BMI patients benefit from NIPS versus traditional maternal serum screening (MSS) for the purpose of common aneuploidy screening.

Methods: 51,737 patients who received NIPS were stratified into standard BMI classes. For each BMI group, the aggregate analytical sensitivity was calculated by summing—the product of (1) the sensitivity for a given FF and depth based on a model of whole-genome sequencing (WGS) NIPS and (2) the BMI-specific probability of observing a patient at that FF. Scaled sensitivities were incorporated into residual-risk calculations to assess impact on patient results reporting.

Results: Due to downward shifts in the FF distribution, NIPS sensitivity drops as BMI increases: non-obese analytical sensitivity for trisomy (T21) is 99.8%, whereas for class III it is 95.4%. Nevertheless, even those patients with class III BMI have expected T21 sensitivity in excess of that obtainable via traditional maternal serum screening (92.9%).

Conclusions: Due to their systematically lower FF, high-BMI patients are subjected a higher “no-call” rate for NIPS methodologies that have a minimum-FF threshold. The alternative of using other screening methodologies such as MSS is suggested by professional guidelines. In either case, a class of patients could be subjected to a lower quality of care. However, we demonstrate that NIPS alone is a superior option for high-BMI patients when using methods that maintain high sensitivity at low FF such as whole-genome sequencing, allowing providers to offer the same high level of care to all of their patients, regardless of body habitus.

P1-124

State-wide utilization of cell-free DNA as a primary or secondary screen: Results from the Victorian Perinatal Record Linkage (PeRL) study

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Objectives: Prenatal screening and diagnostic pathways have increased in complexity since the widespread availability of cell-free DNA (cfDNA) testing. To date, population-based ascertainment of cfDNA testing as a primary or secondary screen has not been possible in our hybrid public/private health care system. We aimed to perform individual record-linkage of women undergoing screening with cfDNA, combined first trimester screening (CFTS), second trimester serum screening (STSS), and/or prenatal diagnosis in 2015 to obtain estimates on the utilization of available clinical pathways.

Methods: All women resident in Victoria, Australia, undergoing a primary screening test or prenatal diagnostic test in 2015 were included. A collaboration between the major private and not-for-profit pathology and ultrasound services was formed to collect cfDNA results across the state, incorporating data from three different cfDNA platforms. These data were linked with state-wide results for CFTS, STSS and prenatal diagnostic procedures. The small cohort who accessed diagnostic testing and/or secondary screening with cfDNA in early 2016, following a primary screening test in late 2015, were also included. Individual record linkage was performed with LinkageWizTM and statistical analyses with STATA v14.0.

Results: In 2015, 70,693 pregnant women (91% of total) accessed screening with at least one of cfDNA, CFTS and STSS. Of the women screened, 24.1% used cfDNA as their primary screening test (n=17,045); 69.6% had CFTS only; 5.0% had STSS, and 1.3% had CFTS and cfDNA. Of the 1,486 women with an increased risk CFTS result (> 1 in 300), 174 (11.7%) had secondary screening with cfDNA; 18/49 had an abnormality confirmed on diagnostic testing. There were 624 women without an increased risk CFTS result who had secondary screening with cfDNA; of 18 having diagnostic testing, one trisomy 21 was confirmed.

Conclusions: Our population-based linkage study provides the first comprehensive assessment of cfDNA utilization as a primary and secondary screening test in Australia. In Victoria, one quarter of women who have prenatal screening choose cfDNA screening as their primary screening test. Only a minority of women with a high risk CFTS result use cfDNA as a secondary screen. In 2015, secondary screening with cfDNA was responsible for detecting one case of trisomy 21 that would have been missed by CFTS alone.
Clinical experience with noninvasive prenatal testing (NIPT) for five common microdeletions

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Objectives: A subset of microdeletions are amongst the most frequent chromosomal aberrations. Most microdeletion are de novo and not associated with maternal age. The most common microdeletion, DiGeorge/velocardiofacial (VCF) syndrome (22q11.2 deletion), has an incidence of 1 in 4,0001. Historically, microdeletions were only diagnosable through invasive testing. Expansion of NIPT has the potential to allow for microdeletion screening earlier in the pregnancy, thereby optimizing clinical management. In 2014, we started offering microdeletion testing for selected microdeletions: DiGeorge/VCF syndrome (22q11.2 deletion), Prader-Willi/Angelman (15q11.2 del), 1p36, Wolf-Hirschhorn (4p16.3 del), and Cri-du-Chat (5p15.2 del). We present our clinical experience for these five microdeletions.

Methods: Maternal blood samples from over 129,000 singleton pregnancies were submitted to the CLIA-certified Illumina Laboratory (Redwood City, CA) for testing with the Verifi™ Prenatal Test, including microdeletion analysis, during the study period. Cell-free DNA was extracted from maternal plasma and sequenced post library preparation. Whole genome sequence data was computationally processed with quantitative scores for each microdeletion determined based on sequence coverage of the respective target regions. Outcome information was requested in all cases with a positive microdeletion result.

Results: The average maternal age and gestational age were 33.5 years and 13.3 weeks in the whole study cohort; 32.4 years and 16.2 weeks in the screen positive cohort. A total of 208 samples were positive for a microdeletion, a screen positive frequency of 0.16% (see image). Clinical outcomes were available for 95 (46%) cases, with 33 concordant cases. 22q11.2 deletion had the highest concordance. The overall observed false positive (FP) and false negative (FN) frequencies were 0.04% and 0.004%, respectively. Of the 5 FN cases, 3 had deletions that were smaller than the limit of detection for our assay.

Conclusions: Screening for common microdeletions is now possible with NIPT. Clinical outcome information reveals good test performance, with low FP and FN frequencies and high positive predictive values (PPVs). Here, the overall observed PPV was 36.7%; for 22q11.2 deletion, the PPV was 90.9% (potential range: 37.1%–96.8%). As expected, cases with reported ultrasound anomalies had a higher PPV (>99.9%, potential range: 50%–100%). The clinical information determined in this study, particularly PPV information, could be valuable in pre-test counseling and incorporated into counseling of positive cases.
P1-125 Table.

<table>
<thead>
<tr>
<th>Screening Positive Frequency</th>
<th>All</th>
<th>22q11.2 deletion (DiGeorge/VCF)</th>
<th>15q11.2 deletion (Prader Willi/Angelman)</th>
<th>1p36 deletion</th>
<th>4p16.3 deletion (Wolf-Hirschhorn)</th>
<th>5p15.2 deletion (Cri-du-Chat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (%)</td>
<td>0.16</td>
<td>0.14</td>
<td>0.12</td>
<td>0.10</td>
<td>0.08</td>
<td>0.06</td>
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</tbody>
</table>

P1-126

Clinical experience with noninvasive prenatal testing (NIPT) for rare autosomal trisomies

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Objectives: A whole-genome sequencing approach for NIPT has the advantage of allowing analysis of all 24 chromosomes. Our laboratory began offering rare autosomal trisomy (RAT) screening, in addition to the common aneuploidies, in 2017. Although autosomal trisomies other than 21/18/13 are less common, genome-wide NIPT found that about one-third of all chromosome aberrations are other than trisomy 21/18/13¹. Traditionally, these conditions were identified by invasive testing or after birth. As the rate of diagnostic testing decreases with more patients opt for screening², identifying RATs can be valuable for pregnancy management. This study presents our initial clinical experience with RAT screening.

Unedited draft - unpublished
Methods: Maternal blood samples from over 10,000 singleton pregnancies were submitted to the CLIA-certified Illumina Laboratory (Redwood City, CA) for the Verifi™ Plus Prenatal Test. Cell-free DNA was extracted from maternal plasma and sequenced post library preparation. Whole-genome sequence data was computationally processed with quantitative scores for each chromosome determined using chromosomal sequence coverage and fetal fraction. Classification thresholds for each chromosome were derived to maximize specificity while accounting for differences in prevalence for each RAT.

Results: 43 (0.4%) screen positives were identified (see image). The average maternal age (35.0 years) and gestational age (12.4 weeks) of the screen positive cohort were similar to the whole study cohort. More cases in the screen positive cohort were noted to have indications of abnormal ultrasound (1.8x) or history suggestive of increased risk for chromosome aneuploidy (5.5x) compared with the whole study population. Clinical outcome information was available in 8 cases (18.6%): 2 confirmed positives (full trisomy 9; segmental 9p duplication), 2 false positives, 3 miscarriages, and 1 elective termination without confirmatory testing; >99% of pregnancies are ongoing.

Conclusions: The screen positive frequency of 0.4% observed here is consistent with previous studies. The most common trisomy identified was trisomy 22, followed by trisomies 7 and 9, which is slightly different than the pattern reported previously. While RATs are most commonly associated with early miscarriage, they have also been associated with in utero fetal demise, intrauterine growth restriction, true fetal mosaicism, and uniparental disomy. Results obtained through NIPT early in pregnancy can be valuable for clinical management. Ongoing outcome collection will provide more insight into the biological aspects of RATs.

P1-126 Table.
Performance of first trimester screening of preeclampsia based on uterine artery pulsatility index and maternal risk factors in routine clinical use

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Objectives: To evaluate the diagnostic performance of first trimester pre-eclampsia screening in routine clinical assessment based on a combined predictive algorithm including uterine artery pulsatility index and maternal risk factors, and to evaluate the effect of aspirin use in the reduction of incidence of preeclampsia, using this screening model.

Methods: Retrospective cohort study including patients participating in a preeclampsia screening program on a tertiary healthcare center located in Santiago, Chile. Singleton pregnancies, with fetal ultrasonography performed between 11\(^{0/7}\) – 13\(^{6/7}\) weeks, with crown-rump length between 45 and 84 mm, confirmed fetal viability, and with uterine artery pulsed doppler velocimetry were eligible. A convenience sample of 1492 patients was obtained. Individual risk of developing early and late onset pre-eclampsia was calculated using a combined predictive algorithm previously described in literature. A positive screening was defined as a risk estimation over the 95th percentile. Pregnancies were followed up until delivery.

Results: Early onset predictive model detected 8 (20%) of the total pre-eclampsias observed in the cohort, and 2 of 6 (33,3%) pre-eclampsias presenting at 34 weeks of gestation or less. Late onset predictive model detected 7 (17,5%) of all pre-eclampsia observed and 6 (17,6%) of 34 pre-eclampsia presenting after 34 weeks of gestation. When both predictive models were used in combination, 105 patients (9,3% of the cohort) tested positive. With this combination, 32,5% of all preeclampsia’s, 33,3% of early onset and 32,4% of late onset pre-eclampsia were identified.

Conclusions: Under routine clinical use, combined screening of pre-eclampsia in first trimester of pregnancy using uterine artery doppler and maternal risk factors, predicted one third of early and late onset pre-eclampsias. When using this method, we observed no benefits of using aspirin in high risk pregnancies. For clinical purposes, more sensitive predictive models of preeclampsia are necessary in first trimester of pregnancy.
Improved detection of sex chromosomal aneuploidies in sequencing-based NIPT

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Objectives: The Non-Invasive Prenatal Test (NIPT) is quickly evolving to become a first-tier test for the detection of fetal chromosomal aneuploidies. Current implementations either apply targeted testing for the most common trisomies (chromosomes 13, 18 and 21) or analyse all autosomal chromosomes. Although fetal gender can be predicted reliably, the current genome-wide implementation shows significant limitations for the detection of sex chromosome aneuploidies (SCAs) due to an inherent correlation between the fetal fraction and reported estimates for chromosome X and Y. We aimed to improve the SCA detection.

Methods: We introduce fetal fraction based normalisation of read counts on chromosomes X and Y. We used seqFF to estimate the fetal fraction, allowing normalisation in a gender-independent approach. Bin-specific linear regression between the fetal fraction and the read counts, normalized using the current state of the art approach, was performed over a set of reference samples, allowing normalisation of each 50kb bin individually. Next, 95% confidence intervals were calculated for the expected values of normal (euploid) samples, male/female twins and fetuses with an aneuploidy of chromosome X (X0) or Y (XXY or XYY).

Results: Based on the 95% confidence intervals, distinct SCA categories could be defined; we obtained a clear separation of all true aneuploidy states. Since implementation, we identified 5 XXY cases, of which 2 were confirmed by an invasive prenatal test (amniocentesis); in 2 other cases, the parents chose not to undergo an invasive prenatal test. In addition, we identified 2 X0 cases, of which 1 was confirmed.

Conclusions: In conclusion, we provide an improved normalisation strategy of NIPT data, giving the technology the capability to more accurately detect fetal SCAs in both male and female fetuses. Early detection of SCAs allows to fine-tune the pregnancy management (e.g., fetal echocardiography in case of X0) and enables early postnatal treatment interventions and counseling, leading to a better outcome.

Non-invasive prenatal diagnosis (NIPD) for CF: Implementation, uptake, outcome and implications

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Unedited draft - unpublished
Objectives: Much published evidence suggests that NIPD for monogenic disorders is likely to be welcomed by potential users and that uptake may be high. Since 2013, our accredited public health service genetic laboratory has offered non-invasive prenatal diagnosis (NIPD) for monogenic disorders. To date we have delivered over 480 tests for 51 conditions. Whilst the majority of early testing was amplicon-based NIPD for de novo or paternally inherited mutant alleles, in October 2016 we introduced relative haplotype dosage analysis (RHDO) for definitive diagnosis of the recessive condition cystic fibrosis (CF). Here we investigate test performance, and explore uptake and pregnancy outcomes.

Methods: A retrospective review of laboratory and patient records was performed to determine the outcome of all cases referred for CF NIPD using RHDO. Testing was performed using a capture based NGS method analysed with an R script to perform relative RHDO and incorporation of quality control checks in our bioinformatics pipeline to reduce analysis time. Genotyping results were determined within turnaround times for reporting. Pregnancy outcomes were determined from referring centres. Invasive testing rates for CF over the last 5 years were determined from laboratory records.

Results: There were 20 referrals since October 2016 (Table). Results were issued within 5.75 days (3 to 11). Five were affected, nine were carriers, four carried only wild-type alleles and two were inconclusive. Case 11 was inconclusive due to a paternal recombination close to the mutation site, invasive testing showed an affected fetus. Case 13 was inconclusive for the maternal allele but as the low risk paternal allele was detected invasive testing was not required. Prior to 2016 we performed approximately three invasive prenatal diagnoses per annum, compared with 20 NIPD in 16 months since offering a definitive CF NIPD service.

Conclusions: Timely, accurate NIPD for definitive prenatal diagnosis of CF is possible in a public health service laboratory. RHDO is able to detect recombinations, which is reassuring as this is a concern when using a linkage approach. The service is popular with parents as evidenced by the significant increase in referrals compared with invasive testing done in the years prior to availability of NIPD. To date, NIPD has accurately predicted the genotype. Several pregnancies are ongoing and outcomes will be reported. High uptake will impact on the cost of prenatal services as the range of testing expands.
Experience with SNP based NIPT testing of common microdeletions in India


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Objectives: The scope of non-invasive prenatal testing (NIPT) has expanded to include screening for clinically relevant subchromosomal anomalies such as the 22q11.2 deletion, Angelman, Prader-Willi, 1p36 deletion and Cri-du-chat syndromes. These conditions can be screened with high sensitivity and specificity using NIPT as demonstrated in validation studies. Unlike Down syndrome, the incidence is not altered by maternal age. Most of these abnormalities have significant clinical manifestation and no pre-existing antenatal test baring an occasional suspicion on ultrasound. Prenatal detection may help in early management and intervention. We present the experience Medgenome laboratory (India) since introduction of microdeletion screening in May 2017.

Unedited draft - unpublished
**Methods:** About 200 pregnant women opted for microdeletion testing in addition to common aneuploidy screening. For microdeletion screening, cell-free DNA was isolated, the libraries prepared and selectively amplified by massively multiplexed PCR using primers specifically designed for the five microdeletions, 22q11.2, Angelman, Prader-Willi, 1p36 deletion syndrome and Cri-du-chat at 10752 SNPs covering the areas of microdeletions. The enriched library was sequenced using HiSeq 2500 and the data was analysed by Next-Generation aneuploidy testing using SNPs (NATUS) algorithm. Fluorescent in situ hybridization probes at 22q11.2 and Chromosomal microarray (CMA) to further confirm the results. Genetic counseling was provided.

**Results:** Of the 200 samples, two screened high risk for 22q11.2 microdeletion syndrome. On invasive confirmatory testing by Fluorescent in situ hybridization probes at 22q11.2 and Chromosomal microarray (CMA), one sample was a true positive and the other sample was a false positive. No other microdeletion has been detected so far. As microdeletion testing is performed only after aneuploidy screening, three cases of Trisomy 21 and one of Trisomy 13 were also detected.

**Conclusions:** NIPT was successfully able to identify/screen for a case of 22q11.2 deletion. In cases, where there was no prior history or ultrasound abnormalities this is a useful screening tool given that 22q11.2 deletion syndrome is the second most common chromosomal abnormality. NIPT can be especially useful in creating a strong suspicion of the possibility of microdeletion syndromes especially in the absence of other prenatal findings.

P1-131

Single-nucleotide polymorphism based noninvasive prenatal testing – An Indian study

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Unedited draft - unpublished
Objectives: Noninvasive prenatal testing (NIPT) has revolutionized prenatal screening for chromosomal aneuploidies in many countries. Its implementation has been sporadic at best in India. Medgenome laboratories in India started offering the Panorama non invasive prenatal test (Natera Inc) from August 2015. To evaluate the performance of the test in Indian conditions, we evaluated the 516 pregnancies as a part of a research study.

Methods: Ten leading institutions across India participated in the research study after approval from their respective review boards. 516 pregnancies were selected based on selected inclusion and exclusion criteria, after informed consent. These pregnant woman had tested intermediate to high risk on conventional first and second trimester screening. Laboratory testing was performed at Medgenome laboratories, Bangalore using validated protocol from Natera, Inc that involved interrogation of 13,328 SNPs on five chromosomes, analysis by the cloud-based proprietary NATUS algorithm. Results were confirmed either by invasive diagnostic testing or by clinical evaluation after birth. Statistical analysis was performed.

Results: Of 511 samples, results were obtained in 499 (97.7 %). Of these, 480 (98.2 %) were low risk and 19 were high risk, which included 13 Trisomy 21, 2 Monosomy X, 1 each of Trisomy 13 and 18, 1 XXX and 1 XXY. A sensitivity of 100% was obtained for detection of trisomy 21, 18, 13 and sex chromosome abnormalities. The specificity ranged from 99.3-100% for abnormalities tested. Taken together the positive predictive value for trisomy 21, 18, 13 and Monosomy X was 85.7 %. The average fetal fraction was 8.2%, which is lower than the average observed elsewhere.

Conclusions: This is the first report of systematic study of clinical experience with SNP based NIPT in India, and demonstrates comparable performance in all aspects of testing to the results in the countries where NIPT has been widely implemented.

P1-132

Determination of fetal fraction in maternal plasma samples: Comparison of assay performance

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Unedited draft - unpublished
Objectives: Accurate determination of fetal fraction is important for applications utilising cell free fetal DNA (cfDNA) to avoid false-negatives due to low or absent cfDNA and to establish limits of detection. However, there is no standardisation or guidelines regarding fetal fraction measurement and different assays can provide very different estimations. In order to determine the most accurate assay here we compare six different approaches, and discuss their respective advantages and limitations.

Methods: Three fetal fraction measurement assays were developed: one exploiting the Human Leukocyte Antigen (HLA) complexity, SRY digital PCR (dPCR) and a Single Nucleotide Polymorphism (SNP) bait-based assay. In addition, the ZFX/ZFY, Y chromosome read count (RAPIDR algorithm) and FF MASTR kit assays were also used. Spike-in DNA samples of various percentages were created for two mother-child pairs and tested on all six assays. Fourteen maternal plasma samples from pregnant women carrying chromosomally normal fetuses were double-centrifuged and cfDNA was extracted. CfDNA was tested using four of the fetal fraction measurement assays. Results were compared using Friedman tests and Pearson correlation.

Results: The Friedman test revealed significant differences among assays both when spike-in and maternal plasma samples were tested. Correlation analysis revealed strong positive correlations between fetal fraction measurement and actual spike-in percentage for all assays, with the FF MASTR and SNP bait-based assays showing the strongest positive correlation in combination with proximity to the line of agreement. The Ychr read count assay showed the strongest correlation, but was further away from the line of agreement and constantly over-estimated the spike-in percentage. The other three assays showed less strong positive correlations.

Conclusions: SNP based assays were the best performing in terms of accuracy, sensitivity and informativity. We hypothesise that the differences in fetal fraction estimation produced by the various assays reflect biases introduced during amplification processes. Other assay considerations, such as number of loci tested, DNA input volume, labour intensity, turnaround time and add-on or embedded format are discussed. Our results highlight the importance of the fetal fraction measurement being an integral component of the test performed, rather than an additional test, and optimisation for the specific cfDNA application and laboratory.
The attitude of pregnant women to NIPT and the analysis of its effectiveness in different groups of patients

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\textsuperscript{3}Centre of Fetal Medicine, Saint-Petersburg, Russian Federation

Objectives: In Saint-Petersburg the first trimester screening (FTS) was performed for all pregnancies since 2012. Invasive procedures for fetal kariotyping are suggested for all high risk (more than 1/100) pregnancies by City Genetic Service. New and modern non-invasive prenatal test (NIPT) using fetal DNA in maternal blood is now only commercial (mostly foreign) suggestion. This study investigates the different reasons of pregnant women to undertake NIPT and its results in 537 NIPT cases with known outcome (2015-2017).

Methods: This study includes Panorama, Prenetix, Prenatest and Veracity NIPT methods.

Results:

<table>
<thead>
<tr>
<th>Groups</th>
<th>n (%)</th>
<th>Down syndrome cases, n</th>
<th>Down syndrome detection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>174 (32%)</td>
<td>5 (1 missed abortion before 11 w.)</td>
<td>2.8%</td>
</tr>
<tr>
<td>FTS risk 1/100 and more</td>
<td>37 (6%)</td>
<td>5</td>
<td>14%</td>
</tr>
<tr>
<td>FTS risk 1/101 to 1/1000</td>
<td>43 (8%)</td>
<td>3</td>
<td>7%</td>
</tr>
<tr>
<td>FTS risk less 1/1000</td>
<td>126 (24%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Women’s anxiety</td>
<td>163 (30%)</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

Conclusions: Two Down syndrome babies were born after negative NIPS results. Their mothers did not have their FTS results, but one of them had US markers after 16.5 weeks. One case of trisomy 18 with negative NIPT results in the first trimester was revealed after US screening at 20 weeks revealed multiple markers of trisomy 18 follower by the invasive procedure and confirmation of trisomy 18. So the highest effectiveness is in the women of high and borderline risk groups after FTS. Further investigations are needed to collect more information about women attitudes towards NIPS and to shape out the groups of

Unedited draft - unpublished
P1-134 Table.

<table>
<thead>
<tr>
<th>Groups</th>
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P1-135

The role of health economics evaluation in pricing and reimbursement decision making for the non-invasive prenatal testing (NIPT) in Europe

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4Ariosa Diagnostics Inc., Roche Sequencing Solutions Inc., San Jose, CA, United States

Objectives: Health Technology Assessment (HTA) agencies and governmental bodies have previously found that Non-invasive Prenatal testing (NIPT) performance justifies the integration into trisomy screening and have recommended incorporating NIPT into prenatal testing programs. Although it is widely recognized that offering NIPT to all pregnant women regardless risk and age is more beneficial than limiting to high risk, policy makers initially recommend introducing NIPT as a second line test due to budget impact concerns. Economic analysis is needed to evaluate the financial impact of NIPT implementation in the public health systems for the general pregnancy population.

Methods: A decision-analytic model was developed to assess the clinical and economic impact of adopting NIPT for screening of common fetal aneuploidies. The model compares conventional screening approach such as first trimester combined screening with three scenarios of NIPT implementation: 1) Primary NIPT screening; 2) Contingent NIPT screening; 3) NIPT for increased risk population (advanced maternal age, prior/family history of trisomies or a positive result from traditional screening testing). We selected Belgium as a base case for the analysis due to the recent approval of NIPT reimbursement. The model includes only the direct medical cost of screening, diagnosis and procedure-related complications.

Results: Four European experts in prenatal screening reviewed the model’s clinical validity. Modelled results were compared to published trisomy screening models and tested via
sensitivity analyses. Results derived from the model demonstrated that NIPT: (i) can bring substantial clinical benefit (less fetal trisomy cases missed, less unnecessary invasive procedures, and less unaffected pregnancy fetal losses), (ii) significantly reduces the costs for invasive testing, as well as (iii) reduces procedure-related complications in all scenarios. The cost per trisomy detected was €63,016 for conventional screening versus €66,633 for Primary NIPT scenario, for a difference of €3,617.

Conclusions: Economic evaluation for NIPT has significant impact on pricing, reimbursement and patient access. The Belgium Government recently decided to offer primary NIPT to all pregnant women with reimbursement price of €260. According to our model this scenario would have only minor cost increase but associated with significantly better clinical outcomes. This model was customized in other European countries to inform the policy makers about the affordability of NIPT as a viable option within their unique health care systems.

P1-136

Prenatal DNA test as first-line screening - benefits, opportunities and limitations

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Objectives: At recent years genomics-based non-invasive prenatal testing is adopted as a complementary prenatal screening test for detection of common aneuploidies for different terms. Some technologies with fraction measurement allow to perform a prenatal DNA test as first-line screening stage and detect aneuploidy previous to 12 weeks and include only an estimate of five kinds of chromosomes. Lack of clinical data on the fetus does not allow to determine the minimum test period. The clinical definition of the prenatal DNA test in the early period is debated.

Methods: In this research we estimated an optimal feasible minimum period for prenatal DNA testing.

Results: The cohort included 450 pregnant consulted and willing to implement NIPT. In 7 cases aneuploidy was detected early 11 weeks and confirmed by CVS. With a fetal size greater than 25 mm in only two cases the fraction was insufficient for analysis. In 17 cases by US examination an undeveloped pregnancy was established. In 14 cases karyotyping was performed, chromosome anomalies were established in 9 case, only 3 belonged to the aneuploid group 21,13,18, X,Y. After an early negative NIPT in 6 cases in I or II trimester aneuploidy of 8, 9, 16 and 22 were diagnosed.

Conclusions: Prenatal DNA-screening could be implemented and covered by the public healthcare system as a first-linescreening test for all pregnant women in order to detect fetus genetics status and rule out aneuploidy in first trimester. The prescription of the DNA test with
the detection of only five kinds of chromosomes is not desirable before the 9th week of gestation. As previous to this period, a high level of aneuploidy is retained for other types of chromosomes. The effectiveness of DNA screening in the early period may be higher when the range of tested chromosomes

P1-138-LB

Hypoxia-related bilateral massive adrenal calcifications: A case report of autopsy and placental examination

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²Pathology Department, Tunis, Tunisia
³Embryo-Foetopathology Department, Tunis, Tunisia

Objectives: Adrenal calcifications may be associated with adrenal hemorrhage, maternofetal infection, tumor or Wolman disease. Hypoxia that may be related to septicemia or coagulation disorders is the major pathogenic factor to be considered during antenatal period. We present a case of hypoxia-induced bilateral massive adrenal calcifications in a stillborn in order to emphasize the usefulness of the placental examination in clarifying the underlying mechanisms and causes.

Methods: The fetal autopsy and placental histopathological examination results following stillbirth were retrospectively analyzed in an attempt to explain the massive bilateral adrenal calcifications.

Results: A 28 year-old woman, gravida 2 para 1, with a history of previous unexplained fetal death was admitted because of recurrent stillbirth at 28 weeks of gestation. After abortion, a complete fetoplacental examination was performed. It showed a macerated female fetus who presented with severe intrauterine growth restriction and thymic hypotrophy, placental hypoplasia (187.95g; normal 290-305), multiple pale and red infarcts and trifurcated cord with marginal insertion. Histology demonstrated thymic lymphocytic depletion, bilateral adrenal massive calcifications and severe placental vascular lesions including large areas of infarction, diffuse villous hypotrophy and increased syncytial knots. Coagulation screening tests were considered.

Conclusions: The case report shows that placental examination is necessary, in addition to fetal autopsy, to investigate the underlying mechanisms and causes of adrenal calcifications.

Unedited draft - unpublished
Coexistent lobar holoprosencephaly and Chiari II malformation: The value of the neuropathological examination after pregnancy interruption in view of the challenging prenatal diagnosis

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¹Fetopathy Unit, Bizerte, Tunisia
²Department of Obstetrics and Gynaecology, Bizerte, Tunisia
³Department of Pediatrics, Bizerte, Tunisia

Objectives: Chiari II malformation, usually associated with myelomeningocele, constitute one of the most common severe central nervous system malformations. Holoprosencephaly is a less common but equally complex brain malformation. Both malformations are fundamentally different with respect to the embryonic mechanism and morphology. We describe coexistent Chiari II malformation and holoprosencephaly that occurred in a severely polymalformed fetus in order to raise the challenges in prenatal diagnosis and highlight the usefulness of the neuropathological study after pregnancy interruption.

Methods: We describe coexistent lobar holoprosencephaly and Chiari II malformation in a fetus with trisomy 18, born at 24 weeks of gestation to a 42-year-old G4P3 woman. The prenatal ultrasound data were consistent with myelomeningocele associated with ventriculomegaly, suggesting Chiari II malformation, and bilateral talipes equinovarus deformity. Fetopathological examination findings are detailed with a special focus on neuropathological abnormalities.

Results: The fetopathological examination showed intra-uterine growth restriction, characteristic craniofacial dysmorphism and malposition of the extremities, open-sacral myelomeningocele, hypoplastic bilobed lungs, ventricular septal defect, right abnormal retro-esophageal subclavian artery, crossed fused renal ectopia, adrenal hypoplasia, common mesentery, bicornuate uterus and single umbilical artery. The neuropathological study confirmed the Chiari II malformation and identified a lobar holoprosencephaly. The main macroscopic findings related to lobar holoprosencephaly included the presence of the interhemispheric fissure along the entire midline, olfactory bulbs, hypoplastic corpus callosum connecting the hemispheres with a bridge of cortical tissue, joined thalami and dilated lateral ventricles with absence of the septum pellucidum.

Conclusions: Prenatal diagnosis of the most differentiated form of holoprosencephaly (lobar holoprosencephaly) is difficult. The diagnosis becomes more challenging when this entity is associated with other brain abnormality, especially Chiari II malformation. Thus, it is necessary to achieve a neuropathological examination following termination of pregnancy to establish a correct diagnosis.
TRPV6 variants interfere with the maternal-fetal calcium transport through the placenta causing transient neonatal hyperparathyroidism

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¹Prenatal Diagnosis and Medical Genetics, Mount Sinai Hospital, Toronto, Canada
²Division of Cell Signaling, Okazaki Institute for Integrative Bioscience (National Institute for Physiological Sciences), National Institutes of Natural Sciences, Okazaki, Japan
³Division of Pediatrics, Department of Developmental and Urological-Reproductive Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan
⁴Division of Human Genetics, Children’s Hospital of Philadelphia, Philadelphia, PA, United States
⁵GeneDx, Gaithersburg, MD, United States
⁶GeneDx, Gaithersburg, MD, United States
⁷The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynecology, Mount Sinai Hospital; University of Toronto, Toronto, ON, Canada
⁸The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynecology, Mount Sinai Hospital; University of Toronto, Toronto, ON, Canada
⁹Division of Cell Signaling, Okazaki Institute for Integrative Bioscience (National Institute for Physiological Sciences), National Institutes of Natural Sciences, Okazaki, Japan
¹⁰Kitasato University, Kanagawa, Japan
¹¹Department of Neonatology, Tokyo Metropolitan Children's Medical Center, Tokyo, Japan
¹²Department of Neonatology, Tokyo Metropolitan Bokutoh Hospital, Tokyo, Japan
¹³Tokyo Metropolitan Children's Medical Center, Tokyo, Japan

Objectives: To find the role of the TRPV6 gene in the maternal-fetal transfer of calcium through the placenta.

Methods: DNA analysis of the TRPV6 gene in 6 newborns with prenatal onset of transient neonatal hyperparathyroidism (TNHP).

Results: We identified three missense variants at the outer edges of the second and third transmembrane domains that alter the localization of the TRPV6, one recurrent variant at the S2-S3 loop, and two in the fourth ankyrin repeat domain that impair TRPV6 stability. Compound heterozygous loss of function mutations for the pathogenic frameshift allele and the allele with intronic c.607+5G>A mutation resulting in the most severe phenotype. These results suggest that TNHP is an autosomal recessive disease caused by TRPV6 mutations that affect the placental maternal-fetal calcium transport.

Conclusions: The TRPV6 gene has a major role in controlling the materno-fetal transport of calcium through the placenta resulting in Neonatal Hyperparathyroidism in fetuses/newborns.

Unedited draft - unpublished
with homozygote/compound heterozygote mutation in the TRPV6 gene. Once the baby is born and being fed, the condition disappears/improves substantially. To our best knowledge, this is the first known autosomal recessive condition which affect the placental support of the fetus.

P1-141-LB

**A case of aneurysmal malformation of the vein of Galen resulting in severe intrauterine growth restriction and stillbirth**

Sihem Darouich\(^1\), Lassaad Mkaouar\(^2\), Amira Ayachi\(^2\), Mechaal Mourali\(^2\)

\(^1\)Fetopathology Unit, Bizerte, Tunisia  
\(^2\)Department of Obstetrics and Gynaecology, Bizerte, Tunisia

**Objectives:** Vein of Galen aneurysmal malformation (VGAM) is an uncommon congenital arteriovenous malformation. It usually presents in the antenatal period with congestive heart failure, but scarcely with intrauterine growth restriction (IUGR). We describe a unique case of VGAM that was prenatally diagnosed due to severe IUGR and heart failure, and was associated with poor outcome.

**Methods:** We report on antenatal and autopsy findings in a stillborn presenting with VGAM.

**Results:** A 23-year-old woman, was referred to our institution at 32 weeks of gestation because of severe asymmetric IUGR. Repeated ultrasound confirmed IUGR that was associated with cardiomegaly and oligohydramnios. Color Doppler demonstrated midline hypoechogenic mass in the pineal region of the brain with high velocity flow, suggesting VGAM which was confirmed by fetal MRI. The outcome was rapidly fatal. Fetopathological examination showed a female fetus of 28 weeks of gestation, macerated, presenting with hydrops, thymic and adrenal hypotrophy, congestive cardiomegaly and hepatomegaly. Neuropathological examination exhibited a ruptured VGAM and ex-vacuo tri-ventricular dilatation. Histology revealed severe anoxic visceral damage.

**Conclusions:** The case report suggests that prenatally diagnosed VGAM carries a poor prognosis, especially when it results in severe IUGR.

P1-142-LB

**Fetal non-isolated diastematomyelia: Correlation between prenatal imaging and fetopathological examination findings**

Sihem Darouich\(^1\), Souhir Bouzguenda\(^2\), Nadia Aloui\(^3\), Rim Ben Hmid\(^4\), Mohamed Badis Channoufi\(^4\), Aida Masmoudi\(^2\)

\(^1\)Fetopathology Unit, Bizerte, Tunisia  
\(^2\)Department of Obstetrics and Gynaecology, Bizerte, Tunisia  
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\(^4\)Department of Neurosurgery, Bizerte, Tunisia

Unedited draft - unpublished
Objectives: Diastematomyelia, also known as split cord malformation, is a congenital spinal anomaly characterized by a longitudinal splitting of the spinal cord. It may be isolated or associated with other spinal dysraphisms such as myelomeningocele or visceral abnormalities, especially urogenital malformations. The most common site of diastematomyelia is the thoracolumbar region. We present a well-documented case of fetal diastematomyelia associated with myelomeningocele and Arnold-Chiari malformation type II in order to achieve a correlation between imaging and autopsy findings.

Methods: We report on a fetal case of diastematomyelia. The imaging and fetopathological examination findings are detailed.

Results: Routine ultrasound in a 22-weeks pregnant woman revealed fetal brain and spinal abnormalities. There were severe hydrocephalus, thoracolumber myelomeningocele, abnormal appearance of spinal curvature and widening of the spinal canal associated with bony spur traversing the spinal cord on the same site, suggesting diastematomyelia. MRI was performed and confirmed the Chiari malformation type II and myelomeningocele as well as the diastematomyelia. The two hemicords were separated by a bony septum. The pregnancy was terminated. The fetopathological examination confirmed the neurological abnormalities. No visceral malformations were observed.

Conclusions: We report on a diastematomyelia of poor outcome that it is associated with myelomeningocele and chiari malformation type II. The ultrasonographic findings of diastematomyelia are widening of the spinal canal and echogenic bony spur traversing the spinal canal. Prenatal knowledge of spinal cord anomalies is important for antenatal counseling as it allows distinguishing between isolated form of diastematomyelia that has an excellent prognosis with surgical repair and the complex entity that is associated with spina bifida and chiari malformation type II and justify termination of pregnancy.

P1-143-LB

Hysterosalpingographic evaluation of tubal patency after methotrexate therapy for ectopic pregnancy

Ahmed Elmilgy Aboelroose

Lecturer of Obstetrics & Gynecology, Ismailia, Egypt

Objectives: Worldwide, ectopic pregnancy (EP) stills is the first cause of maternal death in early pregnancy. Sexually transmitted infections and tubal surgery are responsible for the majority of the tubal damage seen in ectopic pregnancies. Methotrexate has contributed to alleviating
some of the disease burden of ectopic pregnancy, where it affords approximately 25% of women a nonsurgical and fertility-preserving treatment option. The present study aims at evaluation of the tubal patency after methotrexate therapy in the treatment of undisturbed tubal ectopic pregnancy.

**Methods:** It is a descriptive study evaluating tubal patency in patients suffered from undisturbed tubal ectopic pregnancy and received methotrexate therapy. It was conducted on 56 patients all received methotrexate for management of undisturbed ectopic pregnancy attending outpatient clinic in Suez Canal University hospital for follow-up. Hysterosalpingography was done to assess tubal patency after methotrexate therapy by at least two months. Full history was taken from patients.

**Results:** The present study revealed that 71.4% of patients (40 cases) have patent tubes and 28.6% have blocked tubes. Moreover, 21.4% of patients have ipsilateral tubal block only, 3.6% have contralateral tubal block and 3.6% have bilateral tubal block, from many risk factors of tubal block the significant risk factors (p value < 0.05) were history suggestive of PID, acute PID hospitalization, and history of septic miscarriage.

**Conclusions:** Methotrexate affords approximately 25% of women a nonsurgical and fertility-preserving treatment option. The tubal patency after methotrexate therapy for the management of undisturbed tubal ectopic pregnancy was high (71.4%) and there are some statistically significant risk factors for tubal block as history suggestive of PID, acute PID hospitalization, history of septic miscarriage, and IUCD usage.

P2-1

**High maternal BMI does not challenge cell-based noninvasive prenatal diagnostics**

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²ARCEDI Biotech ApS, Aarhus, Denmark  
³Dept Obstet Gynecol, Aarhus University Hospital, Aarhus V, Denmark  
⁴Aarhus University Hospital, Aarhus, Denmark

**Objectives:** Emerging technologies that source intact fetal cells from maternal blood have been proposed as superior alternatives to noninvasive prenatal tests from cell free fetal DNA (cffNIPT). These technologies are called cell-based noninvasive prenatal diagnostics (cbNIPD). Increased maternal BMI affects DNA fetal fraction in the blood thus limiting the use of cffNIPT on pregnant women with high BMI. Still, the influence of BMI on the number of fetal cells and cbNIPD technology has not previously been explored. The objective of this study was to investigate the relationship between pre-pregnant BMI and the number of fetal cells in venous blood samples.

*Unedited draft - unpublished*
Methods: Data consists of 91 blood samples from pregnant women at high risk of trisomy 21 (>1:300 in the Danish combined first trimester screening program) who opted for invasive testing in the Central Region of Denmark. Blood samples were drawn as part of a larger validation study of the cbNIPD method conducted by ARCEDI Biotech ApS, a Danish biotech company that holds proprietary technology for the enrichment and analysis of fetal extravillous trophoblast cells. The 91 subjects were subcategorized into four BMI weight classes according to WHO classification (<18.5 = underweight; 18.6-24.9 = normal; 25-29.9 = overweight; 30-39.0 = obese).

Results: Mean BMI was 23.9 and ranged between 17 and 38.7. The mean number of fetal cells was 12.6, with a range of 1 to 43 cells in one sample. Analysis of variance (ANOVA), comparing the mean number of fetal cells in the four BMI subgroups showed a decrease in the number of fetal cells with increasing pre-pregnant BMI. However, this was not significant (p= 0.14). Kruskal-Wallis non-parametric test also showed no significant correlation (p=0.07). Importantly, using ARCEDI’s fetal cell enrichment technology, all the samples, even from the obese group rendered fetal cells.

Conclusions: Increasing pre-pregnant BMI tends to decrease the amount of fetal cells in high risk pregnancies, but not significantly. The current data shows that cbNIPD should not be hampered by an increased BMI because every pregnancy, irrespective of the BMI, has rendered fetal cells for downstream genetic analysis.

P2-2

Novel microfluidic technology to enrich fetal cells from maternal blood

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3Department of Obstetrics and Gynecology, Toho University Medical Center Sakura Hospital, Chiba, Japan

Objectives: Fetal cells circulating in maternal blood provide an ideal source of fetal genome for non-invasive prenatal diagnosis (NIPD). Centrifugation is usually the first step to treat a maternal blood sample, but the big problem is that the change of the cell densities after the blood collection makes the gradient fractionation impossible. To overcome this problem, we have developed a microfluidic chip that can pass 5-10 ml blood samples within 1-2 hours. The chip was designed to remove most of erythrocytes and enrich NRBC, so that the subsequent FACS can purify NRBC.
Methods: The microfluidic chip consists of a main channel and the number of side channels (lateral flow path) which are crossed at right angles. Blood cells will go straight into the main channel, where a small portion of cells are withdrawn to the sides. Channels are designed to capture most of erythrocytes into the narrow bundles and to enrich NRBC into the wide channels.

Results: The maternal blood was applied to the microfluidic chip, 24 hours after the collection. The blood from expectant mothers with the male fetal gender was used. 1.5x10^6 cells were taken from the 20 bundles of wide channels, where NRBC were enriched. The collected cells were stained with Hoechst, anti-CD45 (PE) and anti-CD235a (FITC). The [Hoechst (+) / CD45 (-) / CD235a (+)] population was collected, and the presence of fetal cells was confirmed by PCR (SRY) detection. We also tested on 48 and 72 hours samples and the similar results were obtained.

Conclusions: We confirmed that our microfluidic chip could capture much more amount of fetal cells than previously thought. In our calculation, there are 10-20 fetal cells in 1ml maternal blood, which is 100 times more than a previous calculation of 1 out of 10 ml. Although the NRBC obtained by our method are the mixture of maternal and fetal, immuno-staining of a fetal specific marker and FISH analysis can detect the structure of fetal chromosome.

P2-3

Fetal fraction following selective reduction in twin pregnancies

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^2Ariosa Diagnostics Inc., Roche Sequencing Solutions, Inc., San Jose, CA, United States

Objectives: The loss of a fetus in a multiple pregnancy is recognized to be a complicating factor when using maternal plasma for aneuploidy screening. Cell-free DNA (cfDNA) from a non-viable embryo or fetus is released into the maternal bloodstream and may lead to an increased chance of a discordant NIPT result; however, the amount and duration of this biological process is not well understood. The objective of this study is to observe changes in fetal cfDNA over time in pregnancies with one non-viable twin.

Methods: We present a cohort of four patients with known euploid twin pregnancies in which reduction to singleton gestation was performed. Maternal blood was obtained prior to the procedure and at sequential time points over a two to five week period. cfDNA was isolated from maternal plasma and polymorphic DANSR assays were used to determine the fetal cfDNA percentage (fetal fraction) in each sample.
Results: Fetal fraction was observed to decrease between time points in all four cases but no consistent pattern was observed. In contrast to the average increase in fetal fraction over time previously reported in singleton pregnancies, no patient had a higher fetal fraction at the end of the serial blood draws than the start, likely reflecting the loss of contributory fetal fraction from the co-twin.

Conclusions: In this small cohort, the fetal fraction of cell free DNA dropped overall in the weeks following fetal reduction. Because a similar pattern may occur in pregnancies complicated by a spontaneous fetal reduction or vanishing twin, a larger study, both in terms of number of pregnancies followed and measurements per pregnancy may provide additional data to more comprehensively describe these patterns.

P2-4

Development of a novel methylation-based fetal fraction estimation assay using multiplex ddPCR

George Koumbaris, Skevi Kyriakou, Achilleas Achilleos, Charalambos Loizides, Louiza Constantinou, Elena Kyri, Kyriakos Tsangaras, Petros Mina, Marios Ioannides, Philippos Patsalis

NIPD Genetics, Nicosia, Cyprus

Objectives: Accurate fetal fraction assessment is very important in non-invasive prenatal testing (NIPT). Affected samples with low fetal fraction have an increased risk for misdiagnosis. We present a multiplex droplet digital PCR (ddPCR) assay for fetal fraction estimation using methylation sensitive restriction enzymes (MSREs) and a robust set of novel fetal-specific differentially methylated regions (DMRs).

Methods: We discovered 38 fetal-specific DMRs which can potentially be used for fetal fraction estimation in NIPT. Eight biomarkers (7 DMRs and 1 reference control) were selected for further analysis. An assay comprising MSRE digestion followed by multiplexed (octaplex) ddPCR was developed for fetal fraction estimation. A chromosome Y multiplex ddPCR assay (YMM) was also developed for fetal fraction estimation in male fetuses. YMM was used to test the robustness of the methylation-based fetal fraction estimation assay in 138 male pregnancy samples. A final validation was performed on 234 pregnancies using FFMM and fetal fraction measurements obtained from the VERACITY NIPT test also showed strong correlation.
Conclusions: We developed a robust methylation-based assay for accurate fetal fraction estimation using a novel set of fetal-specific DMRs. This simple method can be used as an accurate fetal fraction estimation tool in NIPT.

P2-5

Non-invasive prenatal testing of microdeletion syndromes

Kyriakos Tsangaras, Petros Mina, Marios Ioannides, Charalambos Loizides, Achilleas Achilleos, Elena Kypri, George Koumbaris, Philippos Patsalis

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Objectives: The discovery of cffDNA in maternal plasma has greatly facilitated the development of NIPT of fetal aneuploidies. However, sub-chromosomal copy number change detection still remains a challenge. Towards this goal, we employed a proprietary hybrid capture-based technology and novel bioinformatics pipeline for the detection of microdeletion syndromes. By leveraging the inherent high enrichment uniformity and high read depth of this in-solution hybridization NIPT method we achieved accurate non-invasive detection of fetal microdeletion syndromes. The assay combines multiple depth of coverage-based and fragment size-based ploidy detection engines to detect 1p36, DiGeorge, Wolf-Hirschhorn, and Smith-Magenis microdeletion syndromes with high sensitivity and specificity.

Methods: cfDNA was extracted from 752 unaffected first trimester pregnancy plasma samples and 29 affected prenatal and synthetic samples. Enrichment probes were designed to span the syndromes’ critical regions avoiding low copy repeats and repetitive elements. All samples were enriched using a hybrid capture technology as previously described. Enriched sequencing libraries were analyzed using a proprietary statistical analysis pipeline developed to test for deletions in each of the syndromes.

Results: The assay was able to correctly classify all abnormal and normal samples resulting in 100% specificity and specificity.

Conclusions: Using a proprietary target capture enrichment technology and novel multi-engine copy number detection pipeline we accurately detected all normal and abnormal samples. This novel microdeletion NIPT method overcomes the limitations of other methodologies and increases the number of diseases that can be reliably detected by NIPT, thus offering more choices to couples towards an informed management of their pregnancy.
Development of a novel non-invasive prenatal test for aneuploidies, microdeletions and 50 single gene diseases

Michael Nicolaou, Charalambos Loizides, Marios Ioannides, Kyriakos Tsangaras, Petros Mina, Achilleas Achilleos, Elena Kypri, George Koumbaris, Philippos Patsalis

NIPD Genetics, Nicosia, Cyprus

Objectives: We hereby present a novel non-invasive prenatal test (NIPT) for major aneuploidies, microdeletions and 50 monogenic diseases with moderate and severe phenotypes, including Hematological, Kidney, Ophthalmological, Neurological, Inherited Metabolic Diseases, such as Thalassaemia, Cystic Fibrosis, Phenylketonuria, Tay-Sachs, etc.

Methods: cfDNA was obtained from 300 pregnancies referred for NIPT at 10th-15th week of gestation for identification of 651 causative mutations in 50 disease associated genes. Additionally, a study including another 1000 pregnancies using cfDNA and paternal DNA is ongoing for NIPT for major aneuploidies, microdeletions and 50 monogenic diseases. An enriched sequencing library was prepared using custom TArget Capture Sequences (TACS) as previously described. TACS were designed based on genomic locations of known causative mutations for monogenetic diseases under investigation. Enriched products were sequenced using NGS and the data was processed using a custom bioinformatics pipeline.

Results: For the initial 300 samples, a high number of causative mutations were identified and a selection of those was confirmed using Sanger sequencing. For the ongoing study with 1000 samples, causative mutations were identified and the fetal risk for aneuploidies, microdeletions and monogenic disorders was determined.

Conclusions: This is the first time that NIPT is made available for a high number of single gene diseases together with aneuploidies and microdeletions, opening a new chapter in prenatal screening. The cumulative risk for the fetus is estimated to be as high as 1/125. This novel NIPT is expandable to hundreds of single gene diseases. It can be taken potentially by all pregnant women as early as the 10th week of gestation.

Utility of the non-invasive prenatal diagnosis in the program of prenatal screening of Trisomy 21 in our sanitary area

Ignacio Peral Camacho, María del Mar Viloria Peñas, Esperanza Lepe Balsalobre

University Hospital Virgen de Valme, Seville, Spain

Unedited draft - unpublished
**Objectives:** The emergence of Non-invasive Prenatal Diagnosis (NIPD) is conditioning a change of strategy in prenatal aneuploidy screening programs. Currently, it is not possible to substitute First Trimester Combined Screening (FTCS) for this NIPD due to economic conditions. However, in Public Systems, it is convenient to locate it in our screening programs. A first strategy would be to implement a contingent model where, after a FTCS, a group of pregnant women is selected with an intermediate risk to which a second screening test (NIPD) would be applied. A second strategy would be to apply the NIPD as an alternative to the invasive technique.

**Methods:** Observational, descriptive and retrospective study, in two periods: before and after the implementation of the new strategy (September 2015-June 2016 versus September 2016-June 2017). In the initial period, the usual strategy was applied, indicating an Invasive Technique to all positive FTCS for Trisomy 21 (risk≥1/280). In the second period, only the invasive technique was indicated for those with a risk≥1/50. In pregnant women at risk between 1/51-1/280 and normal ultrasound, NIPD was indicated the invasive technique. The determination of the biochemical markers was performed on the Cobas-6000 (Roche Diagnostics) and the calculation of risk using the corporate software siPACAC.

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>Nº screenings</th>
<th>Positive screenings</th>
<th>RD</th>
<th>FPR</th>
<th>NIPD</th>
<th>Negative invasive tests</th>
<th>Positive invasive test*</th>
<th>Voluntary revocation**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strategy I</strong></td>
<td>2545</td>
<td>97</td>
<td>84%</td>
<td>3,2%</td>
<td>0</td>
<td>48</td>
<td>17</td>
<td>32</td>
</tr>
<tr>
<td><strong>Strategy II</strong></td>
<td>2107</td>
<td>112</td>
<td>87%</td>
<td>4,5%</td>
<td>60</td>
<td>27</td>
<td>16</td>
<td>9</td>
</tr>
</tbody>
</table>

*Referred only to Trisomy 21.

**Conclusions:** The detection rate of both strategies is similar since all the cases submitted to NIPD were true negative. The indication of invasive technique before a positive result of FTCS has gone from 100% to 46%. This new strategy presents high acceptance by the pregnant women improving the problem of the high rate of revocations that have gone from 33% to 8%.
Non-invasive prenatal diagnosis for monogenic disorders: Benefits, challenges and guidance needed

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Objectives: Definitive diagnostic testing based on analysis of cell free DNA in maternal plasma, non-invasive prenatal diagnosis (NIPD), is making prenatal diagnosis safer and more accessible for families at increased risk of monogenic disorders. In the UK, NIPD is offered by two public health sector laboratories and includes testing for FGFR2 and FGFR3 mutations, cystic fibrosis, spinal muscular atrophy, Duchenne and Becker muscular dystrophies and more than 40 other rare diseases. Here we aim to review activity in one laboratory, review uptake, explore the societal and ethical issues arising and discuss the need for clinical guidelines.

Methods: We undertook an audit of laboratory records since 2012 when NIPD was first approved for use in the NHS to determine number of tests done, for which conditions and outcomes.

Results: There has been an exponential increase in testing from ~ 40 tests in 2012 to ~130 in the first 10 months of the current financial year. We are seeing high uptake of tests in families at low risk of recurrence as well as those with recessive risks, with some families requesting NIPD where they would have avoided invasive testing previously. In the current financial year 27% of tests were requested because of a very low, germline recurrence risk. Overall 20% were mutation positive (affected), with a failure rate of 1%. Data collection with regard to pregnancy outcome is ongoing.

Conclusions: Some have questioned (Wilkie, Prenat Diagn 2017) whether offering NIPD for conditions where there is a low recurrence risks is economically justifiable at current prices. Previous research with women who used NIPD in such circumstances found benefits to include: reducing the period of uncertainty; early reassurance; and normalization of pregnancy within the first trimester. Given the increasing availability and the fact that some companies are now offering prenatal screening for new dominant mutations we suggest there is a need for international debate and the development of guidelines to guide more widespread implementation.
Detection of fetal exosome DNA by massively parallel sequencing in the plasma of pregnant women

Sen Lu

BGI Research, Shenzhen, China

Objectives: During human pregnancy, Placental trophectoderm cells can release exosomes into maternal circulation. Trophoblast cells also give rise to cell-free DNA (cfDNA) in the maternal blood, and has been used for noninvasive prenatal screening for chromosomal aneuploidy. We intended to prove the existence of exosome DNA (exoDNA) in the exosomes of maternal blood, and compared exoDNA with plasma cfDNA in terms of genome distribution, fragment length, and the possibility of detecting genetic diseases.

Methods: Maternal blood from 20 euploid pregnancies, 9 T21 pregnancies, 3 T18 pregnancies, 1 T13 pregnancy, 2 pregnancies of beta-thalassemia, and 2 pregnancies with FGFR3 mutations were obtained. Exosomes were enriched from maternal plasma, and confirmed by electronic microscopy (EM), western blotting, and flow cytometry (FACS). ExoDNA was extracted and its fetal origin was confirmed by quantitative PCR. To characterize exoDNA and compare with cfDNA, pair-end whole genome sequencing was performed. Lastly, the fetal risk of genetic disease was analyzed using the exoDNA sequencing data.

Results: ExoDNA span on all 23 pairs of chromosomes and mitochondria, sharing a similar distribution pattern and higher GC content comparing with cfDNA. ExoDNA showed shorter fragments yet lower fetal fraction than cfDNA. ExoDNA could be used to determine fetal gender correctly, and all trisomies, paternal mutations of beta-thalassemia, and de novo FGFR3 mutations were identified correctly with no false positive results.

Conclusions: We proved that fetus originated exoDNA could be identified in the exosomes extracted from maternal plasma. ExoDNA shared some similar features to cfDNA, and could potentially be used to detect genetic diseases in fetus.
Cell free fetal DNA testing in Argentina experience after 4 years, updated information

Jose Pablo Marchili

Buenos Aires, Argentina

Objectives: We have offered cffDNA testing in Argentina since July 2013. Over 3800 patients were tested. Update of the data.

Methods: Samples shipped to Illumina’s CLIA Laboratory in USA to perform the verifi® prenatal test.

Results: Outcome information: 3810 cases, in the last 12 months: 1450; increase over the previous year (1006 samples): 44.13%. Reported results: 3757, cancelations due to logistic issues: 53. Turnaround time for a patient in ARG is approximately 5.3 days, improved in 1 day compared with the last year (5 to 8 days average time). Technical cancelations: 1 (0.026%), a redraw yielded a successful result. The other 52 (1.39%) cancelations were administrative due to sample arrival in lab beyond stability date. In June 2017 we started offering screening aneuploidies for all chromosomes, 509 patients chose this test (13.54%) of the entire cohort.

Conclusions: We see an increased acceptance and demand from patients and OBs. The consistence and performance encourages pregnant women to accept cfDNA test as first line screening and as a second step ruling out common aneuploidies after an increased risk in FTCST, positive serum screening or abnormal ultrasound. As reported worldwide the number of invasive procedures dropped down.
Counselors’ knowledge about the Dutch prenatal anomaly-screening offer including NIPT: Results of a cross-sectional national pre-posttest survey

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⁸Netherlands

Objectives: In the Netherlands, since April 2017, the Non-Invasive Prenatal Test (NIPT) has been offered as a first-tier screening test for trisomy 21, 18 and 13 to all pregnant women within a study context (TRIDENT-2 study). Consequently, the content (e.g. choices) and organization of the counseling for prenatal screening has been changed. Adequate knowledge about prenatal screening and the disorders screened for is a prerequisite for high quality counseling. Therefore, we explored counselors’ knowledge about prenatal anomaly screening before and after a mandatory Continuing Education (CE) about NIPT in March 2017 and the actual implementation of NIPT in April 2017.

Methods: We used a pre-posttest cross-sectional online 36-item survey to assess knowledge of counselors regarding different aspects (e.g. test-characteristics, clinical features of conditions) of the Dutch prenatal anomaly-screening program including NIPT. The first measurement (T0) took place in February 2017, before a mandatory CE for counselors and implementation of NIPT; the second measurement (T1) was in January 2018. We asked the eight Regional Prenatal Screening Centers to invite all ~3000 Dutch counselors (most midwives) to participate. We calculated knowledge scores and compared the findings of T0 and T1. At T0 we included 1739 counselors (~58%) and at T1, 1063 counselors (~35%).

Results: At T0, >75% of the counselors answered 16 of 36 knowledge questions correctly. Knowledge scores were best (>95% correct) on different aspects of counseling (e.g. non-directiveness). Moreover, scores were worst regarding the positive predictive value of NIPT for Patau-syndrome (11% correct) and Down-syndrome (17% correct). At T1, knowledge increased significantly. More than 75% of counselors answered 23 items of the 36 knowledge questions correctly. Knowledge scores were worst on the item regarding the survival rate for children.
having Patau-syndrome (43% correct) and the positive predictive value of NIPT for Patau-syndrome (46% correct). Additional analyses will be presented at meeting.

Conclusions: Counselors’ knowledge about prenatal anomaly screening improved after a mandatory CE about NIPT in March 2017 and actual implementation of NIPT as first-tier screening. We conclude that overall Dutch counselors demonstrate solid knowledge about prenatal anomaly screening. They seem well aware that counseling should be non-directive since all anomaly screening is optional. However, their knowledge may still be improved in some areas such as NIPT test characteristics, such as the positive predictive values and knowledge of clinical features and variability of conditions screened for. Further research is necessary to examine the impact of counselors’ knowledge on prenatal counseling in practice.

P2-13

 Outcome of high risk for digynic triploidy results from SNP-based non-invasive prenatal testing

Trudy McKanna1, Jessica Chaperon1, Allison Ryan1, Herman Hedriana2

1Natera, Inc., San Carlos, CA, United States
2University of California Davis, Davis, CA, United States

Objectives: Most pregnancies with digynic (maternal) triploidy (DT) have small placentas yielding low fetal fraction (FF) and higher rates of ‘no-calls’ on non-invasive prenatal testing (NIPT). However, in those instances with adequate FF, SNP-based NIPT can uniquely identify the additional haplotype and determine parental origin. The objective of this study was to establish a positive predictive value (PPV) for pregnancies suspected to be at high risk for DT by SNP-based NIPT. In addition, when triploidy was ruled out or truth was unknown, possible maternally-derived causes were investigated for potential correlation with high-risk results.

Methods: Retrospective outcome data were collected for SNP-based NIPTs performed between 1/1/2015 and 12/31/2017 and coded as suspected DT. IRB-approved outcomes included: number of fetuses, ultrasound findings, results of cytogenetic testing including parental origin of triploidy, and maternal medical findings. Data were obtained by telephone or email and two-sided t-tests were performed at the 5% significance level.

Results: A total of 39 cases of suspected DT were identified. Outcome data were obtained for 30 (77%) cases (Table); 9 cases were lost to follow up due to patient transfer or use of a reference laboratory. The PPV for DT was 7/30 cases (23%): 3 were cytogenetically confirmed and 4 were considered highly suspicious via ultrasound. The PPV for maternal neoplasm was 8/30 cases (27%). Triploidy cases had significantly higher gestational age versus maternal neoplasms and normal outcomes (P<0.001, both). FF means between normal outcome, maternal neoplasm, and triploidy groups were not significantly different.
Conclusions: In this small retrospective study, PPV was 23% for pregnancies determined to be high risk for DT using SNP-based NIPT. Triploidy was associated with later gestational age, and FF did not differ between normal outcome, maternal neoplasm, and triploidy groups. These SNP-based NIPT data deliver a PPV requiring DT follow-up. Equally relevant is a similar PPV for maternal neoplasms. Given these findings, clinical follow-up may be considered for false-positive DT NIPT results.

P2-13 Table.

Table. Outcomes from suspected DT pregnancies determined via SNP-based NIPT

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Number of cases, N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal fetal and maternal outcome</td>
<td>12 (40.0)</td>
</tr>
<tr>
<td>Maternal neoplasm</td>
<td>8 (26.7)</td>
</tr>
<tr>
<td>Triplody suspected by ultrasound</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>Confirmed triploidy</td>
<td>3 (10.0)</td>
</tr>
<tr>
<td>Complete molar pregnancy</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Early fetal demise</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Ongoing early gestation</td>
<td>1 (3.3)</td>
</tr>
</tbody>
</table>

* 4 lymphoma, 1 colon cancer, 1 Stage IV cholangiocarcinoma diagnosed a year after delivery, 1 ovarian teratoma, and 1 unspecified.

P2-14

Non-invasive prenatal diagnosis (NIPD) for achondroplasia and thanatophoric dysplasia: Accuracy of referrals influences overall test performance

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Objectives: Published research indicates that next generation sequencing (NGS) of cell-free fetal DNA (cfDNA) in maternal plasma accurately detects FGFR3 mutations in fetuses with skeletal dysplasias. Since 2012 our clinical laboratory has used this analysis to deliver a non-invasive prenatal diagnostic service for achondroplasia and thanatophoric dysplasia (TD). At-risk pregnancies are largely identified by sonographic findings, with a minority referred because of an affected parent or small recurrence risk subsequent to a previously affected pregnancy. Here we evaluate the diagnostic yield of NIPD for achondroplasia and TD, assess the accuracy of referrals based on sonographic findings, and explore outcomes after testing.

Unedited draft - unpublished
Methods: We performed a retrospective review of laboratory and referral records to ascertain all NIPD tests performed for achondroplasia and TD since July 2012 using our next generation sequencing panel to detect mutations in the \textit{FGFR3} gene. Records, including ultrasound reports where available, were reviewed to ascertain the diagnostic yield of the test overall, and when sub-divided according to the number of phenotypic features of achondroplasia or TD identified on ultrasound prior to testing, gestational age, and fetal and maternal doppler findings. Pregnancy outcomes were ascertained wherever possible from the referring centre.

Results: Of the 296 tests done using our \textit{FGFR3} panel, 231 tests were done because of sonographic findings; 74 (32\%) had a mutation. Where ultrasound reports were available, the diagnostic yield when only one sonographic feature was present was 21\% (30/141), increasing to 45\% (17/38) in the presence of two, and 71\% (10/14) for three or more features. For achondroplasia, pregnancies tested after 24 weeks’ gestation were more likely to receive a diagnosis than those referred at earlier gestations. Where reported, the presence of normal dopplers, thereby excluding IUGR, was also associated with increased likelihood of a true diagnosis of achondroplasia.

Conclusions: The sonographic features of achondroplasia and TD are well documented, and here we have shown the potential of fetal ultrasound to maximise the diagnostic yield of NIPD in fetuses suspected to have these conditions. High diagnostic yields are obtained when several features are present. This demonstrates how sonographic diagnosis facilitates targeted, safe, accurate molecular diagnosis in conditions difficult to diagnose with ultrasound alone. We suggest the development of clear guidelines for referral to decrease costs and increase benefits, prevent unnecessary NIPD and potentially allow first-line broader spectrum testing for fetuses where the aetiology may be more heterogeneous.

P2-15

Validation of novel bioinformatic algorithm SCAR for noninvasive prenatal fetal sex determination in twin pregnancies

Michaela Hyblova\textsuperscript{1}, Frantisek Duris\textsuperscript{2}, Marcel Kucharik\textsuperscript{1}, Jaroslav Budis\textsuperscript{3}, Gabriel Minarik\textsuperscript{1}, Tomas Szemes\textsuperscript{3}

\textsuperscript{1}Medirex Inc., Bratislava, Slovakia
\textsuperscript{2}CVTI SR, Bratislava, Slovakia
\textsuperscript{3}Geneton Ltd., Bratislava, Slovakia

Objectives: Currently used approaches for determination of fetal sex in noninvasive prenatal testing (NIPT) have shown limitations in correct prediction of fetal sex in cases of twin pregnancies. According to recent information only SNP based tests of NIPT category are able to determine fetal sex for each of the twins. Aim of the study was to test the feasibility and to validate the new bioinformatic algorithm called SCAR to predict sex of both fetuses in twin

Unedited draft - unpublished
pregnancies with utilisation of whole genome coverage genomic scan of circulating DNA from pregnant plasma.

**Methods:** Low coverage whole genome sequencing analysis was performed with use of MiSeq and NextSeq platforms on circulating DNA of 76 pregnant women with twins according to previously published protocol. For each of the fetuses novel sex determination algorithm called SCAR predicted the most probable combination of twin sexes: girl-girl; girl-boy or boy-boy according to calculation based on fetal fraction counted from sequenced fragment lengths and genomic reads mapped on Y chromosome. All predictions were verified after delivery.

**Results:** Among 76 twin pregnancies, 70 were identified correctly and 6 cases were found as uninformative. All of 6 uninformative samples fell to the group with fetal fraction lower than 10%.

**Conclusions:** Novel algorithm SCAR was correct in approximately 92% pregnancies however fetal fraction under 10% critically affected reliable sex determination in boy-girl and boy-boy pregnancies and led to uninformative result of prediction.

**P2-16**

**Selection of additional, coincidental and controversial findings in noninvasive prenatal testing analyses and their clinical validation**

Gabriel Minarik¹, Martina Sekelska¹, Marcel Kucharik¹, Frantisek Duris², Jaroslav Budis³, Michaela Hyblova¹, Tomas Szemes³, Peter Krizan¹

¹Medirex Inc., Bratislava, Slovakia
²CVTI SR, Bratislava, Slovakia
³Geneton Ltd., Bratislava, Slovakia

**Objectives:** Noninvasive prenatal testing (NIPT) is becoming integral part of routine prenatal screening worldwide. Among different analysis approaches whole genomic scan is one of the most commonly used. It has the potential to be used not only for detection of selected common chromosomal aneuploidies, but also in detection of gains or losses of genomic material over the whole genome. The complexity of by this approach potentially detectable genomic events is a problem, especially when it comes to clinical validation. Aim of the study was to test the applicability of Trisomy test in detection of such not routinely targeted genomic events.

**Methods:** The study covers cases with additional, coincidental or controversial findings detected by Trisomy test while routine screening. For analysis of samples whole genome low coverage approach on Illumina sequencing platforms was used. For confirmation of the findings diagnostic methods as cytogenetics, qfPCR, MLPA and aCGH were used on subsequently invasively obtained samples.
Results: Among presented cases are non-standard trisomies, mosaics, gender misinterpretation as well as maternal and maternal/fetal aberration bearing cases. Validation of results of Trisomy test by diagnostic molecular tests reveal that different non-standard findings of the NIPT category tests could be detected correctly, but their confirmation is still strongly dependent on subsequent diagnostic molecular testing.

Conclusions: Because of technical limits of the method and extensive complexity of genomic aberrations that could be detected by NIPT tests their utilization in prenatal screening for non-standard genomic events is possible only if a battery of diagnostic tests is available.

P2-17

Segmental UPD to the rescue: A biological mechanism underlying discordant NIPT results

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Objectives: Trisomy/monosomy rescue, which carries risk for uniparental disomy (UPD), is a familiar biological mechanism in the context of aneuploidy. With the increasing capabilities of non-invasive prenatal testing (NIPT), the detection of subchromosomal events, beyond full trisomies, is now possible. Similar to full aneuploidies, these subchromosomal events may undergo post-zygotic correction, resulting in segmental UPD. Complex rescue mechanisms may help explain discrepancies between NIPT results and diagnostic testing. Here we describe four cases with a subchromosomal deletion detected by NIPT, in which the diagnostic testing revealed UPD for the region of interest.

Methods: Maternal blood samples submitted to Sequenom Laboratories® for MaterniT® 21 PLUS or MaterniT® GENOME testing were subjected to DNA extraction, library preparation, and whole genome massively parallel sequencing as described by Jensen et al. and Lefkowitz et al.1-2

Postnatal placental testing was facilitated by the ordering provider and completed by an external laboratory (case 3) or by Integrated Genetics (cases 1 & 2). Follow up information and pregnancy outcomes were elicited from the clinicians as part of routine, ongoing laboratory protocol for positive cases.

Results: In the cases below (Table 1), NIPT reported a subchromosomal deletion in isolation or as part of a complex finding. Confirmatory microarray testing (amniocentesis or postnatal)

Unedited draft - unpublished
revealed segmental UPD for the precise region reported as deleted by NIPT. All completed postnatal placental testing was consistent with the reported NIPT findings. Collectively this is suggestive of corrective post-zygotic recombination subsequent to a deletion, ultimately resulting in segmental UPD and fetoplacental discordance. Complex somatic mechanisms, like telomere capture stabilization or homolog-templated recombination (Image 1), in which the missing region is replicated from the homologous chromosome, may help explain the discrepancies between NIPT (‘pre-rescue’) and diagnostic testing (‘post-rescue’).

**Conclusions:** Rescue events that carry risk for UPD are important to consider when faced with discordant NIPT results. This is particularly poignant for imprinted chromosomes and autosomal recessive disease genes. These four cases highlight the importance of careful test selection in confirmatory prenatal diagnosis of positive NIPT results, as many of these findings eluded standard karyotype analysis alone. Microarray utilizing SNP technology may be particularly useful to determine both copy number and potential UPD issues alike, which could have additional clinical implications directly impacting pregnancy management.
### Table 1. Case Details

<table>
<thead>
<tr>
<th>Case</th>
<th>NIPT Result</th>
<th>Diagnostic Testing</th>
<th>Placental Testing</th>
<th>Clinical Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Image" /></td>
<td>Amnio SNPArray: 57.4Mb LOH at 13q21.1-q34</td>
<td>Postnatal placental array: 57.14 Mb mosaic (37%) terminal 13q deletion (consistent with NIPT) &amp; allelic mosaicism of 13q21.1-qtt, consistent with segmental UPD for this region.</td>
<td>Fetal anatomy normal throughout pregnancy. Mildly elevated AF-AFP of 2.49 MoM with a normal ACNE. Microsatellite studies maternal segmental UPD for 13q21.1-qtt and biparental inheritance for 13pter-q21.1. Negative custom sequencing panel for evaluation of AR disease genes. Baby doing well and discharged 4 days after delivery.</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Image" /></td>
<td>Amnio Karyotype: 46,XX</td>
<td>Postnatal placental Array: 8.72Mb 1p35.33-p36.23 deletion &amp; 3.44Mb 1p35.23-p36.22 duplication (consistent with NIPT)</td>
<td>2VC, reportedly unremarkable neonatal period</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Image" /></td>
<td>Amnio Karyotype: 45,XX,r(19)</td>
<td>Postnatal Placental Array: 8p11.2 deletion and mosaic 20p11.23 duplication. Normal chromosome 19. (consistent with NIPT)</td>
<td>Small for age (antenatal &amp; postnatal), tetralogy of fallot, supernumerary nipple, digit contracture</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4.png" alt="Image" /></td>
<td>Amnio FISH and Karyotype: 46,XY</td>
<td>EDD Summer 2018</td>
<td>Normal serum screening: NT 1.3mm. Early anatomy: wnl. Considering cord blood to assess for occult mosaicism</td>
</tr>
</tbody>
</table>
P2-18

Has noninvasive prenatal testing impacted termination of pregnancy for Down syndrome?

Eva Pajkrt, Judith Horenblas, Karline van de Kamp, Elisabeth van Leeuwen, Rosalinde Snijders, Alida Knegt

Academic Medical Center, Amsterdam, Netherlands

Objectives: After the introduction of noninvasive prenatal testing (NIPT), it has been suggested that uptake was associated with a decreased tendency to terminate the pregnancy when DS is confirmed. The aim of the study was to investigate the impact of NIPT on termination and live birth rates for DS after its introduction in April 2014.

Methods: A retrospective population based study of all pre- and postnatally diagnosed cases of DS with an estimated due date between 01-01-2012 and 31-12-2017 in the region for which the Academic Medical Centre in Amsterdam provides tertiary care.

Results: In table 1 outcome of pregnancies with DS in association with first trimester screening results. Overall 71% of pregnancies with DS were terminated. In the group that opted for NIPT the live birth rate was 8% compared to a rate of 4% with previous methods of screening.

Conclusions: Termination rates following the detection of DS by NIPT seem unchanged compared to historical termination rates.

P2-18 Table.

<table>
<thead>
<tr>
<th>Screening</th>
<th>n</th>
<th>TOP</th>
<th>PND</th>
<th>Alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuchal translucency &gt;3,5 mm</td>
<td>85</td>
<td>82 (96%)</td>
<td>2 (2%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>High risk on the combined test</td>
<td>83</td>
<td>78 (94%)</td>
<td></td>
<td>5 (6%)</td>
</tr>
<tr>
<td>Fetal malformation visualized</td>
<td>44</td>
<td>40 (91%)</td>
<td>1 (2%)</td>
<td>3 (7%)</td>
</tr>
<tr>
<td>NIPT indicative of trisomy 21</td>
<td>37</td>
<td>33 (89%)</td>
<td>1 (3%)</td>
<td>3 (8%)</td>
</tr>
<tr>
<td>No screening performed</td>
<td>78</td>
<td>0 (0%)</td>
<td>5 (6%)</td>
<td>73 (94%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>327</strong></td>
<td><strong>233 (71%)</strong></td>
<td><strong>9 (3%)</strong></td>
<td><strong>85 (26%)</strong></td>
</tr>
</tbody>
</table>

Unedited draft - unpublished
P2-19

Cell free (cf)DNA screening test failures: A systematic review of failure rates, risks of Down syndrome and impact of repeat testing

Glenn Palomaki, Edward Kloza

Women & Infants Hospital, Providence, Rhode Island, United States

Objectives: To systematically review the published literature regarding cell free (cf) DNA as a primary screen for Down syndrome in the general pregnancy population. Trisomies 18/13 are less common in the first trimester (72% of all trisomies are Down syndrome) and far less likely to be live born (84% Down syndrome) or survive to one year (99% Down syndrome).

Methods: Search the peer-reviewed English-language publications that reported diagnostic results and test failure rates for all subjects. Extract information regarding odds of failure in Down syndrome and euploid pregnancies and gauge the impact of repeat testing. When possible, limit data to Down syndrome only. Analyses utilized a random effects model. Covariates included country of study, measurement of fetal fraction, methodology, aligned reads, plasma volume, proportion collected after 20 weeks, repeat testing, and maternal weight.

Results: Thirty articles were identified for primary analysis. Study’s origin (Western vs Asian with initial tests only) and repeat testing were significant covariates (3.3, 0.6 and 1.2%, respectively, p=0.001). Odds ratio for failure in Down syndrome/unaffected pregnancies was 0.98 (95% CI 0.62 to 1.55, I²=0%). Fourteen additional studies found 83% of failures were repeated and 79% of those were successful. Among a general US pregnancy population of 100,000, initial test failure would occur in 3,286 euploid and 9.7, 5.8 and 1.8 trisomy 21/18/13 pregnancies, respectively. Repeat testing and targeted ultrasound would resolve most failures, with 165 women needing invasive testing.

Conclusions: Lower failure rates in studies from Asia may be related to not routinely testing for fetal fraction and lower rates of obesity. Repeat testing is effective in providing reliable results after an initial test failure. Protocols for cfDNA screening should focus on Down syndrome. In the setting of screening in the general pregnancy population, professional guidelines for follow-up of cfDNA test failures should stress repeat testing and targeted ultrasound to identify structural abnormalities common in trisomy 18/13 pregnancies, rather than a primary offer of invasive testing.
Non-invasive prenatal testing for single-gene disorders: A summary of positive results

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Objectives: The incidence of single-gene disorders in liveborns is approximately 0.36%.¹ Non-invasive prenatal testing (NIPT) for single-gene variants across 30 genes—associated with clinically-serious conditions having a combined incidence of approximately 0.17%²—was introduced commercially in 2017. Testing analyzes circulating cell-free DNA from maternal plasma and maternal and paternal genomic DNA. Validation data showed >99% sensitivity and specificity (each). This study examines the screen-positive cases identified using NIPT for single-gene disorders.


Methods: NIPT for single-gene disorders was performed using paired maternal and paternal blood-draw samples; the first 369 cases reported are included. Upon requisition, self-reported indications for testing were requested and included abnormal ultrasound finding, advanced paternal age, advanced maternal age, family history, and general screening. Only likely pathogenic and pathogenic variants were reported. Confirmatory prenatal or postnatal diagnostic testing was recommended for all screen-positive patients. Follow-up data were obtained from the ordering clinic.

Results: Of 369 NIPT sample pairs, 27 (7.3%) were screen positive, with an average gestational age of 22w3d; 59.3% (16/27) were identified in the second trimester. Pathogenic or likely pathogenic variants were identified in 10 genes: COL1A1 (5 cases), COL1A2 (4), FGFR3 (7), FGFR2 (2), PTPN11 (2), RIT1 (2), NIPBL (1), JAG1 (2), KRAS (1), TSC2 (1). Most cases (26/27 [96.3%]) had a clinical history (abnormal ultrasound [22] or positive family history [4]). Of those, 5 (18.5%) pursued prenatal or postnatal diagnostic testing—all had the NIPT result confirmed. The remainder declined or planned for diagnostic testing on their newborn.

Conclusions: NIPT for single-gene disorders is a novel test that can noninvasively identify clinically-serious conditions. The screen-positive rate for single gene NIPT in this cohort was 7.3%; as expected, many positive variants were found in genes associated with genetic skeletal disorders and identified following abnormal ultrasound findings in mid-pregnancy. If NIPT for
single-gene disorders is provided early in pregnancy, it has the potential to improve pregnancy management and provide parents critical information about their pregnancy at an early stage.

P2-21

**Targeted cfDNA analysis using DANSR assays for determination of fetal RHD status**

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**Objectives:** To develop a targeted cell-free (cfDNA) test, the Harmony® prenatal test, enhancement that allows determination of fetal RhD status in RhD-negative pregnant women.

**Methods:** 12 simulated pregnancy plasma samples with known *RHD* genotype were prepared by titrating non-pregnant, *RHD*-positive cfDNA (fetal source) into non-pregnant, female *RHD*-negative cfDNA (maternal source) to simulate fetal fractions of 5%, 10% and 15%. A 0% sample served as a negative control. Digital Analysis of Selected Regions (DANSR) assays targeting exons 2, 3, 4, 5 and 7 of the *RHD* gene were added to existing DANSR assays and the generated DANSR products were hybridized onto a custom DNA microarray for analysis of fetal fraction and determination of fetal *RHD* status using the fetal fraction optimized algorithm FORTE.

**Results:** In all 12 simulated pregnancy samples, *RHD* sequences were detected. The *RHD* signal in each case correlated with fetal fraction and was therefore consistent with an *RHD*-positive fetal source on the background of an *RHD*-negative maternal source. As expected, no *RHD* sequences were detected for samples with 0% fetal fraction.

**Conclusions:** Targeted cfDNA testing using DANSR assays has the potential to determine fetal *RHD* status and be used as a noninvasive screening method to identify pregnancies at increased risk for RhD immunization.

P2-22

**24 chromosome re-analysis of suspected trisomic NIPT cases that were non-concordant at follow up: Evidence of full concordance with the new software**

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*Unedited draft - unpublished*
Objectives: Serenity is currently validated for NIPT for chromosomes 13, 18, 21, X and Y however in recent months we have extended analysis capability with a view to full karyotypic analysis (Serenity 24). The objective of this study was to review all our current NIPT cases reported as a suspected result, which were subsequently followed up with a negative or discordant result. We reanalysed these cases through Serenity 24 to determine whether: a) there were any total or partial aneuploidies of the chromosomes that were not originally tested; and b) if our new test showed improved levels of detection.

Methods: NIPT cases reported between January 2016 and early 2017 with a suspected aneuploidy of chromosomes 13, 18, 21, X and Y, were reviewed for cases where follow up testing (NIPT, amniocentesis or CVS) or live birth produced a negative or discordant result. 15 cases were identified that fit our criteria; all were initially run through single-end sequencing. Archived plasma samples from these cases were reprocessed and run through paired-end sequencing, given a result for chromosomes 13, 18, 21, X and Y (Serenity Basic) prior to being run through an algorithm for all chromosomes (Serenity 24).

Results: Results from Serenity Basic: 13 cases were euploid and concordant with follow up and outcome data; 1 failed due to low fetal fraction; 1 was discordant with follow up and outcome data. Results from Serenity 24: all 15 cases had full concordance with follow up and outcome data.

Conclusions: We concluded that there were no abnormalities on other chromosomes that were causing false positives in our original test. Moreover, with improvements to our testing procedures through paired-end sequencing, review of thresholds, and advances in our analysis software, the evidence suggests that our test would be unlikely to produce false positives for cases such as these. We thus report improved levels of sensitivity and specificity for the detection of common aneuploidies including sex chromosome abnormalities.
Methods: From September 2015 till April 2017, 4109 samples of pregnant women were analyzed using Trisomy test. For tested trisomies high risk samples detection whole genome low coverage scan was used in association with home-made bioinformatic pipeline and our own biostatistical algorithm.

Results: Of 4109 analysed samples 3847 were reported as euploid and 76 as trisomic. After analysis of the first blood sample, 184 cases were found to be nonreportable, after second blood sample analysis only 42 samples were still unreportable, so no call rate of the test was 1%. Among trisomic samples 55 samples were reported as high risk for trisomy 21, 15 samples as high risk for trisomy 18 and 6 samples as high risk for trisomy 13. Two false negatives and two false positives were recorded in the whole cohort.

Conclusions: Total sensitivity of the method used for detection of all three trisomes was 97.37%. Total specificity of the method was 99.95%. Trisomy test performance based on calculations of its sensitivity, specificity and no call rate is fully comparable with other commercial tests used in NIPT worldwide.

P2-24

Reduction of false positive sex chromosome aneuploidy NIPS calls caused by maternal monosomy X

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Objectives: Monosomy X (Turner's syndrome) is one of the most common aneuploidy in the female population. Its’ prevalence is about 1 in 2000 live birth. In the last few years noninvasive prenatal DNA screening (NIPS) have become more widespread for detection of common aneuploidies including monosomy X. However, according to the clinical trial results false-positive NIPS results for monosomy X are quite common. The aim of this study was to establish maternal contribution to false positive NIPS results and develop the method to distinguish maternal and fetal origin of high-risk monosomy X noninvasive prenatal DNA screening calls including mosaic maternal cases.

Methods: A total of 1196 woman carrying singleton pregnancies have been recruited. Maternal plasma DNA semiconductor massive parallel sequencing was performed to detect common aneuploidies. For the case of high monosomy X risk call, analysis method to distinguish fetal and maternal monosomy X have been additionally applied.

Results: According to NIPS results, 19 patients had a high risk of fetal monosomy X. In 11 (61%) cases, fetal aneuploidy was confirmed by karyotyping. Other 8 cases were false positives. In
three out of 7 cases, additional analysis based on in silico size selection allowed to assume maternal monosomy X. In these cases, FISH analysis confirmed mosaic monosomy X in maternal blood cells. The cause of one false positive case was true fetal mosaicism. Fetus had mosaic 47,XXX/46,XX karyotype. One more maternal mosaic monosomy case was detected for male fetus with abnormal ratio of X and Y fetal fraction.

Conclusions: The prevalence of maternal mosaic monosomy X karyotype is 0.3% (4/1196) - 10 times higher than published before (p<0.05). Additional in silico size-selection and data analysis increases PPV for monosomy X from 58% to 69% for studied population, reducing number of false positive cases with no changes in sample preparation and sequencing protocols.

P2-25

Detection of partial 4-th chromosome deletion and 12-th chromosome duplication with noninvasive prenatal DNA screening

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Objectives: Noninvasive prenatal DNA screening (NIPS) is believed to be highly sensitive for common aneuploidy detection. Since some NIPS technologies use whole genome sequencing, aneuploidies and large CNV of any chromosome could be detected. Although detection of rare aneuploidies large CNV is possible it is not recommended in Obstetricians and Gynecologists guidelines. Majority of NIPS providers do not report this information. This study describes case of detection of partial 4-th chromosome deletion and 12-th chromosome duplication with NIPS.

Methods: 30-year-old woman (gravida 3, para 1) referred to laboratory for genetic counseling and NIPS. Results of 1st trimester screening indicated that the pregnancy was at low risk of chromosomal abnormalities. Blood for NIPS was collected at 13 weeks 4 days of gestation. NIPS was carried on Ion S5 sequencer with in-house developed data analysis pipeline. Molecular karyotyping was performed using CytoScan Optima Array (Affymetrix, Santa Clara, CA).

Results: NIPS results showed low risk for common aneuploidies and high risk for partial 4-th chromosome deletion and 12-th chromosome duplication. Ultrasound examination at 15 weeks of gestation revealed nasal bone hypoplasia and humerus contraction. Amniocentesis was performed at 16 weeks of gestation. Molecular karyotyping confirmed NIPS results and showed 35 Mb distal part of the short arm 4-th chromosome deletion and 28 Mb distal part of the short arm 12-th chromosome duplication. The deletion is described as typical for Wolf-Hirschhorn syndrome, OMIM 194190. Conventional karyotyping of parents revealed mother's balanced translocation 46,XX,t(4,12)(p15.1;p11.2)
Conclusions: This case illustrates that NIPS can also be useful screening technology to improve prenatal detection rates of rare large CNV.

P2-26

Maternal expansion of the FRA10B repeat can cause false positive NIPT results

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Objectives: Genome wide Non Invasive Prenatal Testing (NIPT) analysis suggested a 20Mb deletion starting at 10q25 in eight independent pregnancies, which could not be confirmed by invasive follow-up testing. Since the start of the deletion was exactly at the FRA10B locus in all samples tested, we investigated a possible relation between maternally expanded FRA10B alleles and the deletion.

Methods: Genome wide NIPT was performed using single read sequencing, followed by bioinformatical analysis of all autosomes using the WISECONDOR algorithm (https://github.com/VUmcCGP/wisecondor). FRA10B expansions were detected in cultured blood lymphocytes with bromodeoxyuridine (BrdU). Maternal and/or fetal deletions were assessed using FISH and array analysis.

Results: Four of the eight mothers were available for follow-up testing. In all four the presence of an expanded FRA10B repeat was confirmed. To confirm our hypothesis that these expansions are associated with low-grade mosaic deletions we performed FISH analysis, showing a maternal low mosaic 10(q25->qter) deletion in one case. This deletion was confirmed by array analysis. As approximately 90% of the cell free DNA tested during NIPT is maternal, this low maternal mosaic can be detected during NIPT analysis.

Conclusions: The recurrent deletion we detected at 10(q25->qter) during routine NIPT analysis is caused by low level mosaic maternal deletions, that are a associated with expanded FRA10B repeats. As the carrier frequency of FRA10B in the general population is 1:40, the associated false positives may be encountered at a high rate, especially as NIPT tests becomes more sensitive. FRA10B expansions should be added to the group of maternal variants that may cause false positive NIPT results. They are not associated with any clinical phenotype, therefore invasive follow up procedures are not indicated.

Unedited draft - unpublished
Genome-wide cell-free fetal DNA screening in routine non-invasive prenatal testing practice: a prospective study on over 38,000 clinical cases

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Objectives: Conventional cell-free fetal DNA (cfDNA)-based non-invasive prenatal testing (NIPT) focuses on detection of common aneuploidies, leaving a gap of ~17% of clinically relevant chromosomal abnormalities that would go undetected. Genome-wide NIPT would greatly expand the range of chromosomal rearrangements detectable, suggesting the usefulness of genome-wide in clinical prenatal screening practice. In this study, we expanded conventional cfDNA-based NIPT to cover the entire genome in a large general population of pregnant women, in order to assess the incidence of chromosomal abnormalities not detectable by traditional NIPT.

Methods: 38,095 pregnant women undergoing genome-wide cfDNA-based NIPT were enrolled in the study. Sequencing data were analyzed using algorithms for common fetal aneuploidies, aneuploidies and subchromosomal aberrations. Clinical outcomes were obtained in 37,804 pregnancies.

Results: Clinically relevant chromosomal abnormalities were detected in 630 (1.7%) pregnancies, 560 (1.6%) of which were confirmed by invasive prenatal diagnosis. In 500/560 cases common aneuploidies were involved, 30/560 were rare autosomal trisomies (RAT) and 30/560 were segmental imbalances. A total of 60 fetal conditions would have otherwise been overlooked if only a conventional NIPT had been performed. The specificity for common aneuploidy, RAT and segmental aneuploidies was 99.92%, 99.97%, and 99.97, respectively; the sensitivity was 100%.

Conclusions: The results of this study demonstrate that genome-wide cfDNA analysis represents an enhanced screening tool for prenatal detection of chromosomal abnormalities, allowing identification of clinically relevant imbalances that are not detectable by conventional cfDNA testing. This level of screening provides an improved detection rate as compared to conventional NIPT, with no appreciable decrease in specificity. These findings provide substantial evidence for the feasibility of introducing genome-wide NIPT into routine prenatal diagnosis practice as a first-line test.
Novel approaches to develop critical reference materials to extend noninvasive prenatal testing to monogenic diseases

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Objectives: Highly characterized reference materials are required to advance NIPT testing of Trisomy 13, 18 and 21, deleterious microdeletions and other monogenic diseases. The goal of this study was to develop reference materials that could be used to advance the development of next generation circulating cell free DNA assays (ccfDNA). Ideally reference materials would be patient sourced and be highly characterized for type of disorder and biochemical formulation.

Methods: Patients with high risk pregnancies were recruited for study of antepartum, postpartum and cord blood through a Stanford IRB approved protocol. Two approaches for processing the patient samples were applied. In approach 1, lymphoblastoid cell lines were prepared from maternal peripheral blood mononuclear cells and from fetal cord blood cells. The cells were EBV immortalized and expanded. Cellular genomic DNA (gDNA) was extracted and fragmented to mimic ccfDNA. In approach 2, ccfDNA was isolated from plasma and amplified.

Results: 33 disease cases including fetuses with trisomies, cardiac malformations, sex chromosome aneuploidies and microdeletions along with three control cases were enrolled.
7 immortalized lymphoblastoid cells lines were prepared. gDNA was extracted and fragmented to simulate plasma samples. ccfDNA isolation generally yielded about 2,000 usable genome equivalents of DNA. The size distribution of the amplified DNA showed a similar distribution to native ccfDNA with a major peak around 160-170 bp and minor peaks above 250 bp. Amplified DNA and formulated lymphoblastoid derived ccfDNA was tested in SNP-based and chromosome counting-based massively parallel sequencing assays which generally agreed with the expected results.

Conclusions: By processing antepartum and postpartum maternal blood and cord blood from high risk patients, we were able to formulate validation materials by amplifying ccfDNA and by blending DNA from EBV transformed lymphoblastoid cell lines. This methodology has the potential to advance the development of DNA sequencing methods for genetic diseases by producing essentially unlimited supplies of reference materials for noninvasive prenatal diagnosis of aneuploidies and monogenic diseases.

P2-29

Cell-based non-invasive prenatal diagnosis via endocervical sampling

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Objectives: Ideally, methods to detect fetal genetic abnormalities are non-invasive, conducted early during pregnancy and have an equal sensitivity as invasive diagnostic tests. Contrary to circulating cell-free fetal DNA found in the maternal circulation, trophoblasts offer better opportunities for genome-wide prenatal diagnosis because of the absence of maternal DNA. The paucity of trophoblasts in the maternal blood impedes their isolation and analysis. Limited studies demonstrated how trophoblasts can be isolated non-invasively using a cytobrush located at the os externum. Based on this finding, we explored the possibility of detecting extravillous trophoblasts (EVTs) within endocervical samples using different sampling methods.

Methods: Women between 7 and 12 weeks of gestation who gave their voluntary consent, were included in this study. Three different methods of endocervical sampling were tested, which all collect cells at the outer part of the endocervix. First, a cytobrush, second, a flocked swab (FLOQSwab™), both located at the os externum. Third, a new device (My Sample Device™) flushing the outer part of the vagina. The cervical samples were subsequently labelled indirectly using the HLA-G antibody which is specifically expressed on the membrane of EVTs. One tenth of the final cell suspension was then screened microscopically for detection of EVTs.

Unedited draft - unpublished
Results: Eighteen pregnant women were sampled with either cytobrush (n=12), flushing device (n=2) or flocked swab (n=4). In 10 out of 12 cytobrush samples, 1 out of 2 flushed samples and 3 out of 4 flocked swab samples, putative extravillous trophoblasts were detected. The flushing device gave the largest total cell yield, however, no larger number of putative extravillous trophoblasts was found compared to both cytobrush and flocked swab samples. The flocked swab was judged as being the most operator- and patient-friendly, due to its specific structure which prevents to go deep into the cervix and avoids bleeding.

Conclusions: Our preliminary results indicate the presence of putative extravillous trophoblasts in all three sampling methods. However, since the flocked swab is less invasive for the patient and does not give an increased background of maternal cells, this method will be used in further recruitment of pregnant women, which is ongoing. Further isolation and characterization of extravillous trophoblasts will confirm the fetal origin of the cell and can potentially be used towards a cell-based noninvasive prenatal genetic testing approach early in pregnancy.

P2-30

Utilization of the Illumina VeriSeq NIPT solution in Belgium: Sample reporting rates and clinical outcomes for 5000 samples

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Objectives: The aim of this study was to describe real-life performance of the Illumina VeriSeq™ NIPT Solution at a single laboratory in Belgium, i.e. Algemeen Medisch Laboratorium (AML), Antwerp.

Methods: Data was collected and generated by the Molecular diagnostics department of AML (Antwerp, Belgium). Maternal blood samples (median age=30 years) were collected between August 1st and November 1st, 2017 in Streck tubes. Samples were screened for fetal aneuploidy using the VeriSeq NIPT Solution (Illumina, Inc.) according to manufacturer’s instructions. An automated liquid handling system (VeriSeq NIPT Microlab STAR, Hamilton Company) was used to isolate plasma, extract cell-free DNA, and prepare libraries. Libraries were pooled in 48-sample batches before sequencing on a NextSeq 500 (Illumina, Inc.). Processing was done by only 1 FTE, mean number of samples/day is 76.

Results: Of 5000 samples submitted for testing, 4862 (97.24%) were initially reported. Repeat testing in 138 samples increased the overall reporting rate to 99.68% (4984/5000). Of 16 samples unreported after repeat testing, 10 were because of technical reasons or other aneuploidies in the patient’s genome and 6 failed quality controls. A total of 59 samples had a positive NIPT result: 26 (50.89%) trisomy 21; 6 (11.76%) trisomy 18; 7 (13.72%) trisomy 13; 21
(41.17%) with a sex chromosome aneuploidy. Invasive diagnostic test results were available for 21 cases reported as positive by NIPT: 15 true positives and 6 false positives.

**Conclusions:** In the study cohort reported here, the success rate of NIPT reporting was 99.68%, which means that 0.32% of the samples were not reported. For the majority of non-automatic called samples, potentially underlying biological reasons can be assumed (awaiting confirmation by invasive procedure) and in 0.12% of the cases no result could be generated. Clinical outcome information suggests the test is performing well.

P2-31

**Theoretical performance of targeted sequencing for the detection of microdeletion syndromes in non-invasive prenatal testing**

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**Objectives:** We aimed to investigate the performance of a new commercially targeted enrichment kit comprising a clinically focused exome panel complemented with genome-wide backbone baits to detect five well-defined microdeletion syndromes, in the context of non-invasive prenatal testing (NIPT), based on the counting method.

**Methods:** The targeted enrichment kit used was the OneSeq from Agilent Technologies. The efficiency of our approach was evaluated *in silico* through simulation of different levels of mosaicism to mimic the DNA fetal fraction in maternal plasma. The simulations of different levels of mosaicism were performed combining the .fastq files from a female control with normal karyotype to a patient with microdeletion syndrome (1p36 deletion, Smith-Magenis, Williams, Prader-Willi/Angelman, and DiGeorge). The concatenated files were then aligned to a reference genome using the BWA algorithm to generate the .bam files, which were processed and analyzed using NexusCopyNumber software.

**Results:** All five microdeletion syndromes tested were correctly identified. Our data showed that it is possible to identify deletions between ~ 500 Kb - 4 Mb from 10% mosaicism with great sensitivity and specificity.

**Conclusions:** Although several reports have demonstrated the potential use of whole genome sequencing to identify fetal sub-chromosomal imbalances from maternal plasma DNA, targeted sequencing may be considered a better approach in NIPT because it generates a large number of sequences at a low cost, and enables the enrichment of specific genomic regions taking into account their clinical relevance. Because of the difficulty in obtaining plasma samples from pregnant women screened positive for fetal microdeletion/microduplication syndromes, most of the studies are based on in silico models or artificial samples. Moreover, the targeted approaches tested so far in NIPT are patented, which makes
Mendelian disease genotyping by cell-based noninvasive prenatal testing

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Objectives: Noninvasive prenatal testing (NIPT) based on intact fetal cells isolated from the maternal circulation is a promising pathway in the field of prenatal diagnosis. We have previously shown the feasibility of fetal trophoblast-based NIPT for the detection of fetal copy number abnormalities down to a size range of 1 to 2 Mb (PMID: 27616633 and 27761919). Additionally, with the data presented here, we show that this methodology is also suitable for the detection of single gene mutations.

Methods: Specific genotyping assays were developed for cystic fibrosis, Tay-Sachs disease and sickle cell anemia. These were validated on compound heterozygous lymphoblast cell lines having mutations for one of these disorders, by means of specific multiplex PCR followed by paired-end sequencing. These disease-specific assays were subsequently tested on fetal trophoblasts isolated from blood samples collected from pregnant women. After initial blood processing and trophoblast enrichment, individually picked fetal cells underwent whole genome amplification (WGA). Three different WGA kits (PicoPLEX, Ampli1 and Repli-G) were compared. Concurrent clinical data for these samples were generated by amniotic fluid or chorionic villi sample analysis.

Results: Single lymphoblasts (triplicates) were processed for each WGA method. Most WGA reactions succeeded, except when applying RepliG on fixed cells. Subsequent multiplex PCR was successful for 13 out 15 PicoPlex reactions. All Ampli1 reactions succeeded, except for one CFTR amplicon containing an Ampli1 restriction site. All 9 unfixed cell Repli-G products amplified successfully. Although the PCR success rate in trophoblasts was considerably lower, all products that actually were successfully amplified yielded an adequate sequencing coverage (>1,000 reads/mutation), and the results were concordant with the fetal clinical diagnosis. No mutations were seen when the fetus was not a carrier or affected.

Conclusions: With these data we show that cell-based NIPT is suitable for the detection of point mutations associated with Mendelian disorders. We are currently in the process of optimizing our method further. A more extended WGA kit comparison and more data on the application of this cell-based method on more maternal samples are in progress.
Non-invasive prenatal testing in the screening of trisomy 13 (T13), T18 and T21 based on 19558 pregnant women in a Chinese prenatal diagnosis center

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Objectives: The clinical performance of non-invasive prenatal testing (NIPT) in the Down’s syndrome screening based on 19558 pregnant women in a Chinese prenatal diagnosis center was investigated.

Methods: This was a retrospective analysis of NIPT study in singleton pregnancy (n=19558). The NIPT test is offered routinely as a prenatal screening test for common fetal aneuploidies, including trisomy 13 (T13), T18 and T21 to pregnant women with risk factors of one or more anomalies. Maternal peripheral blood (10 ml) was collected at a gestational age of 12+6 to 22+6 weeks. Sequencing data were analyzed using a proprietary algorithm. Women with positive NIPT results were recommended to receive karyotype analysis and amniotic fluid puncture for further validation. The cases were followed up for 90 days after delivery.

Results: NIPT results were obtained in 99.6% samples. Of these, 19407 (99.5 %) were low risk and 99 were high risk of T13, T18 and T21. A sensitivity of 100% and specificity of 99.8 % was seen in all tested chromosomal abnormalities combined. Taken together, after confirmation, the positive predictive value for Trisomy 21, 18, 13 was 60%, 75%, and 27.3%, respectively. The average fetal fraction was 8.5%, which was slightly lower than the average observed in studies elsewhere. There were one false negatives due to mosaicism of trisomy 21.

Conclusions: In conclusion, NIPT technique is feasible for the prenatal screening of T18 and T21 with higher sensitivity and specificity compared with conventional methods. In conclusion, NIPT technique is feasible for the prenatal screening of T18 and T21 with higher sensitivity and specificity compared with conventional methods.
Objectives: Based on previous reports that fetal-derived DNA molecules in the maternal plasma are generally shorter than those derived from mother. Herein this study, we introduce a novel method called Pre-linear amplification (PLA) based-fetal DNA enrichment method, which can increase cell-free fetal DNA fraction by preferentially amplified the short DNA molecules. We investigate the nature of PLA-based preparation method and it may provide substantial improvement in current non-invasive prenatal testing (NIPT).

Methods: The novel method, which performs 20-30 cycles of linear amplification before conventional exponential amplification on conventional library preparation of maternal plasma, called Pre-linear amplification (PLA) based library preparation method. Short DNA fragments were significantly enriched in PLA libraries by preferentially amplified the short DNA molecules. We validated this method by comparing the size distribution and fetal fraction calculated by Y-chromosome sequences between PLA and conventional NIPT library preparation method. Totally, we recruited 60 pregnancies from first and second trimester to see the fetal DNA enrichment effect and difference.

Results: Among 60 pregnancies recruited, 24 were carrying male fetuses, and the average fetal fraction was 13.1%, with a range of 4.5% to 26.3%. In PLA libraries, the average fetal fraction increased to 17.5%, with a range of 6.6 to 28.7%, showing an averagely 1.2 fold higher than the conventional method. Besides, the lower the fetal fraction, the higher the increase rate was observed, which can benefit the samples collected from first-trimester (Figure 1. a, b). The genome-wide size distribution in male and female fetus both shows that PLA libraries presented higher ratio of short DNA molecules. (Figure 1. c, d).

Conclusions: As previously reported, fetal-derived DNA molecules in maternal plasma are generally shorter than those derived from the mother. PLA libraries preparation method affords an effective and convenient way to enrich fetal DNA in maternal plasma sequencing. It can be used to increase the reliability of samples with low levels of fetal DNA fraction and may provide substantial improvement in routine non-invasive prenatal testing.
**P2-35** Image.

**P2-36**

**Validation of a paired-end sequencing-based NIPT assay for 24-chromosome analysis**

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**Objectives:** Rare Autosomal Trisomies (RATs), or trisomies occurring on chromosomes other than 13, 18, 21, and sex chromosomes, have been reported to be found in up to 1.1% of clinical NIPT samples in previously published studies, and are thought to comprise a significant proportion of test failures in whole-genome sequencing (WGS)-based NIPT methodologies. In

*Unedited draft - unpublished*
order to further reduce sample failure rate and provide a more comprehensive prenatal testing service, we sought to develop an assay for copy-number examination for all chromosomes.

**Methods:** In order to develop and validate this assay, we performed a retrospective analysis of 3332 stored plasma samples that had been previously processed through our paired-end sequencing-based NIPT assay for chromosomes 13, 18, 21 and sex chromosomes, the alignment data from which was used to generate aneuploidy calls for all chromosomes. Raw sequencing data from these runs was reprocessed through our new 24-chromosome analysis pipeline, and results were compared with those obtained previously. Discordances between the datasets were further scrutinised and used to refine aneuploidy thresholds.

**Results:** Following initial analysis, there were 75 discordant chromosome calls between the two datasets, of which 67 represented euploid/suspected or suspected/detected thresholding mismatches, and 8 represented euploid/detected aneuploidy classification errors. Of the 8 euploid/detected mismatches, 5 were discordant for chromosome X calls, which previous studies have shown to have a higher incidence of false positive results, and two were on GC-rich chromosome 19. Refining the RAT thresholds reduced the number of threshold-mismatch samples from 67 to 45, while not diminishing sensitivity or creating additional mismatches. When considering all chromosomes, this represents a per-chromosome discordance rate of 0.067%.

**Conclusions:** In this study, an overall aneuploidy detection rate of 1.92% was observed, with 0.72% of overall samples determined to have a positive RAT result. This is consistent with previously published results, which range from 0.3% to 1.1% in overall RAT detection rates. Furthermore, the two methodologies used in this study displayed an overall discordance of <0.1%. The high concordance between bioinformatic methodologies, and with previously published data, suggests strong performance of the assay. Collection of clinical outcome data for this cohort is ongoing, to be published when available, and ongoing assay performance will be monitored through collection of outcome data.

P2-37

**Non-invasive prenatal diagnosis (NIPD) of X-linked and autosomal recessive single gene disorders by relative haplotype dosage (RHDO): A review of 18 months of clinical service**

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**Objectives:** We aimed to launch a service for NIPD of multiple single gene disorders (SGD). Through the Health Innovation Challenge Fund (HICF) funded NIPSIGEN project, we have...
developed and implemented a relative haplotype dosage (RHDO)-based method for NIPD of spinal muscular atrophy (SMA), Duchenne and Becker muscular dystrophies (DMD/BMD), cystic fibrosis (CF) and congenital adrenal hyperplasia (CAH). Diagnostic services for SMA and DMD/BMD were launched in September 2016, followed by the launch of a CF service in December 2017. Since the launch of our services, we have performed NIPD for 48 referrals (UK and international).

**Methods:** The test involves targeted enrichment of thousands of SNPs across multiple genomic regions and massively parallel sequencing (Illumina MiSeq) of cfDNA followed by RHDO analysis. Maternal, paternal and proband genomic DNA samples are tested alongside cfDNA for haplotype phasing and to measure fetal fraction. Our method can test 2-3 pregnancies on a single MiSeq run, thus centralising testing and decreasing costs for use in clinical service. The development of an automated analysis pipeline has increased capacity further.

**Results:** We have reported 16 normal, 18 unaffected carrier and 10 affected pregnancies with an average turnaround time of 11 calendar days. Gestational age has ranged from 8 - 13 weeks. Repeat samples were requested due to a low fetal fraction in 4 cases (8%). We have had no failed samples, however, for 4 cases (8%), a partial result was issued due to persistent low fetal fraction, a recombination event or lack of informative SNPs and therefore follow-up invasive testing was recommended. Of the 48 diagnostic tests, we have so far received postnatal confirmation of 15 results, with no discrepancies.

**Conclusions:** We have shown that NIPD by RHDO is a robust assay for both X-linked and autosomal recessive disorders, which does not require follow-up invasive testing to confirm positive results. Multiplexing of testing and automated analysis reduces costs and increases capacity sufficiently to allow testing to be provided routinely within a clinical setting. The assay could be further extended to increase the availability of NIPD for many monogenic disorders, thus providing accessibility to NIPD for many more couples with a pregnancy at risk of a SGD.

P2-38

**Clinical evaluation of NIPT for women at advanced maternal age: A multicenter retrospective study**

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**Objectives:** The world is facing a serious challenge of birth defects caused by the increase in the number of women with advanced maternal age (AMA). However, there was no sophisticated scheme of prenatal screening and diagnosis for advanced maternal age until now. In present study, we want to explore the clinical effect of non-invasive prenatal testing (NIPT)

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for the women at advanced maternal age (AMA) and discuss the relationship between women age and NIPT effect.

**Methods:** 14035 women with AMA (over than 35 years old at delivery) who accepted non-invasive prenatal testing were recruited for this study. They were from two prenatal diagnosis centers. NIPT were checked by Illumina Next CN 500. All of the AMA women received prenatal genetic counseling, selected prenatal diagnosis and different clinical treatment according to the results of NIPT.

**Results:** 114 cases (0.81%) got the NIPT positive results of T21/T18/T13. 104 cases accepted prenatal diagnosis and 87 cases were proved as true positive. The sensitivity, specificity, PPV and NPV were 100%, 99.88%, 92.55% and 100% respectively. 74 women (0.53%) showed NIPT positive results of SCAs. 46 women (62.2%) accepted fetus karyotype analysis. The PPV for SCAs in AMA women was 41.3%. The risk of T21/T18/T13 for the women over than 40 would significantly increased (OR=2.90, p=0.0069).All of the parameters of the pregnant women over than 40 reached 100%. It was worth noting that PPV had greatly improved.

**Conclusions:** NIPT is a good choice for AMA pregnant women. It can not only achieve satisfactory clinical effect, but also greatly reduce invasive prenatal diagnosis. We will get better effect of NIPT by further manage AMA women stratified by their age.
Development and pilot study of the PRENID-scale: A measure for informed-decision making in first trimester prenatal screening

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Objectives: Pregnant women in the Netherlands can choose between NIPT, the combined test (CT) and not participating in first trimester prenatal screening (FTS). It is unclear whether pregnant women are making informed decisions about this screening offer. To give insight in

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the decision-making process pregnant women go through, an instrument for informed decision-making was developed.

**Methods:** The prenatal informed decision-making scale (PRENID-scale) was developed with medical and psychological experts. The PRENID-scale consists of a knowledge-scale (range 0-25), testing knowledge of trisomy phenotypes and test characteristics, and of a deliberation-scale (range 39-195) measuring the process of thinking about the consequences of (not) engaging in FTS and imagining the consequences of an abnormal result, such as continuing or ending one’s pregnancy. Twenty-three pregnant women, all counselled for the choice between NIPT, the CT and no FTS, were included in the pilot study (M = 30.9 years).

**Results:** Both the knowledge- and deliberation-scales of the PRENID-scale possess a high internal consistency reliability (respectively $\alpha = 0.83$ and $\alpha = 0.89$). Pregnant women choosing the CT had the most extensive knowledge about the different forms of FTS and the syndromes screened for (M = 19.8), whereas pregnant women declining FTS had the least amount of knowledge (M = 14.7) ($p = ns$). Pregnant women opting for both NIPT and the CT deliberated most extensively about their choice (M = 167), whereas women declining FTS had deliberated about their choice significantly less (M = 128.3) ($p = .03$).

**Conclusions:** The PRENID-scale is the first scale extensively measuring deliberation in the low-risk pregnant population. We found that pregnant women who want their pregnancy examined more extensively, had more knowledge and deliberation than women who do not, or less extensively, want their pregnancy examined. An informed decision, based on sufficient knowledge and deliberation was made by 43.5% of the pregnant women, whereas 56.5% of the women had insufficient knowledge. Almost all women deliberated about their choice. Whether women declining FTS make informed decisions according to the PRENID-scale needs to be investigated in a larger study.

P2-42

**Exploring the impact of prenatal diagnosis and Hormonal Replacement Therapy (HRT) on Working Memory (WM) outcomes in boys with 47,XXY (Klinefelter Syndrome)**

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**Objectives:** Although only 25% of cases are ever diagnosed, 47,XXY (Klinefelter syndrome, KS) is the most frequently occurring X & Y chromosomal variation (1:660). Neuroimaging studies have
revealed that boys with 47,XXY possess a thinner cortex in the frontal region of the brain, leading to deficits in executive function (EF), a collection of skills that includes planning, inhibition, and cognitive flexibility. EF also encompasses working memory (WM) capabilities, yet very few studies have explored WM in 47,XXY boys or the impact of prenatal diagnosis and hormonal replacement therapy (HRT) on WM.

Methods: 111 boys with 47,XXY were evaluated using the Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV). In this group, 50 (45%) received HRT prior to 11 years of age; 61 (55%) did not receive any HRT. Twenty in the HRT-group received early hormonal treatment (EHT); 18 received a booster treatment of testosterone between 5-11 years of age (B); 12 received EHT and B (EB). HRT was administered based on the patient’s pediatric endocrinologist’s assessment of phallus size compared to neurotypical boys of the same age. Eighty-seven (78%) were diagnosed with 47,XXY prenatally, while the remaining 24 (22%) were diagnosed postnatally.

Results: On the WISC-IV, boys who were prenatally diagnosed with 47,XXY had higher working memory index (WMI) scores when compared to their postnatally diagnosed counterparts (P=0.0366). Of those prenatally diagnosed, boys who had not received any HRT had lower WMIs than those who received either EHT (P=0.00158), B (P=0.0203), or EB (P=0.00052). Of the prenatally diagnosed boys, none of the EB boys a below average WMI score, compared to the 5.5% EHT and 12.5% booster boys who had below average WMIs. Overall, of the 111 boys, 24% presented with below average WMIs.

Conclusions: To our knowledge, this is the first study suggesting two predictors of improved WM in 47,XXY boys: prenatal diagnosis and HRT. These results support the need for prenatal genetic testing, and demonstrates the need to identify children with 47,XXY as early in life as possible. These result show the potential that prenatal diagnosis combined with HRT possibly corrects some atypical effects that may arise from morphological brain differences within the frontal lobe in 47,XXY boys. Further research is needed to determine optimal timing, long-term effects, and underlying relationship between prenatal diagnosis, biological mechanisms of HRT, and WM outcomes in 47,XXY.

P2-43

Wilson’s disease and pregnancy: A case report

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Unedited draft - unpublished
Objectives: Wilson's disease (WD), also called hepatolentic degeneration, is a disorder of copper excretion metabolism in the body. WD is a rare disease and seen at 1/30,000. The copper toxicity and liver failure may cause hormonal disorders in untreated cases in WD. In these cases, primary amenorrhea and oligomenorrhea are frequently seen. We want to present our rare case of Wilson's disease and pregnancy.

Methods: A 26-year-old woman, gravida 1 parity 0, was referred to our clinic for the first examination of pregnancy. She has a familial WD, the patient were closely followed up and medical tratments were regulated with related medical branches. The patient was taken to the cesarean section for nonreassuring fetal heart rate at the 38th week of gestation. The complication was not observed postoperatively, she was discharged by healing.

Results: WD is a rare and caused accumulation of copper in organs such as brain, liver, cornea and kidney by the ineffective copper excretion with inadequate ceruloplasmin levels. WD has adverse effects on fertility, and also has high abortus rates. In the literature, there are cases of Wilson's disease with successful pregnancy with appropriate treatment. In these patients decrease the chances of success in pregnancy because of endocrine disorders and liver diseases resulting from failure of the copper metabolism.

Conclusions: In such cases, the importance of early diagnosis and treatment has been revealed in our writing and literature review. Through early diagnosis and treatment, our case was spontaneously pregnancy and continued to have a healthy pregnancy without having been exposed to infertility through hormonal imbalances of WD.

P2-44

Perceptions of informed choice in prenatal genetic testing: Views from women in China

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Objectives: This study aimed to explore perceptions of informed choice in prenatal genetic testing in women from two Chinese cities with very different social economic and ethnic backgrounds.

Methods: A Q-methodology study was conducted during June 2016 to February 2017 in two cities of China: a highly developed metropolis city - Shanghai, and an underdeveloped city with a large ethnic minority population, Duyun. A total of 169 women (84 from Shanghai and 85 from Duyun) with at least one child aged three years or younger were recruited. They were asked to rank-order a set of 41 statements on informed choice along a Q-sorting grid. Each participant's distribution of the statements is called a Q-sort (see Figure 1 for an example). Semi-structured interviews were conducted after the Q-sort was completed.

Unedited draft - unpublished
Results: One consensus statement prevailed across all factor arrays: It was strongly agreed that the woman and her husband/partner should make the decision about testing together. Five distinct sets of viewpoints were further identified. Viewpoint 1 revealed the women tended to make a shared decision led by themselves. Viewpoint 2 perceived informed choice as “no choice” because all tests were thought as a “must” for expectant mother. In viewpoint 3 and 4, husband/partner or doctors were expected to make the final decision. In viewpoint 5, husband/partner and other family members were anticipated to join the decision-making.

Conclusions: Informed choice is about how to balance the unbalanced power among various actors. This study reveals the diminishing influence of traditional family values on women’s decision regarding prenatal testing. Only a few participants from Duyun submitted their autonomy to their husband and his family members. Participants from Shanghai, often with better education and higher income, only took their husband and her own family’s advices into consideration, keeping the in-laws out of the decision circle. In contrast to kinships, the role of a small core family seems to emerge as a decisive force in informed decision making in contemporary China.

P2-45

Evaluation of an expanded carrier screening offer in a non-commercial setting

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Objectives: Most expanded carrier screening panels that have been introduced to date have been advertised and offered on a commercial basis. Since May 2016, expanded carrier screening for 50 severe recessive disorders is available in a non-commercial hospital setting in Amsterdam, to facilitate informed reproductive decision-making. The screening aims at couples without a priori increased risk (no family history). Couples can apply for counseling via www.dragerschapstest.nl, or by physicians’ referral. Pre-and posttest counseling is provided by genetic professionals at the outpatient clinic. External geneticists can send in blood samples as well (external requests). Outcome and impact of testing was evaluated.

Methods: A capture-based next generation sequencing strategy is used for testing. Only pathogenic variants are reported (individual reports are issued). CNV analysis is included. Reimbursement is possible for individuals with a high-risk indication (HRI) based on ancestry, consanguinity and/or family history. Partners of couples can opt for parallel (both partners at the same time) or sequential testing. Pre- and posttest questionnaires were completed by
attendees including reasons for testing, knowledge, psychological impact of test-results and satisfaction.

**Results:** In one year, 67 couples (46 HRI) and 19 individuals (17 HRI) were seen. Thirty-three couples (50%) choose parallel testing. Main reason for testing was to avoid severe illness in the child (41%). 122 test-results were completed, including 9 partners tested after positive tested carriers, and 22 external requests. Carrier status of one (n=31 persons), two (n=5), three (n=1) or four (n=1) mutation(s) was identified. 69% tested negative. Fewer carriers were identified in the HRI group (27%) compared to the general risk group (42%). No carrier couples were found. Satisfaction was high but 43% believed the costs were too high.

**Conclusions:** In the first year, about one third of tested individuals were carriers. Although our main goal was to offer couples with no increased risk the possibility to have carrier screening, two-thirds reported a high-risk indication. In the Netherlands carrier screening is not well-known, and we suspect that few people heard from the screening possibility. Our results nevertheless show that although interest in testing from the general public is still relatively low, considering the number of individuals who requested a test, offering expanded carrier screening within regular healthcare is feasible. An update on outcome will be presented at the ISPD Conference.

**Omphalocele: From diagnosis to growth and development at two years of age**

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**Objectives:** To compare the prenatal perspective of omphalocele (i.e. survival of fetuses) with the perspective after birth (i.e. survival of liveborn neonates), and to assess physical growth and neurodevelopment in children with minor or giant omphalocele up to two years of age.

**Methods:** We included fetuses and neonates diagnosed between 2000 and 2012. Physical growth (SD scores, SDS) and mental and motor development at 12 and 24 months were analysed using general linear models, and outcomes were compared with reference norms.

**Results:** We included 145 fetuses and neonates. Of the 126 prenatally diagnosed fetuses, 50 (40%) were liveborn, and 35 (28%) survived at least two years. Nineteen (13%) neonates were diagnosed after birth. Of these 69 liveborn neonates, 52 (75%) survived, and 42 children (81% of survivors) were followed longitudinally. At 24 months, mean height and weight SDS were significantly below 0 in both minor and giant omphalocele (Table). Mental development was
comparable to reference norms in both groups. Motor function delay was found significantly more often in children with giant omphalocele (82%) than in those with minor omphalocele (21%; p=0.002).

**Conclusions:** The prenatal outcome perspective of omphalocele is considerably worse than the postnatal perspective; a multidisciplinary approach in parental counselling is recommended. Considering the large proportion of children with giant omphalocele and delayed motor development, we recommend close monitoring of these children and timely intervention.

P2-46 Table.

<table>
<thead>
<tr>
<th></th>
<th>Minor omphalocele (n=25)</th>
<th>Giant omphalocele (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Height SDS</strong></td>
<td>-0.57 (-1.05, -0.09)</td>
<td>-1.32 (-2.10, -0.54)</td>
</tr>
<tr>
<td><strong>Weight SDS</strong></td>
<td>-0.86 (-1.35, -0.37)</td>
<td>-1.58 (-2.37, -0.79)</td>
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</tbody>
</table>

Data are shown as mean [95% confidence interval]. SDS: standard deviation score.

P2-47

**The impact and relationship of timing of diagnosis and testosterone replacement therapy (TRT) on anxiety disorders in 47,XXY (Klinefelter Syndrome)**

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**Objectives:** 47,XXY (KS) is the most common X & Y chromosomal variation (1:660 males) but is only diagnosed 25% of the time (Savic, 2012). The incidence of anxiety related disorders (ARD) and the potential impact of testosterone replacement therapy (TRT) on anxiety has not been well investigated.

**Methods:** 80 males with 47,XXY were evaluated using the Child Behavior Checklist (CBCL). 40 males received TRT and 40 did not receive TRT. 65% of the boys in the TRT group received early hormonal treatment (EHT) in infancy. The remaining received TRT between 5 and 10 years of age. TRT was based on the patient’s pediatric endocrinologist’s assessment of the size of plallus.
in comparison to neurotypical boys of the same age. 76% were diagnosed prenatally while the remaining 24% were diagnosed postnatally.

**Results:** Untreated boys were clinical above the 97th percentile in internalizing problems (40%), total problems (42.5%), and anxiety problems (27.5%) whereas treated boys were clinical in internalizing problems (22.5%). Prenatally diagnosed untreated boys demonstrated reduced clinical symptoms when compared to postnatally diagnosed children in thought problems \((P=0.006)\). Within the treated group, prenatally diagnosed again performed better with reduced clinical symptoms in affective problems \((P=0.008)\) and anxiety problems \((P=0.008)\). Positive effects of TRT were evident in a reduction of social problems \((P=0.01)\), total problems \((P=0.01)\), externalizing problems \((P=0.03)\), and anxiety problems \((P=0.048)\) in the treated group, regardless of timing of diagnosis.

**Conclusions:** This is the first study to explore anxiety in 47,XXY and find an increased presence of anxiety (27.5%) in untreated boys, as well as postnatally diagnosed boys. This study describes the positive effect of TRT in the neurodevelopment of boys with 47, XXY and demonstrates that treatment seems to reduce anxiety in prenatal boys. Further investigation is warranted to explore individual responses to TRT to develop more personalized and precise treatments. Anxiety related disorders should be considered as part of the behavioral phenotype of 47,XXY and anxiety treatments like therapy and medications may reduce behavioral issues with boys with 47,XXY.

P2-48

**Congenital diaphragmatic hernia: Impact of contemporary management strategies on perinatal outcomes**

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**Objectives:** During the last two decades the prenatal and postnatal management of congenital diaphragmatic hernia (CDH) has been notable for several revolutionary advances, such as improved prenatal diagnosis, postnatal intensive care and surgical management, with concomittant increases in postnatal survival. This study aims to demonstrate the direct impact of these changes in the perinatal management of CDH upon neonatal and one-year survival rates from 1996-2015 in the state of Western Australia. We hypothesized that an improvement in survival rates may have altered women’s decision-making for pregnancy management options.

*Unedited draft - unpublished*
Methods: Western Australia is a geographically isolated state in Australia which houses a single tertiary level perinatal center, with a state-wide comprehensively integrated and computerised health record database. This is a retrospective study of all cases of CDH, including terminations, stillbirths and livebirths, in Western Australia (WA) from 1996 to 2015, identified from 5 independent databases within the WA health network. Detailed information pertaining to pregnancy and survival outcomes were subsequently obtained from review of maternal and infant medical records. To ascertain if new ventilation strategies had affected pregnancy and survival outcomes, data were divided into four 5-year epochs for analysis.

Results: 215 cases of CDH were identified with 164 diagnosed prenatally. There was a decline in livebirth rates for prenatally diagnosed cases from 1996, reaching a nadir of 5.3 per 10,000 births (2006-2010), before increasing to 9.73 per 10,000 births in 2011-2015. There was a corresponding decline in the number of pregnancies terminated, from 8.3 to 4.6 per 10,000 births and an increase in survival of livebirths with CDH, from 38.9% to 81.3% $P = 0.01$; Figure 1), especially in isolated cases (33% to 71%; $P = .05$). This correlated with introduction of gentle ventilation with permissive hypercapnia in 2003.

Conclusions: This is one of the first studies to explore changes in prenatal management of pregnancies complicated by the diagnosis of CDH. Although the mortality of CDH remains significant, the overall survival rate has increased over the last 20 years. This optimistic observation has consequently lead to an increased tendency for women to continue their pregnancy and a concomitant decline in termination rates, especially in isolated cases of CDH. Information from this study is highly applicable in the counselling of women following prenatal detection of CDH.

P2-48 Figure.
Prenatal genetic analysis of a fetus with 2q37.3 microdeletion and 17p13.3p13.2 microduplication

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Objectives: To perform genetic study on a fetus with ultrasound abnormalities of microcephaly and craniosynostosis, and analyze the correlation between phenotype and fetal genotype.

Methods: Umbilical cord blood was taken for G-banded karyotype analysis and single nucleotide polymorphism array (SNP array). G-banded karyotype analysis and fluorescence in situ hybridization (FISH) were performed on peripheral blood from fetal parents.

Results: Karyotypes were normal in both fetus and parents. SNP array detected a 4.682 Mb terminal deletion at 2q37.3 and a 3.651 Mb terminal duplication at 17p13.3p13.2, involving pathogenic genes such as HDAC4, KIF1A and PAFAH1B1 (LSI1). The deletion region covered the region of 2q37.3 microdeletion syndrome, and the duplication region overlapped with partial region of the Miller-Dieker syndrome (MDS). But the ultrasound abnormalities in our case were different from the typical phenotype of these syndromes. FISH result of mother indicated 46XX, t (2; 17), (q37.2; p13.2), suggesting that the fetus inherited the abnormalities from her mother.
Conclusions: The interaction between 2q37.3 microdeletion and 17p13.3p13.2 microduplication may lead to atypical fetal features from 2q37.3 microdeletion or 17p13.3p13.2 microduplication alone.

P2-50

Knowledge of perinatal palliative care following a fatal fetal anomaly diagnosis

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Objectives: A fatal fetal anomaly (FFA) diagnosis and the subsequent discussion on termination of pregnancy for FFA (TOPFA) and perinatal palliative care (PPC) is associated with significant emotional distress and grief reaction. The unavailability of TOPFA in Ireland doesn't preclude parents from acquiring knowledge about and traveling abroad for termination. Whatever the choice, PPC is implemented upon diagnosis. PPC is the planning and provision of supportive care during life and end-of-life care for a baby and family in the management of life-limiting conditions. The aim of this study is to assess the public’s knowledge of services available following a FFA diagnosis.

Methods: A cross-sectional telephone survey of Irish adults was facilitated by IPSOS MRBI. Using census derived quota controls a representative national sample of adults aged eighteen and over were contacted using random digit dialling. 970 participants were approached to complete the survey. Fieldwork was monitored daily to ensure age, gender, social class and region of respondents were a representative sample.

Results: 83.9% (n=814) agreed to participate. Only 60.5% (n=491) knew of the option for PPC. 49.6% (n=404), respondents did not know if PPC could commence at diagnosis, once the baby reached 24 weeks (56.6% n=461) or until the baby was born alive (49.8% n=405). Under 45s (45.5% n=72 v 38.2% n=166; p=0.003) and women (45.4% n=172 v 38.2% n=166; p=0.003) were more likely to know that PPC could commence upon diagnosis. Only 21.8% (n=211) had knowledge that a medical follow up was available following a TOPFA. 30.9% (n=300) were unaware that bereavement care was available following a TOPFA.

Conclusions: The lack of knowledge the public has regarding supports available following a diagnosis of FFA is concerning. The findings suggest the need for public health interventions to improve the awareness of supports and services following a FFA diagnosis.

P2-51

The Irish population knowledge of fatal fetal anomalies

Unedited draft - unpublished
Objectives: Congenital anomalies affect 27.3 per 1,000 births in Europe. Major fetal anomalies account for 2% of births, of which around 15% are fatal anomalies (FFA). Improvements in antenatal screening have increased prenatal diagnosis, which subsequently creates the choice for pregnancy termination or to prepare for an affected baby. Whatever the choice, the need for it to be an informed one is essential. The aim of the study was to assess the public’s knowledge of FFA.

Methods: A cross-sectional telephone survey of Irish adults was facilitated by IPSOS MRBI. Using census derived quota controls a representative national sample of adults aged eighteen and over were contacted using random digit dialling. Fieldwork was monitored daily to ensure age, gender, social class and region of respondents were a representative sample. 970 participants were approached to complete the survey.

Results: 83.9% (n=814) participated. 31.9% (n=260) of respondents knew someone who had a FFA diagnosis. Only 29.6% (n=241) could define FFA. 45.8% (n=373) didn’t know anencephaly was a FFA. 44.3% (n=361) stated that diagnosis could always be made at a 12 week scan with 58.6% (n=477) reporting a diagnosis can always be made at a 20-22 week scan. Under 45s (68.1% n=258 v 44.9% n=195; p<0.001) and professionals (61.8% n=349 v 41.9% n=204; p<0.001) has greater awareness that diagnosis cannot always be made at 12 weeks. 44% (n=358) respondents were unaware that medical intervention was required for survival once born.

Conclusions: The study identifies a lack of accurate knowledge on FFA, its classification, diagnosis and survival. The lack of knowledge highlights the need for improved health information and antenatal education in relation to FFA which in turn would facilitate informed decision-making following a FFA diagnosis.

P2-52

Ethical dilemmas and emotional appeal influence of media commentary on fatal fetal abnormality in Ireland

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Objectives: Antenatal Diagnosis of a fatal fetal anomaly (FFA) confronts parents with their child’s mortality, creating difficult decisions including whether to terminate or continue with
the pregnancy. Media offers an insight into health-related information available to the public. Readers are both active and selective in their interpretations of text however, the influential nature of media is well documented as readers are constrained by the framing of texts. This study analysed information on FFA, termination of pregnancy following FFA (TOPFFA) and perinatal palliative care (PPC) published in the media.

**Methods:** This qualitative study applied a critical discourse analysis which examines the relations between discourse and social and cultural phenomena. It analysed language and visuals of an Irish broadsheet and online journal identifying the discourse abilities to favour or exaggerate certain descriptions of reality and influence the reader.

**Results:** 128 articles (2012-2017) referencing FFA, TOPFFA and PPC were identified. During times of controversy and opportunity for change in Irish Abortion Laws, the media alluded to the legalising of TOPFFA being the preferred choice of the public. This was in addition to implying scepticism about government proceedings. Themes of power and politics, international influence, ethical dilemmas and emotional appeal are imbedded in the discourse, creating political influence and appealing to the emotional side of the reader to influence perceptions and views.

**Conclusions:** Language is not neutral and therefore it is important to analyse the information being delivered to the public. This is of additional relevance as a referendum to adapt Irish Abortion Laws is imminent.

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**Wernicke’s encephalopathy in hyperemesis gravidarum: A case report**

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**Objectives:** Wernicke's encephalopathy is usually associated with clinical conditions such as alcoholism, malignancy, gastrointestinal diseases, chronic peritoneal dialysis and hemodialysis. Wernicke's encephalopathy is a condition that is clinically caused by lack of thiamine, characterized by oculomotor disorder, ataxia and mental status disorder. The deficiency of the thiamine may occur in situations such as pregnancy, lactation and thyrotoxicosis.
Methods: Twelve weeks pregnant patient with hyperemesis gravidarum developed dizziness and double vision complaints. Nystagmus and tongue fasciculations were detected in the neurological examination of the patient. In brain MR, T2 weighted images showed increased intensities in the periakuaduktal gray matter and bilateral medial thalamus. Defined MR findings suggest Wernicke's encephalopathy. Clinical complaints of the patient who had undergone thiamine treatment were observed to disappear.

Results: Hyperemesis gravidarum leads to metabolic disorders such as deficiency of electrolytes, essential vitamins and cofactors. Neurological complications in hyperemesis gravidarum are Wernicke's encephalopathy due to peripheral neuropathy and lack of thiamine due to deficiency of B6 and B12 vitamins. Wernicke's encephalopathy is the most common affected structure. Frequently thalamus, hypothalamus and periakuaduktal gray matter are involved.

Conclusions: In conclusion, Wernicke's encephalopathy should not be forgotten in cases with hyperemesis gravidarum that develop peripheral neuropathy in a patient with these clinical findings. The diagnosis is confirmed by improvement in clinical findings with thiamine treatment at patients suspected of having Wernicke's encephalopathy.
Genetic screening and testing in pregnancies conceived by In Vitro Fertilization (IVF) with pre-implantation genetic screening (PGS)

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Objectives: Although residual risk for aneuploidy during pregnancy is lower after transfer of an embryo with a normal PGS result, these women are still offered standard aneuploidy screening and testing. Which screens or tests they choose to undergo however has not been previously studied. Therefore, our objective was to investigate prenatal screening and diagnostic testing in pregnancies conceived by IVF with normal PGS results from one academic center and several private fertility clinics.

Methods: We reviewed medical records of 83 women who received prenatal genetic counseling between 1/2012 and 4/2017 for a pregnancy following IVF with normal PGS results (including six with preimplantation genetic diagnosis (PGD) in addition to PGS). Following data was extracted: maternal age (MA), donor age (DA) if donor oocytes or embryos were used, ethnicity, parity, gestational age (GA), and family history of chromosomal abnormalities in first or second degree relatives. We recorded which prenatal genetic screen or diagnostic test was performed: first trimester screening (FTS), second trimester serum screening (STS), non-invasive prenatal testing (NIPT), amniocentesis, or chorionic villus sampling (CVS).

Results: 74 women were pregnant with autologous oocytes (mean MA 37) and 9 with donor oocytes (mean MA 42; DA 27). GA at genetic counseling was 15 (8-23) weeks. There were 61 (73.4%) singletons, 21 (25.3%) twins and 1 (1.2%) triplets. 53/88 (63.9%) had ≥1 of the following: 14 (16.9%) FTS, 38 (45.8%) NIPT, 5 (6%) STS, 2 (2.4%) CVS and 3 (3.6%) amniocentesis. 9/13 women (69.2%) with and 44/70 (62.9%) without a family history of a chromosomal abnormality underwent ≥1 test. 28/59 (47.5%) women ≥age 35 and 10/24 (41.7%) < age 35 or pregnant with donor eggs had NIPT.

Conclusions: In this retrospective cohort, most women who were pregnant after IVF with normal PGS results elected to proceed with some form of further prenatal screening or testing during their pregnancy. Only few underwent diagnostic testing including amniocentesis (3 patients) or CVS (2 patients). Given that PGS techniques may not be adequate for the detection of chromosomal mosaicism and other chromosomal abnormalities beyond aneuploidy, prenatal screening or testing for chromosomal abnormalities is still recommended during pregnancy, even after transfer of embryo(s) with a normal PGS result. However, the residual risk for aneuploidy after PGS is poorly defined.
Preventing misdiagnosis in PGD families: Beware of the pitfalls!!

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Objectives: Prior to a PGD cycle for a specific monogenic disease, the genetic laboratory needs to determine the suitability of pre-existing genetic tests or to develop a novel test. Clinical data and genetic reports of the family members are requested to confirm the phenotype and genotype. We present six couples who were referred for PGD, to illustrate that the genetic work-up prior to PGD can yield unexpected results which can change the presumed risk haplotype, recurrence risk or even the need for PGD.

Methods: PGD tests for monogenic disorders have been developed in house, based on microsatellite STR (short tandem repeat) marker haplotypes with or without testing of the causal mutation(s). Since 1995 almost 400 of these locus-specific tests were created according to the ESHRE guidelines. A prerequisite is that the clinical diagnosis in the proband and all informative family members is confirmed by genetic analysis.

Results: In two cases (Muenke syndrome, Neurofibromatosis (NF1)) the presumed familial mutations, based on multiple affected persons, were in fact two independent de novo mutations. In another case (myotonic dystrophy type 1) the disease was identified in both parents instead of only one. In two other cases (NF1, Li Fraumeni syndrome) the de novo mutations in male probands were detected in DNA from blood but not in sperm cells. In the last case recessive inheritance was assumed based on the presence of an affected child with molecularly proven spinal muscular atrophy (SMA). However, the child's mutation was not identified in the father.

Conclusions: A reduced recurrence risk was revealed in three families, reducing the need for PGD or making it technically impossible as the risk haplotype could not be identified (NF1, Li Fraumeni syndrome, SMA). The unexpected genetic events in the three other families may have ended in a misdiagnosis if not resolved during the PGD work-up (Muenke syndrome, NF1, myotonic dystrophy). It therefore remains crucial to confirm genetic reports of family members prior to PGD when setting up the PGD test.
Implementation of target capture enrichment on single/few cells for the robust detection of embryo abnormalities

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Objectives: High throughput non-invasive prenatal testing (NIPT) technologies have demonstrated safe, accurate and reliable results for the detection of fetal abnormalities, relying on the detection and analysis of cell free fetal DNA (cffDNA) in maternal plasma. However, such analysis is often limited by the low abundance of DNA, as in the case of fertilized embryos. Therefore, the development of novel, sensitive approaches which can provide reliable results from single/few cells is necessary.

Methods: Amplified DNA isolated from seven and 17 embryos was obtained from 3-day and 5-day biopsy cases. TArget Capture Sequences (TACS) were designed at a median resolution of 1Mb spanning all chromosomes and were used to perform in-solution hybridization capture enrichment as previously described. Novel bioinformatics algorithms were also developed to determine the ploidy status of the samples.

Results: All samples were correctly classified and all abnormalities were detected including numerical and structural rearrangements. Results obtained were in agreement with array CGH.

Conclusions: Targeted sequencing is the preferred method for applications requiring high read depth. This assay in combination with a novel bioinformatics pipeline can be used for the genome-wide screening of fertilized embryos (PGS/PGD). It can also be used in cases where limited number of cells from affected tissues/individuals are available.

Towards optimization of PGD for recurrent t(11;22) carrier

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Objectives: The t(11;22)(q23;q11) is the most frequent reciprocal translocation in humans. Balanced carriers often have reproductive problems, such as infertility, recurrent pregnancy
loss, or the birth of offspring with congenital malformation syndrome called Emanuel syndrome. Preimplantation genetic diagnosis (PGD) is the useful solution for such reproductive problems. Since all of the t(11;22) carriers have the translocation breakpoints within the small ~500bp regions on 11q23 and 22q11, we can set up the optimal PGD condition using a sample from a single typical t(11;22) family.

**Methods:** Experiments were performed using lymphoblastoid cell lines from a patient with Emanuel syndrome having trisomy of both distal 11q23 and proximal 22q11. Assuming the trophoectoderm biopsy (TE) biopsy, five cells were picked up using cell manipulator, and whole genome amplification was performed. For quantitative analysis of two unbalanced translocation regions on 11q23 and 22q11, we tested oligonucleotide- or BAC-based microarray as well as next generation sequencing (NGS). Translocation-specific PCR was also performed.

**Results:** In general, NGS detected the unbalanced region better than any of the microarray platforms, although the sensitivity was not perfect at the proximal 22q11 region possibly due to many segmental duplications. Translocation-specific PCR worked without any problems.

**Conclusions:** We conclude that NGS combined with translocation-specific PCR is the best way for copy number analysis of TE biopsied samples from couples of t(11;22) carrier.

**P2-59**

**PGD for single gene disorders using qPCR leads to impressive implantation rates**

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**Objectives:** The aim of this study was to summarize and report the clinical experience of couples undergoing preimplantation genetic diagnosis (PGD) at a single IVF center. PGD was performed using a recently published and novel qPCR method.

**Methods:** The PGD laboratory database was queried for single gene disorder (SGD) workups that were registered between March 2014 and April 2017. SGD PGD workups that were referred by a single IVF clinic (RMANJ) were then reviewed. The clinical outcomes for those patients was evaluated. Maternal age, number of SGD disorders tested, retrievals, transfers, number of embryos, aneuploidy rates, and sustained implantation rates were calculated and recorded. Sustained implantation is defined as the ratio of the number of fetal heartbeats observed when a patient is discharged at 9 weeks pregnant to the number of embryos transferred.

**Results:** 187 patients were identified that completed the PGD workup and started an IVF cycle. 95% tested for one disorder; the remaining 5% tested for multiple SGD disorders. 119 patients
had one retrieval, 46 patients had two, and 22 patients had more than two. A total of 1,797 embryos were tested for single gene disorders at an average of 6 embryos per PGD cycle. 158 of 187 patients had embryo transfers. 29 patients did not yet attempt an embryo transfer. 6 patients lacked a suitable embryo for transfer, while others had recent retrievals or may attempt transfers in the future.

**Conclusions:** The PGD testing encompassed wide varieties of genetic disorders including HLA testing, dominant, recessive, and X-linked. The overall sustained implantation rate for patients who had SGD tested embryo transfers was 62.2%. The sustained implantation rate for patients at their first SGD tested embryo transfer was 75.2%. 93% of all transfers were single embryo transfers. 98% of embryos tested were given a diagnosis; 2% were inconclusive due to aneuploidy or recombination. Our results confirm the high call rate and accuracy of qPCR-based SGD diagnosis. The results also validate the safety, high success rate and sustained implantation rate of single embryo transfers.

P2-59 Table.

<table>
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<th>SGD Cycle Age Group</th>
<th>Patients</th>
<th>Retrievals</th>
<th>Average # Embryos Tested</th>
<th>Aneuploid Embryos</th>
<th>Embryo Transfers</th>
<th>Sustained Implantation</th>
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P2-62

**Fetal akinesia: Harnessing the power of next generation sequencing**

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**Objectives:** Fetal akinesia represents a complex and heterogeneous group of disorders for which a specific genetic diagnosis often remains elusive due to the challenges of identifying the primary etiology. In recent years, next generation sequencing (NGS) has led to novel gene discoveries and to the identification of extreme or atypical phenotypes of known disease genes associated with fetal akinesia. The objectives of this pilot project are to 1) describe our Prenatal Neurogenetics Clinic’s experience with NGS for fetal akinesia; 2) propose a diagnostic approach encompassing Clinical and Molecular Genetics, Maternal Fetal Medicine, Fetal Imaging, Neurology and Pathology.

*Unedited draft - unpublished*
Methods: Seventy-two new patients were seen in the Prenatal Neurogenetics Clinic for CNS/neuromuscular ultrasound anomalies in a four year period (2014-2017). Six cases of fetal akinesia were identified for review and are described including the clinical and molecular genetics aspects. All are simplex cases from unrelated families and non-consanguineous unions. Following normal chromosomal microarray and parental testing for Spinal Muscular Atrophy and Myotonic Dystrophy type 1, a comprehensive NGS panel test for akinesia was offered. Genomic DNA samples from frozen amniocytes of four deceased fetuses were examined using a commercially available panel of 153 genes responsible for various neuromuscular disorders.

Results: CMA, SMA and DM1 were negative for all cases. There were four pregnancy terminations, one IUFD and one live born infant. Of the four termination cases, NGS identified a probable genetic diagnosis for two fetuses. 1) A variant of uncertain significance (VUS) was identified in the MYH3 gene (p.Glu1284Asp) in a fetus with lower limb arthrogryposis, for which heterozygous variants are associated with Arthrogryposis, distal, type 2A. 2) A VUS was identified in the BICD2 gene (p.Phe205Ser) in a fetus with severe arthrogryposis. Parental variant segregation analysis is being performed to confirm the variants are de novo (likely pathogenic).

Conclusions: A comprehensive fetal akinesia NGS panel identified a genetic diagnosis in two of four affected fetuses. Our findings support the benefits of a specialty Prenatal Neurogenetics Clinic and the relevance of disease-specific gene panels as a diagnostic tool in pregnancies complicated by fetal akinesia. Establishing a genetic diagnosis in fetal akinesia allows for accurate genetic counselling and molecular prenatal diagnosis in future pregnancies. We propose a multidisciplinary approach to fetal akinesia, with NGS as an essential element, and discuss the considerations of using a custom NGS panel versus whole exome sequencing.

Whole exome sequencing identifies Neu-Laxova syndrome as a cause of recurrent cystic hygroma with severe intrauterine growth restriction

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Objectives: A non-consanguineous Chinese couple was referred to the Prenatal Genetics Clinic in three pregnancies for cystic hygroma or increased nuchal translucency, resulting in a positive

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prenatal screen for trisomy 21. Prenatal ultrasound findings included: cystic hygroma, subcutaneous edema, intrauterine growth restriction, hypoplasia of the cerebellum, renal anomalies and arthrogryposis. After a diagnostic odyssey of almost 10 years, whole exome sequencing revealed a rare and unexpected diagnosis.

**Methods:** Fetal examination showed several common features: severe growth restriction, micrognathia, arthrogryposis and rocker-bottom feet. Neuropathologic examination revealed: delayed brain development, congenital agenesis of the corticospinal tracts and hypoplasia of the hippocampus, cerebellum and brainstem. Conventional cytogenomic analysis did not reveal an underlying genetic cause.

**Results:** Exome sequencing identified a homozygous c.1A>C (p.Met1?) variant in the translation initiation codon of the *PHGDH* gene in all fetuses. Mutations in the *PHGDH* gene cause Neu-Laxova syndrome 1 (NLS1), a recessive disorder. Approximately 70 cases have been identified; however, prenatal diagnosis is challenging and there are few reports which describe the fetal pathology. Apart from micrognathia, the typical facial features, such as hypertelorism and ocular proptosis, were not appreciated on fetal examination. Interestingly, each pregnancy presented with an increased nuchal translucency or cystic hygroma. Although edema is a common finding in NLS1, it is usually identified later in pregnancy.

**Conclusions:** While Neu-Laxova syndrome remains a rare condition, it may be a cause of recurrent increased nuchal translucency/cystic hygroma when testing for aneuploidy is negative, even in the absence of the classic facial phenotype. This finding provides further support that cystic hygroma represents a heterogeneous group of disorders and that exome sequencing is shedding light on the underlying genetic diagnoses in this group.

**P2-64**

**MAGEL2 in the prenatal setting: Beyond fetal akinesia and arthrogryposis**

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**Objectives:** Truncating mutation on the paternal allele of *MAGEL2* is associated with Schaaf-Yang syndrome. The phenotype of affected individuals ranges from lethal arthrogryposis multiplex to mild intellectual disability or autism spectrum disorder. Approximately 30 cases have been reported to date, but data on prenatal phenotype are scarce.
Methods: We performed a file review of six patients (including three from the same family, and two siblings) diagnosed after birth with Schaaf-Yang syndrome in a clinical setting at our centers, compiled prenatal and molecular data, and reviewed the literature.

Results: Prenatal findings in our patients include fetal akinesia (2/6), pyelectasis (2/6), increased nuchal translucency (1/4), intra-uterine growth retardation (1/6), contractures (1/6), and cerebral anomalies (1/6). All were severely affected after birth with progressive contractures and developmental delay. Two had severe hydronephrosis, while another had renal failure. Molecular analyses identified truncating variants in \textit{MAGEL2}: c.1762C>T, c.3043C>T, and c.1996dupC. We reviewed data on 33 cases reported between 2009 and 2017, including 10 with prenatal manifestations. Prenatal features include fetal akinesia (7/10), contractures (4/10), and polyhydramnios (3/10). In addition, two children without reported prenatal features were small for gestational age.

Conclusions: Although Schaaf-Yang syndrome can present prenatally, the features may be mild or absent. Fetal akinesia, contractures, and polyhydramnios are recurrent, but are absent in more than 50% of cases. The syndrome should be considered when fetal akinesia or contractures are associated with growth retardation, pyelectasis or oligohydramnios.

P2-65

\textbf{PIK3CA- Related Overgrowth Spectrum (PROS): perinatal diagnosis by clinical exome sequencing}

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Objectives: “PIK3CA-Related Overgrowth Spectrum” (PROS) syndrome is caused by defect in PI3K-AKT-mTOR pathway. It is a genetic syndrome caused by heterozygous (usually somatic mosaic) pathogenic variants of PIK3CA. It is characterized by overgrowth of several tissues in the body. Three allelic rare syndromes are described; MCAP (Megalencephaly-Capillary Malformation - Polymicrogyria Syndrome), CLOVES (Congenital Lipomatous asymmetric Overgrowth of the trunk, lymphatic, capillary, venous and combined-type Vascular malformation, Epidermal nevi, Skeletal and spinal anomalies) and FH (Fibroadipose Hyperplasia). The phenotypic data show that these previously described diseases entities have considerable overlap and represent a spectrum.
Methods: We report the first pregnancy of a non-consanguineous couple with no familial or personal story of genetic disease. At 22 weeks of gestation, we observed a female fetus displaying a left diaphragmatic hernia with intrathoracic-rise of the stomach, a cystic mass and a right deviation of the heart. CGH-array was carried out on uncultivated amniotic fluid sample at 24 weeks of gestation and was normal. Macrocephaly and polyhydramnios were first observed at 28 weeks. All those features were confirmed by fetal MRI. The parents opted for medical termination of pregnancy at 34 weeks of gestation.

Results: A female death fetus was delivered, weight of 2747 g, head circumference of 34cm, matching with a fetus older than 40 weeks. Fetus showed a left diaphragmatic eventration due to incomplete muscularization of the diaphragm and a duodenal duplication. The intrathoracic rise of stomach, spleen and colon were associated with severe pulmonary hypoplasia. Clinical exome sequencing of 3989 genes on DNA extracted from amniotic fluid showed a de novo heterozygous germline c.1030G>A (p.Val344Met) mutation in PIK3CA gene. Although our case presented already described features (macrocephaly, polyhydramnios), it represents the first report of PROS syndrome showing duodenal duplication and hemidiaphragmatic eventration.

Conclusions: PROS syndrome is caused by heterozygous pathogenic variants in PIK3CA gene. Its clinical signs can be heterogeneous according to the localization of affected tissues. PROS syndrome encompasses unique clinical entities but there is a continuum and overlap between the diagnoses. This case emphasizes the difficulty to distinguish, in utero, between diaphragmatic hernia and diaphragmatic eventration. In addition, it represents the first report of PROS syndrome showing duodenal duplication and hemidiaphragmatic eventration. Our case illustrates how clinical exome sequencing can help in further definition of prenatal manifestations associated with postnatally well characterized genetic syndromes.

P2-65 Figure.
Expanding the phenotype of Meckel-Gruber syndrome: First trimester diagnosis of acrana

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Objectives: To present the case of a family with differing phenotypic presentation of Meckel Gruber syndrome (MKS) in two affected pregnancies, including one with first trimester acrana/anencephaly, to improve prenatal detection.

Methods: Here we describe the phenotypic presentation, genetic analysis and pregnancy outcome of two differing cases of MKS in one family.

Results: The family first presented to our clinic for interconception counseling after termination of pregnancy for presumed MKS given fetal posterior encephalocele, cystic kidneys and post-axial polydactyly of the hands. Sequencing and deletion/duplication analysis of MKS1 and TMEM67 identified a VUS in MKS1 (c.1349 C>T). Their subsequent pregnancy was unremarkable. During their third pregnancy, acrana was identified in the first trimester. They opted to continue this pregnancy and underwent CVS. A multigene MKS/Joubert syndrome panel revealed two pathogenic alterations in the CC2D2A gene. Subsequent ultrasounds identified cystic kidneys and post-axial polydactyly of all extremities. The fetus was delivered by c-section at 36 weeks and passed away at one hour of life.

Conclusions: Pathogenic alterations in CC2D2A have been identified in individuals affected with MKS and Joubert syndrome. However, acrana is considered a rare phenotypic finding in MKS (Mougou-Zerelli et. al, 2009). The discrepant phenotypes in our family add to the literature regarding intra-familial phenotypic variability. The finding of acrana/anencephaly in the most recent fetus also raises the possibility that this finding may occur more frequently in association with CC2D2A variants as opposed to other MKS genes and supports the inclusion of MKS in the differential diagnosis of first trimester apparently isolated acrana/anencephaly.

Exome study in fetuses with severe anomalies: Dual role as a discovery and diagnostic tool

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Objectives: Prenatal ultrasonography identifies an increasing number of fetal malformations. For a significant number of these often lethal phenotypes the underlying causal mutations are not identified. Animal data, however, predict that up to 30% of the protein-coding genes of our genome are implicated in embryonic development. We assess the impact and perspectives of exome sequencing (ES) in the investigation of fetal anomaly phenotypes.

Methods: We performed trio or quattro ES in 20 fetuses who presented with severe malformations including brain malformations, hydrocephalus, hydrops, Fryns syndrome, suspected ciliopathy, agnathia-otocephaly complex and fetuses with multiple major anomalies not distinctive or typical of any described condition. We prioritized variants according to their frequency, disruptive potential and their presence in genes involved in developmental pathways in embryogenesis and correlate findings to the malformation pattern, confirmed by autopsy. The comparison of human and animal morphology was used as an important means to validate novel potential candidate genes.

Results: We obtained a molecular diagnosis in two cases (10%), identified likely pathogenic mutations in 3 candidate genes previously associated with different postnatal phenotypes and 4 candidate genes not related to any human condition. In two of those cases candidate mutations in CENPF and KIF14 were confirmed to cause novel fetal malformation phenotypes increasing the detection rate to 20%. Functional or animal model studies of further 3 candidates are ongoing. We present how candidate gene functions, pathways and corresponding animal data are reflecting the specific fetal malformation patterns we observed, potentially increasing the detection rate of prenatal ES to nearly 50%.

Conclusions: We illustrate the successful application but also challenges of ES when identifying mutations causing fetal anomalies. Investigating distinctive fetal anomaly phenotypes by clinical, genomic and functional studies significantly enhances our knowledge on genes, pathways and mechanisms acting during embryonic and fetal life while increasing the prospective clinical utility of diagnostic prenatal genomic sequencing.

P2-69

Pathogenic variants in E3 ubiquitin ligase RLIM/RNF12 cause a variable X-Linked congenital malformation syndrome with intellectual disability

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Unedited draft - unpublished
Objectives: RLIM, also known as RNF12, is an X-linked E3 ubiquitin ligase acting as a negative regulator of LIM-domain containing transcription factors and participates in X-chromosome inactivation (XCI) in mice. Our current understanding of the functions of human RLIM is sparse and the recognizable clinical prenatal phenotype of RLIM-affected families hasn't been
described before. We present the prenatal (lethal) clinic and genetic details of nine unrelated affected families with pathogenic variants in RLIM.

**Methods:** Genomic DNA was isolated from peripheral blood and/or skin fibroblasts and trio-based whole exome sequencing (WES) of nine unrelated families was performed at different centres according to standard procedures. The data was processed as described in Hu et al. (2016). All detected sequence variants were lifted-over to genome version hg19 and functionally prioritized. SNPeff, ANNOVAR and custom in-house scripts were used to annotate the variant call set with mutation and gene information, protein functional predictions, and population allele frequencies. These annotations were used to identify rare likely-deleterious variants consistent with homozygous, compound heterozygous or X-linked hemizygous variants.

**Results:** Twelve of 41 males died because of congenital diaphragmatic hernia with lung hypoplasia. Diseased newborns had prenatal growth retardation and facial dysmorphism. Urogenital abnormalities included micropenis, cryptorchidism, small or absent testis and hypospadias. Associated findings included omphalocele, liver cysts with ductal plate malformation, renal collection duct cysts, polysplenia. Short distal phalanges with broad stubby thumbs, camptodactyly, syndactyly, preaxial polydactyly, pes planus and nail hypo-/aplasia were noticed. Congenital heart defects included: aortic (isthmus) stenosis, persistent foramen ovale, atrial septal defect and Tetralogy of Fallot. All RLIM variants identified are missense changes co-segregating with the phenotype and predicted to affect protein function.

**Conclusions:** *In vitro* experiments revealed that the identified amino acid changes in the RLIM RING-finger impaired RLIM ubiquitin ligase activity. *In vivo* experiments in rlim mutant zebrafish showed that wild-type RLIM rescued the zebrafish rlim phenotype, whereas the patient-specific missense RLIM variants failed to rescue the phenotype and thus represent likely severe loss of function mutations. RLIM related disorders should be considered when a fetus presents with Fryns syndrome features such as congenital diaphragmatic hernia with lung hypoplasia, urogenital and skeletal malformations.

P2-71

**Clinical exome sequencing in malformed fetuses: Preliminary results of the Brussels experience**

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*Unedited draft - unpublished*
Objectives: Several studies have shown the benefits of Whole-exome sequencing (WES) to provide a definitive genetic diagnosis in malformed fetuses with an inconclusive microarray result. At the same time, WES is still expensive and leads to the discovering of incidental findings and variants of unknown significance, making its interpretation challenging and time-consuming in the context of prenatal diagnosis. Trying to minimize these findings, our study aims to determine the diagnostic yield of clinical exome sequencing in malformed fetuses. By October 2016, we recruited 80 cases. Here, we report the first results, for 42 cases.

Methods: Fetuses (aborted or ongoing pregnancies) with one or several major malformation(s), detailed phenotypic data, and no pathogenic chromosomal rearrangements were included. After genetic counselling, parents signed an informed consent. Clinical exome capture was performed in trio and achieved using the SeqCap EZ Choice XL (NimbleGen, Madison, WI), designed to cover all exons and exon-intron boundaries of 3989 genes known to be associated with Mendelian diseases. Samples were sequenced on a HiSeq 1500/NovaSeq 6000 instrument and bioinformatics pipeline was launched. Variants’ filtering was performed using Highlander (http://sites.uclouvain.be/highlander). Parents were informed about the results during a post-test genetic consultation.

Results: Clinical exome sequencing provided a definitive genetic diagnosis in 35.7% of cases (15 cases out of 42) and, just in one case, a candidate variant was found. The highest diagnostic yield was achieved in fetuses presenting fetal akinesia deformation sequence/arthrogryposis multiplex congenita (87.5%) and features resembling Meckel-Gruber syndrome and its related disorders (100%).

Conclusions: Although this study is a preliminary assessment from a limited number of cases, it provides an initial proof-of-principle regarding the advantages of the approach described herein. In comparison with the largest series reported in the literature using WES, our method presents a higher diagnostic yield. Thus, further recruitments/analyses will be carried out in order to determine accurately its diagnostic rate and benefits over WES approaches. In terms of cost, time and quality, clinical exome sequencing could represent a “technical compromise”,

Unedited draft - unpublished
filling the gaps between single-gene analysis and WES and resulting particularly suitable for disorders with high levels of genetic heterogeneity.

P2-72

A rare arthrogryposis syndrome with multiple anomalies diagnosed by whole exome sequencing

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Objectives: Three to five percent of all pregnancies are complicated by fetal malformation or genetic syndrome. In many cases, cytogenetic analysis does not yield a diagnosis, leaving the possibility of a single gene disorder with 25% or higher recurrence risk. Establishing an accurate diagnosis for an affected pregnancy is imperative to allow complete information for reproductive decision-making. Here we describe a case of a rare arthrogryposis syndrome caused by mutations in the \textit{NEK9} gene. This case illustrates an example of a single gene disorder in a previously undescribed population and demonstrates the value of whole exome sequencing as a diagnostic tool.

Methods: Patient was referred at 23 3/7 weeks gestation with abnormalities noted on fetal ultrasound and non-invasive prenatal screening low risk for trisomy 13, 18, and 21. Ultrasound imaging demonstrated fixed joints, skin edema, microphthalmia, shortened long bones, small chest, hemivertebrae, cardiomegaly, and hepatomegaly. Amniocentesis revealed normal lysosomal storage disease testing and microarray with a variant of unknown significance: del2q23.1 which partially overlaps the \textit{MBD5} gene. 2q23.1 deletion syndrome is associated with neurodevelopmental abnormalities and subtle minor dysmorphic features. As the anomalies were not explained by this syndrome, whole exome sequencing was ordered on banked DNA from amniocytes.

Results: Whole exome sequencing results returned after the pregnancy was terminated. Compound heterozygous mutations were identified in the \textit{NEK9} gene, both classified as variants of uncertain significance given the paucity of reports of pathogenic \textit{NEK9} variants. One mutation in our case, c.136G>T, has never been reported; the other, c.1432del, has been reported once. To date, \textit{NEK9} mutations are documented in three families: a consanguineous Saudi family homozygous for missense variant c.2042G>A, and two consanguineous Irish Traveler families with multiple affected individuals homozygous for nonsense variant c.1489C>T. All affected patients had arthrogryposis; phenotypes in the Irish Traveler individuals closely mirror our case.

Conclusions: This case illustrates a diagnosis of an extremely rare single gene disorder in the pregnancy of a non-consanguineous German couple. To our knowledge, this is the first case reported outside of Saudi and Irish Traveler populations. This provides further evidence toward
arthrogryposis with other anomalies as a recessive disease associated with \textit{NEK9} gene mutations. Furthermore, this case demonstrates whole exome sequencing as a valuable tool for investigating etiology when multiple fetal anomalies are identified without diagnosis on more standard tests such as microarray. This information allowed comprehensive counseling for this couple regarding diagnosis, recurrence risks and future reproductive options.

\textbf{P2-73}

\textbf{The sonographic "honeycomb" appearance of congenital fetal myofibromatosis: Case report and review of the literature}

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\textbf{Objectives:} Infantile Myofibromatosis (IM) is a benign neoplasm of infancy with a broad clinical presentation. Most cases are presented before the age of two and are rarely encountered in newborns. The following manuscript describes a rare case of third trimester prenatal finding of Multi-Centric Fetal Myofibromatosis its distinct sonographic "Honeycomb" appearance and a review of the English literature.

\textbf{Methods:} We present a case of diffused Multi-centric IM with multi-organ involvement diagnosed in a 27 year old pregnant woman at 34 weeks of gestation detected on a routine follow up examination. Detailed sonogram performed at our institute revealed multi-organ involvement with masses detected predominantly in the lower extremities, heart, the abdominal cavity and the neck. Figure 1 represents the progressive nature of the disease of a soft tissue tumor mimicking "honeycomb" appearance.

\textbf{Results:} The couple opted delivery and not pregnancy termination. A male infant weighting 2475 gr (50th percentile) was born at 34+6 weeks. After delivery the newborn showed pronounced dysmorphic features such as upslant eyes, he also presented with diffused edema, multiple subcutaneous masses and microcephalus head. Pathological exam from an abdominal wall lesion revealed skeletal muscle containing spindle cell tumor with central necrosis, consistent with infantile myofibromatosis. Surgical removal of the lesion was not an option due to the extent of the disease and after one course of tyrosine kinase inhibitors treatment, the infant died from cardiac failure.

\textbf{Conclusions:} Only seven cases of prenatal diagnosis of IM have been previously reported between 1999-2014 all confined to one fetal organ. The gestational week at diagnosis ranged from 13 to 38 weeks of gestation. In 6/7 cases IM was identified in the third trimester (30-32 weeks) suggesting a progressive nature for this disease. Hence, the role of the sonographer, who performs the third trimester scan and encounters sonographic findings that raise the
suspicion of a solitary tumor should include IM in the differential working diagnosis. Urgent clinical decisions should then be taken due to the progressive nature of this disease.
Prenatal diagnosis of SMC1A associated familial X-linked Cornelia de Lange syndrome in a phenotypically normal mother with somatic mosaicism

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Objectives: Cornelia de Lange syndrome (CdLS) is a well-known malformation syndrome resulting from heterozygous pathogenic variants in NIPBL, RAD21, or SMC3 or from hemizygous pathogenic variants in HDAC8 or SMC1A genes. The SMC1A gene is implicated in approximately 5% of the CdLS and has an X-linked inheritance. Phenotypic variability has been noted in both males and females with SMC1A associated CdLS. We report a unique family with SMC1A associated CdLS from a rare pathogenic variant in the SMC1A gene with a severely affected 3-year-old son, prenatal diagnosis in the current pregnancy and somatic mosaicism in the mother.

Methods: A 24-year-old healthy female, gravida 2 para 1, was seen at the Maternal Fetal Center, Valley Children’s Hospital, at 19 weeks gestational age for genetic counseling and level-II ultrasound. Family history was significant for her 3-year-old son postnatally diagnosed with CdLS and confirmed to have a pathogenic variant in the SMC1A gene (c.2368C>T p.Arg790Trp) in May 2014. The few reported individuals in the literature with SMC1A somatic mosaicism have also had mild clinical phenotypes. As our proband’s mother was phenotypically normal,
this was considered to be a *de novo* occurrence and the family had previously been counseled accordingly.

**Results:** The level-II ultrasound of her current pregnancy detected micrognathia in her male fetus. Karyotype and SNP microarray analysis on amniotic fluid cells revealed a normal male. Targeted Sanger sequencing confirmed the hemizygous familial pathogenic variant in the *SMC1A* gene (c.2368C>T p.Arg790Trp) in the fetus. Targeted Sanger sequencing of the maternal peripheral blood raised concerns for somatic mosaicism in the mother which was confirmed with NextGen sequencing.

**Conclusions:** To date, very few familial *SMC1A*-related CdLS have been identified, with somatic and/or germline mosaicism reported in only 2 families. This family is interesting and unique in several aspects: this is the first report of a phenotypically normal mother with somatic and gonadal mosaicism for *SMC1A* associated CdLS. The prenatal finding of isolated micrognathia that led to prenatal diagnosis of *SMC1A* associated CdLS is also distinctive. The considerations for genetic counseling were complex due to the enormous psychosocial and financial implications for this family as the previously assumed ‘*de novo*’ CdLS state was reclassified to a ‘familial’ state.

P2-75

**The utility of WES results in accelerating prenatal diagnosis in subsequent at-risk pregnancies**

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**Objectives:** The purpose of this study is to retrospectively review the outcome of targeted prenatal known familial variant testing in at risk pregnancies after diagnostic whole exome sequencing (WES) in a family member or prior pregnancy.

**Methods:** Prenatal testing was performed for 67 families with a definitive or a possible molecular diagnosis from a cohort of 9,000 families that underwent WES testing. Targeted PCR-based assays were used to detect single nucleotide variants (SNVs), small insertions and deletions, and larger exonic deletions. Sample types, mode of inheritance, molecular diagnoses and clinical outcomes in these families were systematically evaluated. Fetal samples were collected by either amniocentesis (48%) or chorionic villus sampling (52%).

*Unedited draft - unpublished*
Results: Among the sixty-seven families, 56 (84%) received a definitive molecular diagnosis and 11/67 (16%) had a possible diagnosis by WES. Thirty-six (54%) pregnancies were tested for variants related to autosomal recessive disorders, fifteen (22%) for autosomal dominant disorders, twelve (18%) for X-linked disorders, and four (6%) were tested for multiple variants associated with a combination AD/AR/X-linked conditions. In 14/67 families (21%), the tested fetal samples shared the same genotype as previously reported in the affected proband in the family. The results were negative for fifty-two pregnancies (78%), and inconclusive in one family (1%).

Conclusions: This study systematically reviews the use of diagnostic WES results in subsequent at-risk pregnancies in a family. Our results support that the clinical utility of WES extends beyond providing a diagnosis for the tested proband.

P2-75 Figure.

Figure 1: Comparison of results of prenatal known familial testing with respect to (A) sample type; (B) WES molecular diagnosis; (C) mode of inheritance; (D) Outcome of testing. CVS =chorionic villus sampling, AD = autosomal dominant, AR = autosomal recessive, XL = X-linked.

P2-76

Prenatal diagnosis of COQ9 exonic deletions via custom oligonucleotide array following whole exome sequencing

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Unedited draft - unpublished
Objectives: Primary coenzyme Q$_{10}$ (CoQ$_{10}$) deficiency is a genetically heterogeneous autosomal recessive inborn error of metabolism that presents with multisystem organ disease ranging from lethal neonatal encephalopathy to milder adult onset forms. To date, only a few families with pathogenic variants in COQ9 as the cause of primary CoQ$_{10}$ deficiency have been reported in the literature. We present a case of CoQ$_{10}$ deficiency due to homozygous deletion of exons 4 and 5 of COQ9 in a consanguineous family, fortuitously detected by a sequencing platform, which presented unique challenges for prenatal diagnosis in subsequent pregnancies.

Methods: A 23 y.o. G2P0100 Cuban woman presented at 11 2/7 weeks gestation for genetic counseling due to a prior daughter affected with primary CoQ10 deficiency type 5. The family desired early prenatal diagnosis to inform their pregnancy management decisions. The affected proband, born at 31 2/7 weeks, presented with severe metabolic crisis, respiratory failure, and bilateral congenital contractures of fingers, wrists, and elbows. Due to the suspicion for a terminal/irreversible inborn error of metabolism requiring maximal life-sustaining therapy, intensive care was withdrawn and the infant passed on day of life 1. A comprehensive genetics workup included whole exome sequencing (WES).

Results: Analysis on read depth and junction reads detected an apparently homozygous pathogenic deletion involving exons 4 and 5 of COQ9, interpreted as causative of the phenotype. The family was lost to follow-up and parental carrier status was not confirmed. Fetal diagnosis was challenging because genome-wide chromosome microarray could not guarantee exon-by-exon coverage of COQ9, and fetal WES is neither clinically validated nor cost effective for detection of the familial COQ9 deletion. A chorionic villus sample was sent for karyotype and, in parallel, samples of maternal, paternal, and proband DNA were were analyzed on a custom-designed oligonucleotide array for COQ9.

Conclusions: This confirmed the presence of heterozygous deletions in each parent and the homozygous deletion in the proband. Fetal testing was negative for the familial deletion, consistent with an unaffected, non-carrier fetus. This is the first case of primary CoQ$_{10}$ deficiency type 5 due to exonic deletions in COQ9. It demonstrates the ability of whole exome sequencing to detect exon-level deletions and the challenges of targeting such deletions for prenatal diagnosis in an accurate, time efficient, and cost-effective manner.

P2-77

The value of low dose computed tomography in skeletal abnormalities of the fetus

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Unedited draft - unpublished
Objectives: To review the use of fetal Low Dose Computed Tomography (LDCT) in the evaluation of skeletal abnormalities and the contribution in diagnosis as well as in counseling and decision making towards management of the pregnancy.

Methods: Between May 2016 and November 2017 we scanned thirteen pregnant patients with skeletal abnormalities on prenatal ultrasound (pUS) on a Somatom Force CT scanner (Siemens, Erlangen, Germany) under tube current and kilovoltage modulation at a carefully adjusted milliampere level.

Results: Gestational age ranged from 17 weeks 4 days until 29 weeks 2 days. Five patients showed only skeletal abnormalities and eight showed additional abnormalities on pUS prior to CT. Ten pregnancies were terminated after multidisciplinary counseling. All fetuses underwent a conventional autopsy with a variable number having additional post-mortem work-up by CT scan, magnetic resonance imaging or X-ray. Three pregnancies continued, 2 got lost to follow up and the third died after delivery. The median total dose length product of the prenatal LDCT was 47.1 mGy*cm (range:21.9 - 180.6). The median fetal dose, calculated on virtual antropomorphic phantoms, was 2.06 mSv (range:0.77 – 5).

Conclusions: Prenatal LDCT demonstrated the skeletal abnormalities suspected on pUS to the full extent. Post mortem work-up confirmed 9/10 of our prenatal findings, in the last case chondrodysplasia punctuata was the final diagnosis. We demonstrate cases in which LDCT raised the diagnosis of altered bone density, demonstrating the utility and need for standardized scan protocols based on gestational age and body habitus. Current scanners allow us to perform prenatal LDCT at a very low dose but of diagnostic quality. The low dose protocol reduces the potential radiation detriment of the fetus. The CT scan allowed for optimized counseling and prenatal management.

P2-79

Perinatal outcome of antenataly diagnosed Holoprosencephaly

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Objectives: To determine the Perinatal outcome in fetuses with antenatal diagnosis of Holoprosencephaly.

Methods: This retrospective observational study was conducted in the Obstetrics and Gynecology Ultrasound Unit of the Maternal and Fetal Medicine Department, Women’s Specialized Hospital at King Fahad Medical City, Riyadh, Saudi Arabia during a period between 2006 to 2016. Patients included were any woman with ultrasound diagnoses of holoprosencephaly. Data was retrieved from electronic database of maternal fetal medicine (MFM) department and Neonatal Intensive Care unit (NICU).

Unedited draft - unpublished
Results: During the study period, 70 fetuses were diagnosed antenatally with holoprosencephaly. Mean maternal age was 30.2±7.4 years; the mean gestational age at the diagnosis was 28.2±5.5 weeks. There were 23(32.9%) Primigravida and 47(67.1%) multigravida. Positive family history of congenital anomalies was in 8 (11.6%). Consanguinity was found in 24(34.3%). Patients with diabetes were 8.3%. In patients who had amniocentesis the most common chromosomal abnormality was trisomy 13. Intrauterine growth restriction present in 24(34.3%). Craniofacial abnormalities are most common association. Associated extra craniofacial anomalies included cardiovascular, thoracic, skeletal, gastrointestinal and genitourinary. Most common extra craniofacial associated abnormality was cardiac 21(32.3%).

Conclusions: Outcome of Holoprosencephaly depends on severity of the abnormality. Severe form (Alobar) is associated with high perinatal mortality. Parents need appropriate counseling and support with Multidisciplinary involvement.

P2-80

Contribution of genomic copy number variations detected by microarray in fetuses with congenital heart defects

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Objectives: To assess the detection rate and additional diagnostic yield of microarray analysis in fetuses with congenital heart defects (CHD), compared with karyotyping, and to evaluate the contribution of copy number variations detected by microarray in fetuses with CHD.

Methods: We studied 84 fetuses with various phenotypes of congenital cardiac defects. Cases were divided into isolated and non-isolated CHD groups according to whether there were extracardiac ultrasound findings. Both conventional karyotyping and chromosomal microarray analysis were performed. Detection rate and increased diagnostic yield were calculated, and compared with the pathogenic results detected by karyotyping.

Results: Pathogenic CNVs were detected in 20 cases by microarray with a detection rate of 23.8%. Eight cases were overlooked by conventional karyotyping but identified by microarray, indicating the additional value of 9.5%. Microarray improved diagnostic efficiency in both isolated and non-isolated CHD groups (10.3% vs. 11.5%). The incidences of trisomy and gross chromosomal structural anomaly were significantly higher in non-isolated than in isolated CHD. However, there was no statistical difference in prevalence of microscopic rearrangements.

Unedited draft - unpublished
between the two groups. We also found that 15q11.2 microdeletion was significantly enriched in CHD cases over controls.

**Conclusions:** The present study demonstrated the improved detection rate of microarray in fetuses with CHD. The results suggest that microarray is recommended not only for isolated cases but also for non-isolated cases in prenatal CHD cases. We also propose 15q11.2 deletion, 5q35.2 deletion, and 4q terminal deletion as the potential CHD-causing genetic loci. Genes encompassed in these regions, including *UIMC1, ZNF346, FGFR4, TRIML2*, and *ZFP42*, might function as candidate genes to play important roles in human heart development.

P2-81

**Prenatal ultrasound findings and outcome of isolated omphalocele**

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2Department of Neonatology, Leuven, Belgium
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**Objectives:** To study the outcome of simple and isolated omphalocele from the time of diagnosis.

**Methods:** We conducted a retrospective review of all the cases of antenatal detected omphalocele over a 14-years period. Prenatal follow-up, perinatal outcome, pediatric surgery related complications and longterm follow-up of all cases of omphalocele were collected from our electronic database. Referring physicians were contacted for complementary information after obtained informed consent from all patients.

**Results:** Of the 106 patients with omphalocele, 83 patients were excluded because of chromosomal anomalies (*n*=40, 38%) associated malformations (*n*=28, 26 %), regression (*n*=1, 1%) or loss to follow-up (*n*=14, 13%). 23 patients(22%) presented with an isolated omphalocele. A termination of pregnancy was performed in 9 cases (39%), 3 fetuses died in utero (13%), and there was another loss of follow-up in 3 patients (13%). Survival rate was 35% (*n*=8), whilst it was 3,6 % (*n*=1) in those with associated anomalies. 25% of survivors (*n*=2) delivered prematurely. 1 patient suffered from a short bowel syndrome (1/8 = 13%), 1 from hypertension.

**Conclusions:** Associated chromosomal and structural abnormalities play an important role in decision-making. Less than 10% (10/106=9.4%) of infants with antenatally diagnosed omphalocele were alive in the neonatal period. Short bowel syndrome was seen in 1 patient, 11,1%.
P2-82

Prenatal ultrasound findings and outcome of isolated gastroschisis

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Objectives: To study the outcome of simple and isolated gastroschisis from the time of prenatal diagnosis.

Methods: We conducted a retrospective review of all the cases of antenatal detected gastroschisis over a 14-years period. Prenatal follow-up, perinatal outcome, pediatric surgery related complications and longterm follow-up of all cases of gastroschisis were collected from our electronic database. Referring physicians were contacted for complementary information after obtained informed consent.

Results: Of the 32 cases of gastroschisis, 7 cases were excluded because of associated anomalies (n=3, 9.4%), or loss to follow-up (n=4, 12,5%). The 25 (78%) remaining cases resulted in an ongoing pregnancy. In 5 cases (20%) associated anomalies were detected postnatally. Median gestational age at delivery was 35 weeks (range 30-37 weeks) with preterm delivery (<37 weeks) in 24/25 (96%) patients, mainly because of planned c-sections (n= 20, 80%). 20 babies (80%) underwent primary surgery. Average stay at the neonatal department was 46 days. There was no neonatal mortality. 3 patients (3/25, 12%) suffered from a short bowel syndrome postnatally.

Conclusions: The majority of fetuses (78%) with antenatally diagnosed gastroschisis survived to delivery, with 12% severe morbidity afterwards.

P2-83

Size-inferred analysis of fetal and maternal signals in noninvasive prenatal testing

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Objectives: Nowadays, noninvasive prenatal testing (NIPT) is an integral component of obstetric practice. The primary aim of prenatal testing is screening for fetal aneuploidies such as trisomy...
of chromosome 21 (Down syndrome) or monosomy X (Turner syndrome). However, signals from maternal DNA aberrations such as mosaicism, copy number variations, duplications or deletions can be attributed to fetus, thus resulting in false positive or false negative results. Therefore, it is important for NIPT to reliably detect such artefacts.

**Methods:** We developed a method to distinguish maternal and fetal signals in NIPT results. The method is based on the length of circulating cell free DNA fragments, which is a mixture of both fetal and maternal origin. The method is purely computational in nature and does not require any additional data apart from those obtained through regular NIPT (such as from trisomy detection).

**Results:** We tested the presented method on real samples collected for NIPT of common fetal aneuploidies. We showed that the method could detect all positive (i.e., trisomic) fetal signals as well as two IVF samples with trisomy T18 and one false positive T13 sample.

**Conclusions:** The presented method demonstrated that it is possible to distinguish between fetal and maternal signals in NIPT results. This method is thus able to reduce the number of false positive and false negative results which further improves the quality of the rapidly advancing NIPT.

P2-84

**The prenatal screening of Trisomy 21 in women over 40 years old**

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**Objectives:** In the 70s, the first prenatal programs of chromosomal abnormalities were based on the epidemiological marker, maternal age. The prevalence of Trisomy 21 increases linearly with age up to 35 years and exponentially from this moment. This strategy proved inefficient due to the significant percentage of T21 in pregnant women under 35 years of age and the high number of invasive techniques derived from it. In the 80s and 90s, the first screening programs based on biochemical markers appear and, later, in combination of these with echographic markers.

**Methods:** Type of study: retrospective, descriptive. Patients: Pregnant women from our sanitary area who were combined screening in the first trimester between October 2004 and March 2013. Only simple pregnancies were considered. Markers: the biochemical parameters studied were the free beta fraction of human chorionic gonadotropin (β-HCG) and the pregnancy-associated plasma protein (PAPP-A) determined by electrochemiluminescence in an Immulite device (Siemens). The ultrasound marker used was the translucency nerve (TN) measured by transvaginal ultrasound, following the specifications established by the Fetal
Medicine Foundation (FMF). Software calculation of risk: PRISCA TYPOLOG V.4.0. The data processing was done using Microsoft Office Excel.

Results:

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<td>75.7%</td>
<td>3.6%</td>
<td>2.4‰</td>
</tr>
<tr>
<td>≥40</td>
<td>0.9129</td>
<td>1.3572</td>
<td>1.2480</td>
<td>100%</td>
<td>26.3%</td>
<td>17‰</td>
</tr>
</tbody>
</table>

Conclusions: The prevalence of T21 increases with age, passing in our population from 2.4‰ in women under 40 years to 17‰ in an age equal to or greater than 40 years. The DR and the FPR are higher in the group older than or equal to 40 years (DR 100%, FPR 26.3%) than in the group of those under 40 years (DR 75.7%; FPR 3.6%). In pregnant women older than 40 years with positive combined screening, it would be appropriate to implement a contingent strategy using non-invasive prenatal diagnostic techniques to reduce TFP and minimize the performance of unnecessary invasive techniques.

P2-85

Assisted reproduction techniques and prenatal screening of chromosomopathies

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Objectives: In Spain, around 2% of pregnancies come from assisted reproduction techniques (ART). Numerous studies show an increase in the prevalence of chromosomal anomalies in gestations obtained by assisted reproduction. In our sanitary area is implanted since 2005, a Program of Prenatal Screening of Chromosomopathies. This program is carried out through a combined first-trimester screening (CFTS). The correct management of these pregnancies in the screening programs, through the use of correction factors in the calculation of risk, is essential to obtain quality results.
Methods: The Chromosomopathies Prenatal Screening Program is carried out through a decentralized CFTS in two steps, with determination of Free Beta Fraction of Human Chorionic Gonadotropin (BHCG) and Plasma Protein A Associated with Pregnancy (PAPP-A) between week 8-12 and ultrasound between week (10-14) to date the gestation by the cranio-caudal length and measurement of the nuchal translucency. The determination of the biochemical markers is carried out in the INMULITE-2000 analyzer (Siemens). The measurement of the CRL and NT is performed by sonographers trained according to the standards of the Fetal Medicine Foundation in Toshiba Famio. The risk calculation by software PRISCA V4.0 (TYPOLOG).

Results: During the study period, a total of 24841 CFTS were performed, of which 993 were gestations from ART. A total of 114 chromosomopathies were diagnosed: 66 Trisomies 21, 16 Trisomies 18, 4 Trisomies 13, 8 Monomies X and another 20 chromosomal anomalies. The prevalence of chromosomal abnormalities was 0.25% for spontaneous pregnancies and 0.77% for gestations obtained by assisted reproduction. The overall detection rate was 80% for a false positive rate (FPR) of 4.6%. In the group of pregnancies from ART, the detection rate reached 100% (FPR 5.4%).

Conclusions:

1. In our population, the prevalence of chromosomal abnormalities is higher in pregnancies from ART, coinciding with the data collected in the literature.
2. The detection rate for CFTS was higher in non-spontaneous pregnancies, which could be explained, as some authors show, by an increase in BHC-G concentrations and a decrease in serum PAPP-A concentrations of these pregnant.
3. It should be assessed if the increase in the rate of FPR observed would be assumable in terms of cost-effectiveness and risk of complications, taking into account the higher risk of aneuploidies of these pregnancies.

P2-86

Accuracy of ultrasonographic methods of fetal weight estimation in Chinese and Hispanic fetuses

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3NYU School of Medicine/Brooklyn Campus, Brooklyn, NY, United States
4Newark Beth Israel Medical Center, Newark, NJ, United States

Objectives: To determine the most accurate ultrasonographic method of fetal weight estimation for Chinese and Hispanic fetuses.
Methods: A 2 year retrospective cohort study was performed including all pregnant patients (n=250, 22% Hispanic, 56% Chinese, 22% White) receiving fetal weight estimations within the division of Maternal Fetal Medicine at New York University Langone Hospital – Brooklyn. Accuracies of fetal weight estimation methods described by Hadlock (BPD-HC-AC-FL), Hadlock (AC-FL), Hadlock (BPD-AC-FL), Hadlock (HC-AC-FL), Hadlock (BPD-AC), Warsof (BPD-AC), Shepard (BPD-AC), and Merz (BPD-AC) were considered. Difference in gestational age at time of delivery as compared to gestational age at time of ultrasonographic fetal weight estimation was corrected by adding 30 grams to estimate fetal weight per day until date of delivery.

Results: The most accurate method of fetal weight estimation for Chinese and Hispanic fetuses was determined to be Warsof (BPD-AC) as compared to Hadlock (BPD-HC-AC-FL), Hadlock (AC-FL), Hadlock (BPD-AC-FL), Hadlock (HC-AC-FL), Hadlock (BPD-AC), Shepard (BPD-AC), and Merz (BPD-AC) with an overall accuracy of 92% ($p = <0.001$), difference in estimated fetal weights between White and Chinese or Hispanic fetuses using Warsof (BPD-AC) estimation method ($p = 0.001$) and difference when comparing estimated fetal weights among Chinese and Hispanic fetuses using Warsof (BPD-AC) versus Hadlock (BPD-HC-AC-FL) ($p = 0.03$).

Conclusions: Utilizing the fetal weight estimation method of Warsof (BPD-AC) may more accurately estimate fetal weights for Chinese and Hispanic fetuses as compared to the more widely used method of Hadlock (BPD-HC-AC-FL).

P2-87

PAGE study - post-mortem cohort

Mark Kilby1, Elizabeth Quinlan Jones2, Denise Williams3, Sue Hamilton4, Dom McMullan5, Tamas Marton6, Eamonn Maher7, Ruth Eberhardt8, Jenny Lord8, Matthew Hurles8

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2University of Birmingham, Birmingham, United Kingdom
3Birmingham Women’s NHS Foundation Trust, Birmingham, United Kingdom
4WMGRL, Birmingham Women's and Children's Foundation Trust, Birmingham, United Kingdom
5West Midlands Regional Genetics Service, Birmingham, United Kingdom
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7Department of Medical Genetics, University of Cambridge and Cambridge NIHR Biomedical Research Centre, Cambridge, United Kingdom
8The Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Objectives: Several cohort studies and the preliminary assessment of ~500 trios of the PAGE study have noted that whole exome sequencing (WES) increases the diagnostic yield in fetuses with structural anomalies and normal microarray results. One of the limitations of prenatal WES is variability in fetal phenotyping using ultrasound. This makes prenatal genotype-phenotype correlation challenging. The use of multidisciplinary clinical review panel increases

Unedited draft - unpublished
yield but the inclusion of information from post-mortem examination improves phenotyping. In the PAGE2 study, we are sequencing fetus-parent trios in pregnancies complicated by fetal anomaly associated with perinatal loss and with postnatal post-mortem examination to aid phenotyping.

**Methods:** Between May 2015 - December 2017, WES was undertaken in 27 fetus-parent trios, selected from a retrospective cohort of structurally abnormal fetuses (on prenatal ultrasound) and associated with fetal or early neonatal loss. All gave consent for post-mortem examination (PM) and WES. In 15 cases (55.6%), fetal DNA was taken at termination of pregnancy (TOP) and in 12 (44.4%) DNA was harvested at PM. Pathogenicity was assigned after consideration of the fetal phenotypes. Data were analyzed and findings were classified using ACMG guidelines following multidisciplinary team discussion. Causative pathogenic variants were validated and results explaining the phenotypes were reported.

**Results:** The median gestational age at delivery was 23 weeks (95%CI 21.5 - 28.8) and 5 pregnancies were consanguineous. Demise was associated with TOP (55.6%); stillbirth (29.6%) and early neonatal deaths (14.8%). A genetic diagnostic abnormality was made in 37% (10/27) of cases and VUS were identified in 7.4% (2/27). The greatest yield was in those with multisystem anomalies (5/10)(Table). Reported variants were classified as de novo (4), autosomal recessive (3), compound heterozygous (2) and X-linked (1) encompassing nine different genes.

**Table:** Anatomical system of fetal anomaly at PM

<table>
<thead>
<tr>
<th>System</th>
<th>Total</th>
<th>Diagnosed WES anomaly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Fetal akinesia sequence</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Brain</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Skeletal</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Abdominal</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Hydrops fetalis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Multisystem</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>27</strong></td>
<td><strong>10 (37%)</strong></td>
</tr>
</tbody>
</table>

**Conclusions:** In this relatively small, selected series, with well-defined phenotypes from PM, WES indicated an overall pathologic detection rate of 37%. The use of a multidisciplinary clinical review panel facilitates genetic diagnosis in these pregnancies. The collection of data are ongoing but currently the largest diagnostic yield is in those fetuses with multisystem anomalies both on prenatal USS and PM.
Prenatal diagnoses using single-cell DNA analysis of circulating fetal cells using a single cell-based droplet digital PCR system

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Objectives: The aim of the present study was to develop a method that does not require strict purification steps to directly extract genetic information from living fetal cells in a heterogeneous mixture containing maternal peripheral mononuclear cells.

Methods: Peripheral blood (10 mL) was collected from normal pregnant women. Peripheral blood mononuclear cells were labeled with anti-CD45 and anti-CD14 magnetic beads and then sorted using magnetic-activated cell sorting. All collected CD45⁻CD14⁻ cells were transferred to a single cell-based droplet digital PCR reaction system to assess rare fetal cells. In this system, a hexachloro-6-carboxyfluorescein-labeled RPP30 probe was used as an internal control and a 6-carboxyfluorescein-labeled SRY probe as a target. Threshold lines for the two probe signals were generated by analyzing control male and female human B-cell lines and 1X phosphate buffered saline.

Results: Peripheral blood from ten pregnant women (maternal age, 35.4 ± 4.4 years; gestational age, 29.4 ± 4.0 weeks; fetal sex, 3 male, 7 female) were applied to our single cell-based droplet digital PCR system. The average number of analyzable droplets per sample was 1,283,250 ± 161,911. No droplets were positive for both probe signals in the samples from pregnant women carrying female fetuses. Droplets positive for both probe signals were detected in the samples from pregnant women carrying male fetuses. These latter droplets were considered direct assessments of genetic information from single male fetal cells circulating in maternal blood.

Conclusions: The method described in this study allows extraction of genetic information from rare target cells in a crudely purified cell population. Proof-of-concept experiments confirmed the feasibility of single-cell analyses of circulating fetal cells without cell fixation or whole genome amplification. By optimizing cell sorting and encapsulation methods, as well as generating a more effective minute PCR environment in each droplet, more genetic information, such as single nucleotide and insertion-deletion variants, could be obtained from circulating fetal cells.
**Improved antenatal prediction of twin anemia-polycythemia sequence by a delta middle cerebral artery - peak systolic velocity – A new antenatal classification system**

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²Leiden University Medical Center, Department of Obstetrics and Fetal Medicine, Leiden, NH, Netherlands

**Objectives:** To investigate the predictive value of delta Middle Cerebral Artery- Peak Systolic Velocity (MCA-PSV) > 0.5 MoM compared to the currently used cut-off MCA-PSV criteria (>1.5 MoM in the donor and <1.0 in the recipient) for the diagnosis of twin anemia-polycythemia sequence (TAPS).

**Methods:** This retrospective study involved a consecutive cohort comprising uncomplicated monochorionic (MC) twins and twins with a postnatal TAPS diagnosis between 2004 and 2017 in a Dutch national fetal therapy center. The accuracy to predict postnatal TAPS for delta MCA-PSV > 0.5 MoM and MCA-PSV cut-off values was assessed using 2x2 tables.

**Results:** In total, 45 uncomplicated MC and 35 TAPS twins were analyzed. The sensitivity and specificity of cut-off MCA-PSV to predict a postnatal TAPS diagnosis were 46% (95% CI, 29-63%) and 100% (95% CI, 92-100%), respectively; positive predictive value was 100% and negative predictive value 70% (95%, 64-76%). Delta MCA-PSV showed a sensitivity of 83% (95% CI, 66-93%) and a specificity of 98% (95% CI, 88-100%); the positive predictive value and negative predictive value were 97% (95% CI, 81-100%) and 88% (95%, 88-94%), respectively. There was a high correlation between delta MCA-PSV and inter-twin Hb difference (R= 0.725, p <0.01).

**Conclusions:** This study shows that delta MCA-PSV > 0.5 MoM has a higher diagnostic accuracy compared to the currently used cut-off MCA-PSV criteria. We therefore propose a new antenatal classification system for TAPS (Table 1).
P2-90 Table.

<table>
<thead>
<tr>
<th>Antenatal Stage</th>
<th>Findings at Doppler ultrasound examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Delta MCA-PSV &gt; 0.5 MoM without signs for fetal compromise&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Delta MCA-PSV &gt; 0.7 MoM without signs for fetal compromise&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stage 3</td>
<td>As stage 1 or 2, with cardiac compromise of donor, defined as critically abnormal flow&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stage 4</td>
<td>Hydrops of donor</td>
</tr>
<tr>
<td>Stage 5</td>
<td>Intra-uterine demise of one or both fetuses preceded by TAPS</td>
</tr>
</tbody>
</table>

<sup>a</sup> Newly proposed criterion for the former TAPS stage 1 of MCA-PSV > 1.5 MoM in the donor and < 1.0 MoM in the recipient

<sup>b</sup> Newly proposed criterion for the former TAPS stage 2 of MCA-PSV > 1.7 MoM in the donor and < 0.8 MoM in the recipient

<sup>c</sup> Critically abnormal Doppler is defined as absent or reversed end-diastolic flow in umbilical artery, pulsatile flow in the umbilical vein, increased pulsatility index or reversed flow in ductus venosus.

Table 1. New antenatal criteria for TAPS

P2-93

Results disclosure and patient management in the era of expanded carrier screening

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<sup>1</sup>Counsyl, Chicago, IL, United States
<sup>2</sup>Counsyl, South San Francisco, CA, United States

Objectives: Although professional organizations have recommended carrier screening for conditions like cystic fibrosis for years, advances in technology have allowed for the adoption of expanded carrier screening (ECS), increasing the scope of information available to patients and providers alike. Guidelines highlight the importance of timely results delivery in the context of genetic counseling.<sup>1</sup> We report on an automated results delivery system that includes tele-genetic counseling for patients undergoing carrier screening.

Methods: Upon results availability, providers are notified of their patients’ results. If results indicate a potentially affected individual or a high-risk couple, patients will only receive these results in the context of genetic counseling, and an automated email directs them to schedule a consult. For other patients with results that are negative, positive for carrier status, or uncertain, the patient is prompted via email to access results through a secure portal where they may watch tailored informational videos, request “on-demand” genetic counseling,
schedule a consultation, or decline everything above. Summaries of consultations are sent to the ordering provider.

**Results:** Over an eight-year period, 278,318 carrier screening results were issued through the system. Of these, 41,050 patients (15%) elected genetic counseling, with some electing multiple consultations, resulting in a total of 43,343 consultations. Half of all consultations were for patients who received negative results. Approximately 32% of all consultations completed were on-demand. Median consultation time was ten minutes (interquartile range: 5-15 minutes) for all results. The median patient satisfaction rating for consultations was 4.9/5.0.

**Conclusions:** Combining web education, counseling, and automated notifications, we implemented a service that efficiently manages results disclosure. Approximately half of patients choosing to schedule a consultation had negative results, demonstrating a desire for post-test genetic counseling irrespective of test results. We describe an efficient and scalable means of manifesting medical guidelines on post genetic testing patient management, which we believe is important to quality care as genetic testing uptake grows among the general population.

P2-94

The impact of the political climate on the practice of prenatal genetic counseling in Poland

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²SWPS University of Social Sciences and Humanities, Poznań, Poland

**Objectives:** Our aim is to describe the goals and desired outcomes of prenatal genetic counseling according to Polish geneticists. We will argue that the counselors’ opinions vary widely. In particular, we observe significant discrepancy between the declared importance of nondirectiveness and the diverse meanings attributed to it. Nondirectiveness is incompatible with some of the declared and exhibited behaviours of genetic counselors that mirror personal moral values. This incompatibility is reinforced by the current political climate in Poland. Together with the lack of uniform professional standards for genetic counselling, this results in narrowing the range of reproductive choices available to clients.

**Methods:** We will present empirical data gathered from our research on the practice of genetic counseling in Poland. To check the reliability and validity of the evidence we will combine several sources. We will present the results of a survey of Polish genetic counselors’ opinions and attitudes concerning the professional role of a genetic counselor that we conducted between 2016 and 2017. We will challenge and supplement the survey results with findings from an observational study of counseling sessions conducted in two Polish genetic centers. Finally, data from key informants will be used to deepen and interpret our findings.

*Unedited draft - unpublished*
Results: We will demonstrate that in Poland there is no uniform professional standard regarding the practice of genetic prenatal counseling. Polish genetic counselors are unanimous in declaring nondirectiveness as the guiding principle of genetic counseling. Yet, they are considerably divided in their opinions concerning the actual scope of reproductive choices that should be available to the couple receiving a diagnosis of fetal abnormality. We will argue that this chasm has been induced by the current political climate in Poland.

Conclusions: The lack of professional standards may have considerable effect on the practice of genetic counseling. Poland is a telling example of a country where reproductive choices have been restricted not by changing the law but through the changing political climate and the conformist behavior of professionals that followed.

P2-95

The implementation of novel service delivery model of “on-site” independent genetic counseling via telehealth in eastern Washington State

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²Obstetrix Maternal Fetal Medicine of Spokane, Spokane, WA, United States
³Genetic Support Foundation, Olympia, WA, United States

Objectives: To summarize our experience in the implementation of an “on-site” reproductive genetic counseling service via telehealth for all women presenting at a Maternal-Fetal Medicine (MFM) practice. This MFM practice, like many, has found recruitment and retention of genetic counseling staff difficult and does not have the volume to support a full time genetic counselor. Genetic Support Foundation provides dedicated genetic counseling services on a part time basis, to meet the unique needs of this MFM clinic. The service is not affiliated with any commercial or hospital based laboratory, thus preserving patient autonomy and provider choice regarding best choice of labs.

Methods: Independent genetic counseling via telehealth was introduced in the Obstetrix MFM practice in Spokane, WA in March 2017. All new patients are offered concurrent genetic counseling with a board certified genetic counselor (GC) who is present “on-site” via a shared HIPAA compliant video platform. The GC reviews family history and testing options and is available for add-on patients with newly identified anomalies. Patient elections are immediately communicated to and facilitated by the MFM health care team. Laboratories are selected based on both insurance coverage and provider preference. All results are communicated to the patient by the GC.

Results: From March 2017 thru January 2018, three hundred fifty-two patients were seen for genetic counseling. All genetic testing was declined by 51% (n=180) and at least one test was elected by 49% (n=172) of patients. Of those electing testing 91% (n=156) had screening
and/or diagnostic testing for aneuploidy, 43% (n=74) had carrier screening for one or more conditions and 3% (n=5) had single gene/panel testing. Samples were sent to seven different testing laboratories for a total of 320 individual tests.

**Conclusions:** We describe an independent genetic counseling service that is fully integrated into a MFM practice via telehealth. The service is well accepted by patients and valued by the health care team. It provides a solution to the difficulty in recruiting and retaining genetic counseling staff and improves the efficiency of the practice. It also preserves autonomy by allowing selection of the laboratories deemed to be most appropriate based on insurance coverage and provider assessment.

P2-96

**Outcomes of web-based genetic counselling for non-invasive prenatal screening**

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²Genea, Sydney, NSW, Australia

**Objectives:** Given worldwide concerns that women are not fully counselled on Non-invasive Prenatal Screening (NIPS/T) and the lack of Medical Professionals trained to provide accurate information, we designed a web-based education portal. We wanted to determine if there was interest in web-based education, if it would help women have a more meaningful discussion with their Doctor and enable her to make an informed decision on prenatal diagnosis. A Doctor portal was also designed to see if web-based education was helpful in providing updated information quickly to the Doctor and to increase the number of Clinicians who can effectively counsel on NIPS.

**Methods:** The Genetic counsellor was filmed providing general prenatal diagnosis counselling to camera with detailed information given on Non-invasive prenatal diagnosis, including limitations. Two separate videos were designed, one targeting Doctors and outlining information that should be raised in their counselling session and one designed for patients. Pre and post-test questionnaires assessing basic knowledge on prenatal diagnosis and Down syndrome were designed and placed on the webpage. A Decision Aid was also designed for patients. All data was collected and analysed over twelve months. The site was not advertised to ascertain if this type of information was actively sought by patients.

**Results:** There were 25,451 views of the Portal over twelve months in ten different languages. 54% of patient viewers were new with the average session time 8.33 minutes compared to the Doctor portal (29.6% new viewers and average view 3 minutes). Almost all patients had knowledge errors in the pre-video questionnaire whilst 100% had correct answers after viewing the video. All patients showed increased confidence that they “definitely” had enough
information to make a decision and to ask questions of their health provider. All reported the video “definitely” helped with their understanding.

**Conclusions:** There appears to be an interest in Web based education and it seems to increase patients understanding and knowledge around NIPS. Patients reported they all had increased confidence to be able to discuss their options more fully with their Doctor. Web based education may be beneficial to ensure patients have access to accurate information and help women make the appropriate choice for herself. The high return rate indicates that women can continue the education at a convenient time or watch multiple times to enhance understanding. The Doctors appear to use the site to quickly find an answer and return later.

**P2-97**

**Shifting paradigms: Twin gestations in the age of cell-free DNA**

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\(^2\)Integrated Genetics, Shawnee Mission, KS, United States
\(^3\)Sequenom Laboratories, San Diego, CA, United States

**Objectives:** Prenatal screening options for aneuploidy changed significantly with the introduction of cell-free DNA (cfDNA) based screening tests. These cfDNA options have dramatically shifted the testing paradigm in aneuploidy evaluation during pregnancy. Given the unique biological factors in twin pregnancies such as zygosity and chorionicity, there are some known limitations related to any testing method. Nevertheless, screening and diagnostic options are still typically discussed with patients who have twin pregnancies. The current study evaluates patient decisions regarding screening and diagnostic options in twin pregnancies before and after the introduction of cfDNA.

**Methods:** This study includes patients with twin gestations referred for genetic counseling during two time periods: 1802 patients seen in 2009-2010 and 1603 patients in 2014-2016. Patients who previously had aneuploidy screening or diagnostic testing were excluded. The 2009-2010 patients were offered the option of maternal serum screening (MSS) and diagnostic testing such as chorionic villus sampling and amniocentesis. The 2014-2016 patients were offered MSS, cfDNA, and diagnostic testing. Patients were informed of their risks for aneuploidy in a twin gestation and offered information about benefits, risks, and limitations about their testing options, including unique factors of testing in twin pregnancies.

**Results:** During 2009-2010, 47% of patients had MSS, 20% chose diagnostic testing while 2% wanted both tests. During this time period, 17% of patients declined all testing offered. During 2014-2016, 9% of patients had MSS, 40% chose cfDNA, 11% pursued diagnostic testing and 13% declined all testing. In both time periods, the remaining patients were undecided. There was a significant decrease in the uptake of MSS and diagnostic testing (p<0.001) from 2009-2010 to
2014-2016, attributed to the increased utilization of cfDNA. There was also a significant decrease in women who declined all testing once cfDNA became available (p=0.001).

Conclusions: Twin pregnancy aneuploidy evaluation is more challenging due to decreased detection rates for aneuploidy screening and increased risks for losses related to diagnostic options. While aneuploidy screening in twin gestations is not as accurate as singleton gestations, studies have provided evidence of the screening efficacy of cfDNA in twin pregnancies. This study clearly demonstrates that the vast majority of patients with twin pregnancies prefer screening options. Once cfDNA became available there was a significant shift away from both MSS and diagnostic testing, which demonstrates the value for twin gestation patients seeking first trimester aneuploidy evaluation.

P2-97 Table.

<table>
<thead>
<tr>
<th></th>
<th>2009-2010</th>
<th>2014-2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>cfDNA</td>
<td></td>
<td>40%</td>
</tr>
<tr>
<td>MSS</td>
<td>47%</td>
<td>9%</td>
</tr>
<tr>
<td>DX</td>
<td>20%</td>
<td>11%</td>
</tr>
<tr>
<td>Both / Multiple</td>
<td>2%</td>
<td>7%</td>
</tr>
<tr>
<td>tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Declined all</td>
<td>17%</td>
<td>13%</td>
</tr>
<tr>
<td>Follow-up with OB</td>
<td>14%</td>
<td>20%</td>
</tr>
</tbody>
</table>

P2-98

Counselors’ attitude about NIPT as a first-tier aneuploidy screening test and opinions on quality requirements regarding counseling for prenatal screening

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²Dept. of Clinical Genetics, VU University Medical Center Amsterdam, Amsterdam, Netherlands
³AVAG / Midwifery Science VUMC Amsterdam, Amsterdam, Netherlands

Objectives: Since April 2017, the Non-Invasive Prenatal Test (NIPT) is available as a first-tier screening test for Down-, Edwards-, and Patau Syndrome in a study setting (TRIDENT-2), for all Dutch women. Simultaneously, to optimize the quality of prenatal counseling, Dutch policy makers tightened up the quality requirements for counselors: 1) mandatory additional counseling course, 2) counseling for the combined-test, NIPT, and FAS (fetal anomaly scan) in
the same visit, 3) 30 minutes available for counseling, 4) scheduling a separate counseling consultation. We examined counselors’ attitudes and opinions regarding the provision of NIPT as the first-tier screening and the tightened quality requirements.

**Methods:** In 2017 and 2018, a cross-sectional online questionnaire was sent to all ~3000 Dutch counselors by the Regional Prenatal Screening Centers; the first before implementation of the NIPT as a first-tier screening test (T1), and the second nine months after this implementation (T2). Participants had to indicate whether they considered offering NIPT as the first screening test ‘good’ or ‘not good’ and whether they were of the opinion that the four tightened requirements were ‘good’ or ‘not good.’ At T1 we included 1725 participants and at T2 1063 participants. Additional analyses (e.g., subgroup analyses) will be presented at the meeting.

**Results:** At T1, 75% of all participants showed a positive attitude towards NIPT as first-tier screening. At T2, 86% had a positive attitude, 4% a negative attitude and 10% were ambivalent. The majority of the participants had a positive opinion about three out of four quality requirements: mandatory counseling course (T1:84%/T2:74%), counseling combined test, NIPT, and FAS in the same visit (T1:71%/T2:74%), and 30 minutes available for counseling (T1:60%/T2:63%). Before implementation, 35% of the participants were positive about organizing counseling in a separate visit, as 44% of the participants were positive after implementation of this scheduling.

**Conclusions:** After implementation of NIPT as a first-tier test, more counselors showed a positive attitude towards NIPT than before implementation. The majority of counselors were of the opinion that the new quality requirements for counselors are ‘good’ except for organizing the counseling in a separate visit and to a lesser extent the requirement to schedule 30-minutes counseling-visits. However, after implementation, 11% more participants showed positive opinions towards organizing counseling in a separate visit. Because counseling in a separate visit contribute to women’s informed choice, further research is necessary to examine counselors’ barriers and facilitators in organizing counseling in a separate visit.

P2-99

**Utilization of an educational video for pre-test counselling women undergoing cell-free DNA (cfDNA) based test for aneuploidy: experience from a single centre**

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*Unedited draft - unpublished*
Objectives: The development of prenatal tests has outpaced the ability to counsel patients appropriately regarding their options. With the increased complexity of testing options, it is more difficult to adequately support patients through the process of informed consent. Professional societies advocate for robust informed consent processes in respect for women’s autonomy. With that in mind we developed an educational video for pre-test counselling of women considering cfDNA testing. The aim of this study is to assess its comprehension by users and their attitude towards the video tool.

Methods: We created a video that included all 11 recommended pre-test counselling points for cfDNA testing (Sachs et al, 2015). A link to it is provided by email to women addressing the centre for cfDNA test information. They are recommended to undergo the test after watching the video. The day of blood draw, before meeting a genetic counsellor, women were asked to answer if ‘after watching the video, did they still need to review specific topic/s with counsellor?’ After meeting the counsellor, they were invited to fill a facultative questionnaire exploring their screening modalities and their attitude towards the video.

Results: 254/311 women filled the questionnaire (81.7%). Mean maternal age was 35.9. Video was watched >1 times in 21% of the cases, by smartphone (37%) or computer (35%) and with husband (60%) or alone (31%). 31% enquired clarifications and the educational level in this group is similar to that of the overall population of respondents (p=0.17). The most requested topics to review were: sex chromosome aneuploidies (38.2%), fetal sex (28.9%) and no results (26.3%). 41% requested to review more than 1 topic. The most appreciated and unpleasant aspects of video support were the time convenience and its 18 min length, respectively.

Conclusions: Our survey shows a positive attitude of women/couples regarding first-line test information provision by online video. Advantages of pre-counselling provision of standardized information, such as by video or other methods, besides the guarantee of topic coverage, is the time convenience and better use of counsellor’s time to review and deepen only specific unclear topics. Limitations of this study is the lack of information about patients who may not have been able to see the video and the lack of comparison with the traditional one-on-one counselling method regarding effectiveness of the message against counsellor’s time.

P2-101

Isolated congenital diaphragmatic hernia: Novel genes identification and genotype-phenotype analysis

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3BCNatal. Hospital Clinic/Sant Joan de Deu. Universitat de Barcelona, Barcelona, Catalunya, Spain

Unedited draft - unpublished
Objectives: Congenital diaphragmatic hernia (CDH) occurs as an isolated defect in 60% of cases. CDH prognosis assessment is mainly based on lungs size and liver position. Isolated CDH genetics is heterogeneous and poorly understood. Whether genetic lesions are also outcome determinants is not well-know. The objectives of the study are to identify isolated CDH genetic causes, to fine map the mutational burden in these cases and to search for a correlation between the genotype and the disease severity and outcome.

Methods: Targeted massively parallel sequencing of 143 genes including human and mouse CDH causative genes and biologically related candidate genes in a cohort of 120 fetuses with isolated CDH and detailed outcome measures. For genotype-severity correlation, we searched for an enrichment of variants affecting lung development genes in severe CDH patients. For genotype-outcome analysis, we searched for an increased lethality with variants number.

Results: Pathogenic and likely pathogenic variants were identified 10% of the cohort. These variants affect ZFPM2, GATA4 and NR2F2 which are known CDH causative genes; and new genes namely TBX1, TBX5, GATA5 and PBX1. In addition, mutation burden analysis of variants of uncertain significance identified LBR and CTBP2 as two new candidate genes with enrichment in rare but predicted pathogenic variants. We observed neither significant burden of variants hitting lung development genes in severe CDH group nor was the lethality associated with overall variants number.

Conclusions: Targeted massive parallel sequencing is a useful tool for isolated CDH genetic causes identification. The genotype of the CDH patients does not seem to correlate with the outcome. The analysis of a larger number of patients may confirm genes involved in lung hypoplasia.
normal. An LGA 34week female fetus was diagnosed with BWS (hypoglycemia, large tongue, ear creases and limb overgrowth). The second pregnancy, with elevated HCG, AFP and an omphalocele resulted in premature birth of a male baby with a cleft palate who sadly died. The consecutive pregnancy was positive only for elevated HCG. The family, counseled of the recurrence risk, continued the pregnancy. The 34 weeks daughter had BWS (cleft palate, microretrognatia and hypoglycemia).

Methods: The family was lost to follow-up for many years. Then the now 24-year-old eldest daughter was referred to our clinic to discuss her reproductive options. She stated that both she and her sister were also clinically diagnosed with Mayer Rokitansky Küster Hauser (MRKH) syndrome – having no uterus and fallopian tubes. Prior to her visit, she had undergone both methylation testing of the imprinting center 2 (IC2) and chromosomal microarray - which came back normal. In an attempt to address both the familial BWS and MRKH – sequencing of the CDKN1C gene and WES were preformed (for both sisters and the

Results: The sequencing results revealed a c.367_385del p.AlaSefs*143 variant in the CDKN1C gene. Both sisters and the mother were carriers. This variant, not previously described in the literature, was classified as Likely pathogenic. Unfortunately, the WES did not divulge additional variants that could elucidate the cause of MRKH in the sisters. The eldest sister in now planning on PGD for BWS with surrogacy.

Conclusions: BWS is an uncommon syndrome, and the familial form rarer. The majority of cases are diagnosed after birth on the basis of physical findings. Prenatal diagnosis is feasible due to characteristic manifestations. Nevertheless, due to the variable phenotype a high index of suspicion should be maintained even with the lack of sonographic findings, especially if an older child was already diagnosed with the syndrome, since recurrence risk can be as high as 50%. An early, accurate molecular diagnosis will facilitate reproductive choice in families. The additional phenotype of MRKH should be treated with caution as to its connection to BWS.

P2-104

Preterm placental calcification as an ultrasonographic marker for assessing the risk of stillbirth

Kuo-Hu Chen

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Objectives: Stillbirth is a major issue in antenatal care, and its etiology remains much unknown. The prospective cohort study investigated the previously un-identified risk factor of third-trimester stillbirth to determine the role of Grade III preterm placental calcification (PPC) on assessing the risk of stillbirth.

Unedited draft - unpublished
Methods: In a tertiary teaching hospital, antepartum ultrasonography was performed at 28 weeks’ gestation to establish a diagnosis of PPC. Pregnancies with multifetal gestations, major fetal congenital anomalies, termination, cord accidents, apparent intrauterine infection, and antepartum co-morbidities were excluded. 15,122 eligible pregnancies were enrolled and classified as stillbirth (n=99) and livebirth (n=15,023) groups. Kaplan-Meier survival analysis and logistic regression analysis were used to assess the cumulative stillbirth risk and odds ratios, respectively.

Results: Between stillbirth and livebirth groups, there were no significant differences in maternal age, parity and BMI, but significant differences in PPC (35.4% Vs 6.3%, p<0.001) and smoking. The peak occurrence of stillbirths was at 30 and 37 weeks, with a bimodal distribution of 11 and 17 stillbirths, respectively. For pregnancies with or without PPC, the incidences of stillbirths per-1,000-births were 35.9 and 4.5, respectively. The cumulative stillbirth risk was higher for pregnancies with PPC. Logistic regression analysis revealed the risk of stillbirth (adjusted OR:7.62; 95%CI:5.00-11.62) was much higher for pregnancies with PPC after adjusting with smoking and other demographic factors.

Conclusions: Grade III PPC is highly associated with unexplained stillbirth, and identified an independent risk factor. As an ominous sign, the presence of PPC may precede this negative outcome and can serve as a marker when noted on ultrasonography, and requires closer surveillance for fetal well-being. Appropriate information and suggestions should be provided to affected pregnant women to facilitate earlier intervention or referral.

P2-104 Table.

<table>
<thead>
<tr>
<th>Smoking</th>
<th>Stillbirths</th>
<th>Total births</th>
<th>Stillbirth Rate (per 1,000 births)</th>
<th>Crude Odds Ratio (95% CI)</th>
<th>Adjusted Odds Ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>36</td>
<td>2203</td>
<td>16.3</td>
<td>3.39 (2.25-5.12)</td>
<td>3.16 (2.08-4.80)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>No</td>
<td>63</td>
<td>12919</td>
<td>4.9</td>
<td>—</td>
<td>—</td>
<td>Reference</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade III Preterm placental calcification</th>
<th>Stillbirths</th>
<th>Total births</th>
<th>Stillbirth Rate (per 1,000 births)</th>
<th>Crude Odds Ratio (95% CI)</th>
<th>Adjusted Odds Ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>35</td>
<td>974</td>
<td>35.9</td>
<td>8.20 (5.20-12.45)</td>
<td>7.62 (5.00-11.62)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>No</td>
<td>64</td>
<td>14148</td>
<td>4.5</td>
<td>—</td>
<td>—</td>
<td>Reference</td>
</tr>
</tbody>
</table>

CI, confidence interval.

*p < 0.001, calculated by logistic regression analysis.

Adjusted odds ratio was measured with adjustment of other variables including age, body mass index, and parity.

— indicates no data.
Polycystic ovarian syndrome and subsequent abortion: A nationwide population-based study

Kuo-Hu Chen

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Objectives: This nationwide population-based study aims to explore the relationship between polycystic ovarian syndrome (PCOS) and subsequent unexplained abortion by examining data retrieved from 1998-2012 Taiwan National Health Insurance Research Database. Odds ratios (ORs) for risk of abortion were calculated by logistic regression analysis with adjustment for occupation, urbanization, economic status and co-morbidities.

Methods: ICD9-CM codes 256.4X, 632, 634.X, 637.X, and 646.3X were used separately for the diagnoses of PCOS and abortion, which were further confirmed by records of blood tests, ultrasonography, or pathologic report of surgeries to ensure the accuracy of the diagnoses. Women diagnosed at < 15 or > 45 years of age, and those diagnosed with abortion prior to PCOS were excluded. Women with antoimmune disorders, maternal or fetal chromosomal abnormalities were also excluded. During pregnancy, each woman with a previous diagnosis of PCOS was age-matched to 5 women without PCOS.

Results: Among 6,947 eligible women with a valid PCOS diagnosis, 1,837 (26.44%) had subsequent pregnancies. Abortion occurred frequently among women with a history of PCOS as compared to those without PCOS (33.91% vs. 4.22%, p<0.0001). Logistic regression analysis revealed that PCOS was associated with abortion (adjusted OR=11.59; 95%CI:10.02-13.42). Among 1,837 affected patients, 715 (38.92%) had used medications for PCOS and 156 (8.49%) were treated with metformin, an oral hypoglycemic agent (OHA). There was no significant difference in the incidence of abortion between medication and no-medication sub-groups (p>0.05). The use of OHA did not reduce the risk of abortion (adjusted OR=1.02; 95%CI:0.72-1.45).

Conclusions: A history of PCOS is a significant and independent risk factor for development of unexplained abortion. Using OHA or medication for PCOS does not reduce the risk of abortion.
# Table 1. Characteristics of women with and without a history of PCOS

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Case Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women without PCOS</td>
<td>Women with PCOS</td>
</tr>
<tr>
<td></td>
<td>($n = 9,185$)</td>
<td>($n = 1,837$)</td>
</tr>
<tr>
<td>Age of PCOS diagnosed (y/o)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-25</td>
<td>27.2134 (5.0324)</td>
<td>27.2134 (5.0336)</td>
</tr>
<tr>
<td>26-35</td>
<td>3400 37.02</td>
<td>680 37.02</td>
</tr>
<tr>
<td>36-45</td>
<td>5305 57.76</td>
<td>1061 57.76</td>
</tr>
<tr>
<td>46-55</td>
<td>450 5.23</td>
<td>96 5.23</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td>&lt;0.0001**</td>
</tr>
<tr>
<td>White collar</td>
<td>5064 55.13</td>
<td>1133 61.68</td>
</tr>
<tr>
<td>Blue collar</td>
<td>1685 18.35</td>
<td>306 16.66</td>
</tr>
<tr>
<td>Retired and others</td>
<td>2436 25.52</td>
<td>398 21.67</td>
</tr>
<tr>
<td>Urban</td>
<td>5811 63.27</td>
<td>1255 68.32</td>
</tr>
<tr>
<td>Suburban</td>
<td>2792 30.40</td>
<td>469 25.53</td>
</tr>
<tr>
<td>Rural</td>
<td>582 6.34</td>
<td>113 6.15</td>
</tr>
<tr>
<td>Insurable wage (Economic)</td>
<td></td>
<td>&lt;0.0001**</td>
</tr>
<tr>
<td>&lt;$20000 NTD</td>
<td>2235 24.33</td>
<td>387 21.07</td>
</tr>
<tr>
<td>$20000-40000 NTD</td>
<td>3930 42.79</td>
<td>795 43.28</td>
</tr>
<tr>
<td>$&gt;40000 NTD</td>
<td>1389 15.12</td>
<td>391 21.26</td>
</tr>
<tr>
<td>Retired and others</td>
<td>1631 17.76</td>
<td>264 14.37</td>
</tr>
<tr>
<td>Co-morbidities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>110 1.20</td>
<td>28 1.52</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>244 2.66</td>
<td>76 4.14</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>12 0.13</td>
<td>3 0.16</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>84 0.91</td>
<td>13 0.71</td>
</tr>
<tr>
<td>Chronic pulmonary disease</td>
<td>1339 14.58</td>
<td>281 15.30</td>
</tr>
</tbody>
</table>

* $p < 0.001$, ** $p < 0.0001$, by chi-square test or student t test, as appropriate

Data are expressed as the number (%) or mean ± standard deviation, as appropriate

NTD: New Taiwan Dollar (current exchange rate is US$ 0.0321 = NTD 1.00)

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P2-106

Progression of gestational hypertension to pre-eclampsia: A cohort study of 20,103 pregnancies

Kuo-Hu Chen

*Unedited draft - unpublished*
Objectives: To investigate previously un-identified risk factors for progression of gestational hypertension (GH) to pre-eclampsia (PE) by considering Grade III preterm placental calcification (PPC) and excessive weight gain (≧10 kgw) at 28 weeks’ gestation.

Methods: At a tertiary teaching hospital, obstetric ultrasonography was performed at 28 weeks' gestation to establish a diagnosis of grade III PPC. Weight gain during pregnancy was recorded at the same time. Pregnancies complicated with chronic hypertension, major fetal congenital anomalies, termination before 24 weeks gestation, and abortion before 20 weeks gestation were excluded. In the prospective cohort study, 20,103 pregnancies were enrolled and categorized as normal blood pressure (NBP; n=18,223) and GH-PE (n=1,880) groups. According to severity of the diseases, the GH-PE group was further divided into GH (n=1,088), PE (n=792), and severe-PE (n=209) groups.

Results: There were significant differences between NBP and GH-PE groups in known factors, including maternal age, BMI, parity, multi-fetal pregnancy, and co-morbidities (all p<0.001), which increased GH-PE risks. Regarding progression of GH to PE and severe-PE, there was much greater frequencies of excessive weight gain (51.2% and 49.0% vs. 9.3%) or PPC (63.2% and 61.6% vs. 12.1%) in severe-PE and PE groups than GH group. Logistic regression analysis revealed that PPC was a significant and independent risk factor for progression of GH to PE (OR:13.71; 95%CI:10.25-18.33) and severe-PE (OR:12.42; 95%CI:8.89-17.35), as well as excessive weight gain (OR:8.92; 95%CI:6.67-11.92 and OR:10.25; 95%CI:7.30-12.40).

Conclusions: Being a pathologic implication, the presence of PPC or excessive weight gain during pregnancy may precede progression of GH, and can serve as a warning that requires enhanced monitoring for maternal and fetal well-being. Based on the findings of PPC and excessive weight gain, at-risk pregnant woman should be counseled to facilitate early intervention or referral. In addition, avoiding excessive weight gain during pregnancy may reduce the risk of GH progression to PE.
P2-106 Table. Table 1. Comparison of pregnancies complicated with pre-eclampsia (PE) and gestational hypertension (GH)

<table>
<thead>
<tr>
<th></th>
<th>PE group (n = 792)</th>
<th>GH group (n = 1,088)</th>
<th>Statistics</th>
<th>OR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>p-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>30.48 ± 3.60</td>
<td>29.60 ± 3.50</td>
<td>&lt;0.001**</td>
<td>0.98</td>
<td>0.95-1.02</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</strong></td>
<td>23.16 ± 1.59</td>
<td>22.02 ± 3.24</td>
<td>&lt;0.001**</td>
<td>1.34**</td>
<td>1.24-1.45</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>544 (68.7)</td>
<td>659 (60.6)</td>
<td>&lt;0.001**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>192 (24.2)</td>
<td>308 (28.3)</td>
<td>0.50**</td>
<td>0.49-0.51</td>
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<tr>
<td>2 or more</td>
<td>56 (7.1)</td>
<td>121 (11.1)</td>
<td>0.44**</td>
<td>0.28-0.70</td>
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<td><strong>Multi-fetal pregnancy</strong></td>
<td>0.441</td>
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<td>No</td>
<td>751 (94.8)</td>
<td>1040 (95.6)</td>
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<tr>
<td>Yes</td>
<td>41 (5.2)</td>
<td>48 (4.4)</td>
<td>1.18</td>
<td>0.77-1.81</td>
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<tr>
<td><strong>Excessive weight gain</strong></td>
<td>&lt;0.001**</td>
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<tr>
<td>No</td>
<td>404 (51.0)</td>
<td>987 (90.7)</td>
<td>reference</td>
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<tr>
<td>Yes</td>
<td>388 (49.0)</td>
<td>101 (9.3)</td>
<td>8.92**</td>
<td>6.67-11.92</td>
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<tr>
<td><strong>Smoking</strong></td>
<td>0.541</td>
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<tr>
<td>No</td>
<td>648 (81.8)</td>
<td>902 (82.9)</td>
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<tr>
<td>Yes</td>
<td>144 (18.2)</td>
<td>186 (17.1)</td>
<td>1.68*</td>
<td>1.23-2.30</td>
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<tr>
<td><strong>Preterm placental calcification</strong></td>
<td>&lt;0.001**</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>No</td>
<td>304 (38.4)</td>
<td>956 (87.9)</td>
<td>reference</td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>488 (61.6)</td>
<td>132 (12.1)</td>
<td>13.71**</td>
<td>10.25-18.33</td>
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<td><strong>Co-morbidities</strong></td>
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<tr>
<td>Cardiovascular disease</td>
<td>19 (2.4)</td>
<td>24 (2.2)</td>
<td>0.782</td>
<td>1.09</td>
<td>0.59-2.00</td>
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<td>Diabetes mellitus</td>
<td>66 (8.3)</td>
<td>85 (7.8)</td>
<td>0.682</td>
<td>1.07</td>
<td>0.77-1.50</td>
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<td>Hyperthyroidism</td>
<td>40 (5.1)</td>
<td>58 (5.3)</td>
<td>0.787</td>
<td>0.95</td>
<td>0.63-1.43</td>
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<td>Connective tissue disease</td>
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<td>127 (11.7)</td>
<td>0.479</td>
<td>1.11</td>
<td>0.84-1.46</td>
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<td>Thromboembolic disease</td>
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<td>22 (2.0)</td>
<td>0.710</td>
<td>1.13</td>
<td>0.60-2.12</td>
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<td>Polycystic ovarian syndrome</td>
<td>89 (11.2)</td>
<td>84 (7.7)</td>
<td>0.009*</td>
<td>1.51*</td>
<td>1.11-2.07</td>
</tr>
<tr>
<td>Renal disease</td>
<td>42 (5.3)</td>
<td>54 (5.0)</td>
<td>0.741</td>
<td>1.07</td>
<td>0.71-1.62</td>
</tr>
<tr>
<td><strong>Neonatal outcomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational length (weeks)</td>
<td>34.74 ± 3.64</td>
<td>36.16 ± 2.70</td>
<td>&lt;0.001**</td>
<td></td>
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<tr>
<td>Baby birth weight (g)</td>
<td>2240.05 ± 903.13</td>
<td>2530.71 ± 784.52</td>
<td>&lt;0.001**</td>
<td></td>
<td></td>
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<tr>
<td>Preterm delivery</td>
<td>0.984</td>
<td></td>
<td></td>
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<tr>
<td>No</td>
<td>384 (48.5)</td>
<td>527 (48.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>408 (51.5)</td>
<td>561 (51.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low apgar score&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>456 (57.6)</td>
<td>923 (84.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>336 (42.4)</td>
<td>165 (15.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as the number (%) or mean ± standard deviation (SD), as appropriate
*<p><0.01, **<p><0.001, by chi-square test, Student’s t-test, or logistic regression analysis, as appropriate
<br>*Logistic regression analysis of odds ratio (OR) and 95% confidence interval (CI) for the occurrence of pre-eclampsia (PE) compared with the reference group
<br>b Weight gain ≥10 kg during pregnancy, measured at 28 weeks gestation
<br>c Weight gain ≥10 kg during pregnancy, measured at 28 weeks gestation
P2-107

The poor ovarian responders whose SHBG increased on HCG injection day will get more oocytes and better embryos in controlled ovarian stimulation using medroxyprogesterone acetate

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Objectives: To investigate whether serum SHBG levels are related to IVF outcomes for poor ovarian responders whom in controlled ovarian stimulation using medroxyprogesterone acetate (MPA). To investigate whether serum SHBG levels are related to IVF outcomes for poor ovarian responders whom in controlled ovarian stimulation using medroxyprogesterone acetate (MPA).

Methods: 654 poor ovarian responders in MPA protocol from June 2015 to December 2016 were retrospectively analyzed. According to the changing of SHBG between late follicular phase and the day of hCG priming, it was divided into SHBG decreasing group and SHBG increasing group. Patients aged 20-51 with regular menstrual cycles and the presence of other risk factors for poor ovarian response were included. Basal concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin (PRL), testosterone (T) and SHBG were determined. Free androgen index (FAI) was calculated. IVF stimulation parameters, fertilization and clinical pregnancy rates were evaluated.

Results: There was significant differences in the number of oocytes retrieved, MII oocytes, cleavage rate of oocytes, high quality embryos and advanced embryos rate on the third day between the two groups. The ovulation induction effect of SHBG increasing trend group on HCG day was significantly better than that of SHBG decreasing group. The increase of SHBG on the day of HCG injection had significantly positive association with the number of oocytes retrieved and the number of f high quality embryos.

Conclusions: Our study provides evidence that SHBG can be considered as a predictor of IVF outcomes. The increase of SHBG on the HCG injection day have a positive correlation with part of the stimulation parameters including number of oocytes retrieved and the number of blastocysts in in patients with diminished ovarian reserve using medroxyprogesterone acetate(MPA) combined with gonadotropin (Gn )to controlled ovarian stimulation.
P2-108

First trimester prediction of foetuses growing under the 10th centile at the time of delivery based on clinical and biophysical parameters: A retrospective cohort study

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³Clinica Davila, Santiago, Chile

Objectives: Determine if an algorithm using clinical and biophysical parameters in the first trimester can predict a foetal growth under the 10th centile at delivery.

Methods: Retrospective cohort study in Clínica Dávila, Chile. Singleton pregnancies with first trimester control, 11-14-week uterine artery Doppler pulsatility index (UaPI) and delivery between 32 and 41 weeks were included. Body mass index (BMI), blood pressure and UaPI were obtained. These variables were correlated with new born weight, and the capacity of these variables to predict a foetal growth under the 10th centile at the time of birth.

Results: 912 patients where obtained, 61 were classified as SGA. The UaPI was 1.70 for SGA, 1.35 for large for gestational age and 1.5 for those with normal growth. These differences were statically significant (p < 0.001). The p95 for UaPI for the whole cohort was 2.00, 16/61 of the SGA group (26.23%). The analysis of covariance showed the correlation between SGA and BMI and mean arterial pressure (MAP) was significant (p= 0.0012, 0.0143 respectively). This data presented a ROC curve of 0.56 for the prediction of SGA.

Conclusions: There is evident correlation between BW and UaPI, BMI and MAP. Their use to predict foetuses growing under 10th centile at the moment of delivery is poor and other biomarkers need to be incorporated for a better prediction.

P2-109

Can PAPP-A and PlGF, measured in enhanced first trimester screening, be used to predict adverse pregnancy outcomes?

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²Genetics Program, North York General Hospital; Department of Paediatrics, University of...
Objectives: To assess if first trimester serum pregnancy-associated plasma protein A (PAPP-A) and placental growth factor (PlGF), measured in a general prenatal screening population, can be used to predict the risk of developing adverse pregnancy outcomes. These outcomes include hypertensive disorders of pregnancy (HDP), preterm birth (PTB) and intrauterine growth restriction (IUGR).

Methods: The study was on women who had an enhanced first trimester screening (eFTS) and delivered at North York General Hospital. EFTS uses all markers for FTS with the addition of PlGF and alpha-fetoprotein. The median multiple of the median (MoM) of PAPP-A and PlGF were compared between pregnancies with and without an adverse outcome. The risks of developing an adverse outcome were compared between women who had and did not have a reduced level of PAPP-A (≤0.49 MoM) or PlGF (≤0.42 MoM). Multivariate analysis including maternal characteristics, medical history and biochemical markers was used to generate prediction models.

Results: The study population consisted of 3130 women including 335 with one or more adverse outcomes. The median MoMs of PAPP-A and PlGF were lower in women who had HDP, PTB or any adverse outcome (p<0.05). The risks of having PTB or any adverse outcome were higher in women who had low PAPP-A. The risks of having PTB, IUGR or any adverse outcome were higher in those who had low PlGF. Multivariate analysis showed that the risk of having any adverse outcome was positively associated with BMI, primigravida and pre-existing hypertension, and negatively associated with the MoMs of PAPP-A and PlGF.

Conclusions: This pilot study used routine prenatal screening data from a general population to assess if PAPP-A and PlGF can be used to identify women with an increased risk of developing adverse pregnancy outcomes. Our results showed that low PAPP-A and/or PlGF were moderately associated with increased risk of adverse pregnancy outcomes. Larger studies containing more information on maternal characteristics and history as well as biophysical examination results are needed to verify prediction models.

P2-111

Oxidative stress in healthy pregnancy and preeclampsia is linked to chronic inflammation, iron status and vascular function

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Objectives: During normal pregnancy, placental oxidative stress (OS) is present during all three trimesters and is necessary to obtain normal cell function. However, if OS reaches a certain level, pregnancy complications might arise. In preeclampsia (PE), a dangerous pregnancy specific hypertensive disorder, OS induced in the ischemic placenta causes a systemic inflammatory response and activates maternal endothelial cells. In this study, we aimed to quantify superoxide concentrations (as a measure of systemic OS) using electron paramagnetic resonance (EPR) and correlate them to markers of systemic inflammation, iron status and vascular function.

Methods: Sixty one women with a healthy pregnancy (HP), 10 non-pregnant controls (NP) and 31 PE patients (32±3.6 weeks) were included. During HP, blood samples for superoxide, neutrophil to lymphocyte ratio (NLR), mean platelet volume (MPV) and iron status were taken at 10, 25 and 39 weeks. Vascular measurements for arterial stiffness (Carotid-Femoral Pulse Wave Velocity (CF-PWV), Augmentation index (Alx), Augmentation Pressure (AP)) and microvascular endothelial function (Reactive Hyperemia Index (RHI)) were performed at 35 weeks. In PE, all measurements were performed at diagnosis. CMH (1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrroldine) was used as spin probe for EPR, since the formed CM radical corresponds to the amount of superoxide.

Results: Superoxide concentration remains stable during pregnancy (p=0.90), but is significantly higher compared to the NP controls (p<0.0001). At 25 weeks, there is a significant positive correlation between superoxide and ferritin concentration. (p=0.04) In PE, superoxide, systemic inflammation and iron status are much higher compared to HP (all p<0.001). During HP, superoxide concentrations correlate significantly with arterial stiffness (CF-PWV, Alx and AP all p<0.04), while in PE superoxide is significantly correlated to microvascular endothelial function (RHI, p=0.03).

Conclusions: During HP there is an increased but stable oxidative environment, which is correlated to ferritin concentration. If superoxide levels increase, there is an augmentation in arterial stiffness. In preeclamptic pregnancies, systemic inflammation and superoxide concentrations are higher and result in a deterioration of microvascular endothelial function. Together, these findings support the hypothesis that vascular function is directly linked to the
amount of OS and that measurement of OS in combination with vascular function tests might be used in the prediction of PE.

**P2-114**

**The prenatal screening for preeclampsia in Kazakhstan**

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**Objectives:** Preeclampsia (PE) is a leading cause of maternal morbidity and mortality with an incidence 6-10% of pregnancies in Kazakhstan. PE screening in the 1st trimester of pregnancy allows to improve prenatal care and reduce adverse outcomes for mother and child. Aim of the study was to establish “Predictor PE test” for early prediction of PE, based on maternal characteristics, biophysical and biochemical markers at 11-13 weeks of pregnancy.

**Methods:** 2244 pregnant women were screened in 2013-2017 year at 11-13 weeks of gestation by using maternal demographic characteristics, medical and obstetric history with uterine artery pulsality index (PI), mean arterial pressure (MAP) and maternal serum pregnancy associated plasma protein-A (PAPP-A) and placental growth factor (PlGF) in multiple of the median (MoM) values. Maternal serum markers PLGF, PAPP-A were measured by Delfia Xpress system PerkinElmer, with risk calculation Program “Predictor PE”. Statistical analysis was performed by STATA13 Program.

**Results:** High risk group for PE among 2244 screened pregnant women was 140 (6.2%), out of them for early PE 46 (32.9%), for late PE 94 (67.1%). In PE group medians of PLGF 0.52±0.05 MoM, PAPP-A 0.68±0.05 MoM, PI was 1.29±0.05 compare with control (p<0.05). Information was available for 118 outcomes of deliveries from 140 pregnancies with high PE risk. Among them 87 (74.0%) had realized PE during pregnancy. Mean level of systolic blood pressure was 154.9±1.53 and diastolic blood pressure 99.82±0.76 mmHg. For woman with PE mean date of delivery was 37.41±0.11 weeks and median weight of newborn 3373.8±40.56 g.

**Conclusions:** A “Predictor PE” test for prenatal diagnosis of PE in the first-trimester of pregnancy demonstrated the better performance for early- rather than late-onset PE. The detection rate (DR) for early PE was 84.4%, for late-onset PE – 71.0%.
A placental clock of human pregnancy: Biomarkers and implications in the pathogenesis of preeclampsia at early gestational ages

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Objectives: The placenta plays a crucial role during pregnancy. We hypothesized that modeling the circulating placental protein abundance, in serial maternal blood collected throughout pregnancy, can be used to estimate the gestational age (GA) of the fetus. We further hypothesized that we can predict impending pregnancy complications, such as preeclampsia (PE), through a characterization of clock alteration.

Methods: Circulating placental proteins, soluble fms-like tyrosine kinase 1 (sFlt-1), chorionic somatomammotropin hormone like 1 (CSHL1), placenta growth factor (PlGF), leptin (Lep), activin A (Inhba), and elabela (Ela), were quantified in serial maternal blood collected throughout pregnancy. An elastic net (EN) algorithm was used for multivariate modeling of ELISA data to establish GA. Logarithmic transformed mean squared errors (MSE) of EN predictions, derived from longitudinal sampling of the same subject, were used for binary classifications to predict impending PE.

Results: An EN-based gestational dating model was developed (R² = 0.76) and validated (R² = 0.61) using blood from normal pregnant women. However, the EN model was weakly (R² = -0.17) associated with GA at the time of sampling in preeclampsia (PE), indicating this timed placenta clock is dysregulated in PE. Deviation was observed in MSE patterns of EN predictions from PE to normal controls (P = 0.01) at the gestation of 16-30 wks. Fig 1.

Conclusions: Our gestational dating EN model indicates that placental protein content is highly orchestrated throughout a normal pregnancy, and that the specific biomarkers may function with mechanisms independently of, and possibly earlier than the well-established biomarkers sFlt-1 and PI GF in the prediction of PE. Thus, significant random deviations from the normal “clock” can be used to predict impending PE. Our placental clock analytics may offer a new paradigm to understand the underlying placental biology and to develop innovative diagnostics for PE.
P2-115

Prenatal carrier screening acceptance rates in adopted individuals

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Objectives: Patients who are referred for prenatal genetic counseling at Integrated Genetics are routinely offered general population carrier screening for Cystic Fibrosis (CF), Spinal Muscular Atrophy (SMA) and Fragile X syndrome. The offering of these tests is based on the physician’s direction along with ACOG and ACMG guidelines. The purpose of this analysis was to determine if the acceptance rates for these same general population carrier screening tests are different in patients who identify themselves as being adopted. We limited the data collection and analysis to patients referred for prenatal or preconception comprehensive genetic counseling.

Methods: We looked at all patients referred for comprehensive genetic counseling in 2014 and 2015 and identified 643 adopted patients. When analyzing the acceptance rates for the testing, we excluded any patients for whom the testing was previously done prior to the genetic counseling session. All patients were given an informational sheet discussing the disorders for
which they were going to be offered carrier testing which included descriptions of the disorder, symptoms, inheritance pattern and carrier frequency. During the genetic counseling appointment, the patients were offered additional information about the option of carrier testing and prompted for a decision about testing.

**Results:** For each of the tests, we found that a significantly higher percentage of patients who were adopted accepted genetic testing as compared to the entire patient population. The acceptance rates for CF were 6.6% higher in the adopted patient group (27.30% compared to 20.68%) with p-value of 0.0018 by chi-square test. The acceptance rates for SMA were 6.8% higher in the adopted patient group (28.38% compared to 21.64%) with p-value of 0.0007. The acceptance rates for Fragile X were 6.8% higher in the adopted patient group (31.57% compared to 24.79%) with p-value of 0.0007.

**Conclusions:** The higher acceptance rates in adopted patients suggest this group is motivated to obtain information about genetic risk factors. While patients were not asked for their reasons for pursuing carrier testing, a lack of knowledge may lead to more anxiety about family history and increased interest in testing for inherited genetic disorders. There are currently no US recommendations regarding genetic testing for adopted patients. Given the increased acceptance rates, there may also be an increased motivation for additional genetic disorders. Expanded carrier screening may be a better offering for the adopted patient population to cover a broader spectrum of disorders.

P2-117 Table.
P2-118

**Conceptualization of type 2 collagen disorders based on their phenotypes and genetic mutations**

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**Objectives:** Type 2 collagen group disorders encompass multiple conditions with similar X-ray findings resulting from mutation in the same gene. These disorders are classified into subgroups (10 conditions in the international classification). However, each condition is extremely rare and difficult to study and treat; therefore, the concept of type 2 collagen disorders has not been established and there are no objective assessment criteria. The pathogenic mechanisms of the disorders also remain unknown. However, the detailed mutations involved in the 10 conditions have not been clarified, making diagnosis difficult.

**Methods:** We investigated the phenotypes and genetic mutations in patients with type 2 collagen disorders (including suspected cases) by genetic testing to improve the diagnosis.

**Results:** This is an interim report of a study involving 272 patients (inpatients and outpatients) with type 2 collagen disorders (including suspected cases). The patients were classified as follows: 1) achondrogenesis type 2 (n = 47); 2) platyspondylic dysplasia, Torrance type (n = 5); 3) hypochondrogenesis (n = 20); 4) spondyloepiphyseal dysplasia congenital (n = 96); 5) spondyloepimetaepiphyseal dysplasia, Strudwick type (n = 16); 6) Kniest dysplasia (n = 16); 7) spondyloepiphysial dysplasia (n = 7); 8) mild spondyloepiphysial dysplasia with premature onset arthrosis (n = 8); 9) spondyloepiphysial dysplasia with metatarsal shortening (formerly Czech dysplasia) (n = 2) etc...

**Conclusions:** We are planning to perform genetic testing in patients who volunteer to participate in our study.

P2-119

**A case of incontinentia pigmenti, an X-linked dominant disorder, resulting in miscarriage of a male fetus**

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*Unedited draft - unpublished*
Objectives: Incontinentia pigmenti is an X-linked dominant disorder resulting from a mutation in the inhibitor of kappa light polypeptide gene enhancer in B cells, kinase gamma (IKBKG) gene. This disorder typically occurs in heterozygous females and can cause various symptoms in the skin, eyes, hair, nails, and central nervous system. The condition is lethal in most hemizygous males, resulting in miscarriage. Herein, we report a case of incontinentia pigmenti resulting in miscarriage of a male fetus, which was diagnosed by genetic testing of the woman and fetus after genetic counseling.

Methods: A 28-year-old pregnant primipara was referred to the prenatal clinic of our hospital with a chief complaint of a nuchal translucency (NT) thickness of 4 mm at 13 weeks and 1 day of gestation. However, examination revealed intrauterine fetal death (IUFD). The woman was previously diagnosed with incontinentia pigmenti. During childhood, she developed retinal detachment and lost sight in her right eye. We suspected that her miscarriage was caused by incontinentia pigmenti and performed genetic testing.

Results: Chromosomal analysis of miscarriage tissue (chorionic villus sample) revealed a 46, XY karyotype. Heterozygous deletion of exons 4–10 in IKBKG (NEMO) was identified in the woman, and hemizygous deletion of the same gene was identified in chorionic villi of the fetus. Based on genetic testing, a diagnosis of incontinentia pigmenti was established in the women and fetus. Because the reason for miscarriage was identified, the woman and her husband felt positive about future pregnancy. We provided useful information to them regarding the expected rate of recurrence and how to care for their new baby (if the baby was female).

Conclusions: Typically, patients with hereditary disease know about their disease but may not fully understand its influence on pregnancy outcomes and the fetus. Appropriate management of the baby’s eyes and skin is essential after birth. Appropriate genetic counseling and health management during pregnancy are important. The woman became pregnant again after timing therapy. The second baby did not show NT thickness or developmental abnormalities until 10 weeks of gestation. However, IUFD occurred at 12 weeks of gestation. The cause of IUFD is currently under investigation using chromosomal and genetic testing. Preimplantation genetic diagnosis may be required if she has another miscarriage.

The utility of expanded carrier screening for a consanguineous couple: A case report

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Unedited draft - unpublished
Objectives: Since its inception in 2008, the use of expanded carrier screening for autosomal recessive conditions has become widespread amongst genetics and primary care clinics. Expanded carrier screening is typically marketed as a preconception tool, used to identify potential risks to future children. The value of expanded carrier screening is especially evident when used for consanguineous couples, due to the increased risk of having a child with a recessive condition. In this case, expanded carrier screening was used to retrospectively identify an underlying genetic cause of death for two children of a consanguineous couple.

Methods: We present a prenatal genetic counselling case of a G4P2A1 female related as a first cousin to her husband. Their history is significant for two infant sons that passed away prior to their immigration to Canada. Reportedly, medical records and photos of the infants were not available, and no diagnosis had been made. The couple described the infants as being “floppy” with progressive muscle weakness, seizures, and no suck reflex. One of the sons had a congenital heart defect. Despite other consanguineous relationships, the family history was otherwise unremarkable.

Results: Parental karyotypes and spinal muscular atrophy carrier testing were both normal. The couple then decided to have Counsyl’s Foresight Carrier Screen, an expanded carrier screen for 176 autosomal recessive and X-linked disorders. Testing on the female partner identified a mutation in \textit{HSD17B4}, confirming that she was a carrier of autosomal recessive D-bifunctional protein deficiency. Subsequent testing on her husband confirmed that he was also a carrier. The symptoms of this condition, including hypotonia, seizures and infant death, aligned with the infants’ reported phenotypes, and provided a very likely underlying genetic cause for their deaths.

Conclusions: This case illustrates the utility of expanded carrier screening in a consanguineous couple with a significant family history. By identifying a likely underlying diagnosis, it not only provided the couple with an explanation for their sons’ premature deaths, but also provided the opportunity for precise recurrence risk counselling and prenatal diagnosis in subsequent pregnancies. Expanded carrier screening may play an important role in preconception and prenatal genetic counselling sessions for consanguineous couples, especially for cases when a diagnosis in the family is unknown.

P2-121

Prenatal surgery and gene therapy in fetuses with Down Syndrome: Views of parents

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Objectives: New technologies for gene silencing and genetic modification, combined with new technologies for prenatal genetic screening/diagnosis, have raised the possibility that

Unedited draft - unpublished
previously irreversible genetic conditions could be mitigated or prevented during gestation. While some observers see this possibility as the fulfillment of prenatal diagnosis, others, especially within the patient advocacy community, are more ambivalent.

**Methods:** We surveyed parents of individuals with Down syndrome (DS) to gather their opinions on the acceptability of surgical and genetic interventions in utero. Participants responded to background questions about their child with DS and were then asked to react to 5 hypothetical scenarios featuring interventions to ameliorate Down syndrome. Two scenarios depicted interventions in the prenatal period; a surgical intervention to correct a bladder obstruction and a gene therapy intervention to improve cognitive function.

**Results:** 532 respondents completed the survey. A scenario depicting surgery to repair a heart defect in a newborn with DS was the most commonly supported intervention. The scenario depicting a prenatal surgery on fetal bladder outlet obstruction received significantly fewer positive responses. The least agreed-with intervention was a hypothetical prenatal intervention that would “silence” the extra chromosome causing DS with a series of injections. Open-ended comments on each scenario help illuminate the ambivalence parents feel towards interventions that they view as changing the personality of their child.

**Conclusions:** These results suggest that, while many parents may see benefits in prenatal interventions for DS, their opinions on these hypothetical options are conflicted and volatile - both as a group and individually— and that the way in which interventions, and especially potential side-effects, are presented significantly impacts theoretical uptake.

P2-122

**Incidental Findings: Two cases of APP gene duplications identified by prenatal chromosomal microarray**

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**Objectives:** Prenatal screening and diagnostic testing for fetal chromosomal aneuploidy, such as Down syndrome, have become commonplace in many jurisdictions. As technological advances now allow high-resolution genome-wide screening for genomic imbalances, there is a higher chance that variants of uncertain significance and incidental findings will be detected. We report two cases of fetal APP gene duplications detected by Chromosomal Microarray (CMA) on amniocytes, predicting a high risk for early onset Alzheimer disease later in life.

**Methods:** A detailed review of two cases was performed. Case 1: A referral was received for a 36-year-old G2P1 female. Fetal anatomy scan at 20 weeks identified a choroid plexus cyst and
mild cerebral ventriculomegaly. Family history identified early-onset Alzheimer Disease in the patient’s first and second-degree relatives. Case 2: A referral was received for a 32-year-old G2P1 female. The patient was identified as a carrier of Duchenne Muscular Dystrophy (DMD), and a Robertsonian translocation. Family history was otherwise negative.

**Results:** Case 1: Non-Invasive Prenatal Testing (NIPT) was ordered and returned positive for Trisomy 21. A call from the laboratory director clarified that Trisomy 21 was unlikely, but the results suggested a small chromosome 21 duplication including the \textit{APP} gene. QF-PCR from amniocentesis revealed normal chromosome 21 results, and CMA confirmed a pathogenic \textit{APP} duplication in the fetus. Parental testing was declined. Case 2: The patient underwent amniocentesis; testing included CMA and molecular analysis for DMD. A pathogenic \textit{APP} duplication was identified in the fetus. Parental testing was declined.

**Conclusions:** These unexpected findings illustrate the importance of informed consent for complex genetic screening in pregnancy. Genetics clinics may need to consider the use of a consent form outlining the potential for incidental findings and variants of unknown significance. Incidental findings complicate the genetic counselling session and may increase anxiety for patients involved. In addition to concerns regarding the pregnancy, patients are now faced with the possibility that prenatal testing may predict future health concerns for themselves and other family members.

P2-123

**The ABCs of LGBTQI2S**

Ronni Teitelbaum, Narina Nagra

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**Objectives:** Have you ever wondered what is the difference between sex, gender and sexual orientation? Do you know what LGBTQI2S stands for? To meet the needs of our patients, it is important to educate ourselves about the communities we serve. As health-care workers, we must strive to provide equitable healthcare for our patients, and we must recognize that each patient is unique with different needs.

**Methods:** I hope to provide an interactive oral presentation on this topic.

**Results:** By the end of the talk, you will have an increased understanding of sexual orientation and gender identity terminology. You will be aware of barriers faced by LGBTI2S patients in a healthcare setting. I will share tips on providing optimum care to LGBTI2S patients, specifically in a prenatal diagnosis setting (how to draw inclusive pedigrees and laboratory considerations). I will also discuss how to create an inclusive workplace environment for staff, patients and visitors.

*Unedited draft - unpublished*
Conclusions: I look forward to sharing my knowledge of this topic at ISPD.

http://info2/committees/sites/aha-committee/welcome/

http://www.moleculargenetics.utoronto.ca/genetic-counselling-fac/2014/10/7/ronni-teitelbaum

P2-123 Image.

High serum FSH is associated with brown oocyte formation and a lower pregnancy rate in human IVF practice

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Objectives: To investigate whether brown zona pellucida (ZP) of oocytes affects the outcome of fertilization, embryo quality and pregnancy rate in in vitro fertilization-embryo transfer (IVF-ET).

Methods: Based on the ZP color of their oocytes, a total number of 703 patients dated from 2012 to 2014 were divided into a normal egg group (group A) and a brown oocyte group (group B), with 629 and 74 cases, respectively. Clinical characteristics, gonadotropin (Gn) days, Gn
dosage, serum hormone levels on the day of human chorionic gonadotropin (HCG) injection, ZP thickness (ZPT) of the eggs, fertilization rate, rescue intracytoplasmic sperm injection (rICSI) rate, good-quality embryo rate and pregnancy rate were compared between the two groups.

**Results:** No significant differences were found in the level of serum hormone including E2, P and LH on the day of HCG injection. Moreover, there were no differences in number of mature oocytes, oocyte fertilization rates and rICSI rates after IVF. However, we observed that the ZPT of brown oocytes was higher than that of normal oocytes. The Gn dosage and FSH levels on the day of HCG injection were significantly higher in brown oocytes group than in normal oocytes group and the good-quality embryo rate and pregnancy rate were lower.

**Conclusions:** Compared with normal eggs, oocytes with a brown ZP were found to have a higher ZPT, lower embryo quality and lower pregnancy rate, which might be due to a high Gn dosage injection and high serum FSH levels during IVT-ET cycles.

P2-125

**Neonatal symptoms, neuroimaging, hearing deficit and severe neurodevelopmental sequelae according to prenatal ultrasound findings in children with congenital CMV infection**

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**Objectives:** To assess the outcome of congenital CMV infection (cCMV) according to prenatal ultrasound results in a setting without universal screening.

**Methods:** Retrospective cohort study of 69 mother-child pairs referred to CHU Sainte-Justine (Quebec, Canada), for cCMV from 2003 through 2017. Positive (VPP) and negative predictive values (NPV) of prenatal ultrasound findings were calculated for mild and moderate to severe neonatal symptoms, neonatal neuroimaging anomalies, hearing deficit and severe neurodevelopmental sequelae (bilateral hearing deficit and/or cerebral palsy with or without mental retardation).

**Results:** Among 30 infants with prenatal ultrasound anomalies, 27 had neonatal symptoms, 10 had neonatal uni/bilateral hearing deficit, and 16 neuroimaging anomalies, resulting in 3 neonatal deaths, and 11 cases of severe neurodevelopmental sequelae. Among 39 cases with normal prenatal ultrasound, 11 had neonatal hearing deficit, and 7 developed severe neurodevelopmental sequelae. Prenatal ultrasound anomalies had a PPV of 33.3%, 53.3%, and 40.7% for neonatal hearing deficit, abnormal neuroimaging, and severe neurodevelopmental
sequelae, respectively. When maternal infection had been diagnosed during pregnancy, the absence of prenatal anomalies had a NPV of 100% for severe neurodevelopmental sequelae.

**Conclusions:** Our data shows that any ultrasound anomaly at the time of cCMV diagnosis carries a high risk of severe neurodevelopmental sequelae. Knowing that the fetus is at risk of cCMV seems to improve the accuracy of prenatal ultrasound.

**P2-126**

**Congenitally infected CMV fetuses following first trimester maternal infection: Neonatal and short-term outcome after late vertical transmission**

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²Department of Fetal Medicine, Leuven, Belgium  
³Department of Fetal Medicine, University Hospitals Leuven, Kortrijk, Belgium  
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**Objectives:** To document the course of neonatal and short-term outcome in infants of patients with first trimester primary CMV seroconversion and subsequent amniocentesis. The primary goal was to assess the residual risk of adverse outcome in pregnancies with negative amniocentesis for CMV.

**Methods:** We conducted a retrospective cohort study on all primary CMV seroconversions discovered in pregnancy from November 2006 to July 2015. Pregnancies were included in cases of seroconversion before 14 weeks of gestation and if subsequent amniocentesis for PCR CMV occurred after 21 weeks of gestation. To retrieve the neonatal and short-term infant outcome, a questionnaire was sent to the patients and the referring physicians. Primary focus was on the auditive, visual, neuromotor and cognitive impairment. The study was approved by the ethical board.

**Results:** The study group included 198 pregnancies. In 44 cases amniocentesis revealed a positive PCR for CMV (early infected group), in 7 cases amniocentesis was negative and neonatal CMV screening appeared positive (late infected group) and 147 children were not CMV infected (control group). CMV infected children, both early and late infected, appeared significantly more frequently symptomatic at birth. In terms of short-term outcome, the prevalence of hearing impairment (12.2%), visual impairment (14.6%), motor deficit (24.4%) and behavioral problems (7.3%) was significantly higher in the early infected group. No late CMV infected children showed short-term symptoms.

**Conclusions:** Late CMV infected children present mild clinical symptoms at birth, such as jaundice and mild petechiae. From our results, we conclude that late infected children show significantly less sequelae, although mild audiological, visual and neurodevelopmental sequelae
are described in the literature. When amniocentesis after maternal primary CMV infection appears negative, mothers can be reassured, but correct counseling and intensive neurosonographic follow-up remains important.

P2-127

The outcome of monochorionic diamniotic twins after assisted conception

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Objectives: We aimed to examine if mode of conception is related to pregnancy outcome in monochorionic diamniotic twin pregnancies.

Methods: Retrospective cohort of monochorionic diamniotic twins included in the first trimester between January 2002 and January 2016. Pregnancies were classified as assisted (defined as ovulation induction, in vitro fertilisation or intracytoplasmic sperm injection) or spontaneous conception. The following outcomes were studied: twin-twin transfusion syndrome, gestational age at delivery, mode of delivery, birthweight, birthweight discordance, neonatal intensive care admission, death of one or both twins (defined as fetal or neonatal loss) and overall survival. Categorical outcomes were compared using Chi square analysis. Continuous variables were evaluated using the Student t-test. Multivariate analysis was performed to adjust for potential confounders.

Results: 558 pregnancies were analysed. The assisted conception group was older (31.5y versus 29.9y; p < 0.001) and more often nulliparous (69% versus 44%; p < 0.001). They delivered earlier (32.6 weeks versus 33.9 weeks; p = 0.007). Death of one or both twins was more frequent (25% versus 13%; p = 0.003), resulting in a lower overall survival (80% versus 91%; p < 0.001). Multivariate analysis assessing mode of conception, nulliparity and maternal age as predictors for death of one or both twins, showed that only assisted conception was an independent predictor (OR 2.2; 95% CI 1.2 – 4.0)

Conclusions: Monochorionic twin pregnancies after assisted conception deliver earlier compared to spontaneously conceived twins and have a lower overall survival rate. The reason for the latter appears to be a higher risk of intrauterine fetal demise, although the cause hereof remains to be elucidated.

P2-128

Unedited draft - unpublished
The investigation in the expression of NRF2 in the placenta shares of sIUGR pregnancies

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Objectives: To investigate NRF2 levels in placental shares of the twins in normal MCDA and sIUGR pregnancies, and to discuss the relationship of NRF2 expression and the pathogenesis of sIUGR.

Methods: Twenty-nine pairs of placentas, from 19 sIUGR (enrolled in sIUGR group) and 10 normal MCDA (enrolled in normal group) twin pregnancies, were collected in this research. NRF2 mRNA and protein levels were detected in placentas of normal and sIUGR groups.

Results: Under the qRT-PCR and Western Blot, we found no significant difference of NRF2 mRNA and protein between the twins in the normal group (P>0.05), while in sIUGR group, the levels of NRF2 mRNA and protein were higher in placental shares of the smaller fetus than larger fetus (P<0.05).

Conclusions: NRF2 was up-regulated in placental shares of the smaller fetus in sIUGR pregnancies, suggesting the activation of antioxidant system in the smaller fetus, which may be involved with the pathogenesis of sIUGR.

P2-129

Limitations of combined first trimester screening in multiple gestations. A report a case of Trisomy 18

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Objectives: In recent decades, there has been an increase in multiple gestations, due to the increase in Assisted Reproduction Techniques (ART). Currently twin pregnancies account for 3% of the total, of these, 2.2% are bicigóticas. In addition, advanced maternal age motivates the use of ART, which causes an increased risk of developing affected pregnancies. Edwards´ syndrome (Trisomy 18), included in the Combined First Trimester Screening (CFTS) programs, is the second most frequent autosomal trisomy, with an incidence that varies between 1/4,000
and 1/8,000 pregnancies. In twin pregnancies, the CFTS has a lower detection rate (75-80%) compared to simple gestations (85-90%).

**Methods**: The determination of the biochemical markers (PAPP-A and β-free HCG) was performed on the Cobas 6000-E 601 analyzer (Roche Diagnostics) and the calculation of risk using the corporate software siPACAC. The genetic diagnosis (conventional cariotipe) was carried out in external laboratories.

**Results**: Pregnancy with 41 years who consults for CFTS. The ultrasound of week 12 establishes that it is a bicorial twin gestation, with a nuchal translucency (NT) for the first fetus of 1.3mm, with multiple of the Median (MoM) of 1.04 and in the second fetus a NT of 1.5mm with MoM of 1.2. The analysis performed with the gestational age corrected resulted in a corrected MoM of PAPP-A of 1.15 and for the BHCG of 0.71. The combined risk was 1/518 for Trisomy 21 and 1/21880 for Trisomy 18, both negative. In morphological ultrasound week 20, we observed a female twin with no anomalies and male twin with malformations.

**Conclusions**: In spite of the extensive experience in the use of CFTS, the appearance of a bicorial twin gestation with affectation of a single fetus by Trisomy 18 is unusual. The management of CFTS in multiple pregnancies presents points of controversy since in the ultrasound of week 12 it is not possible to establish the zygosity, resorting to echographic signs to identify the chorionicity, assuming that the bicorial gestations are bicigotic. Due to the low prevalence of these aneuploidies, is necessary a careful interpretation of the risk reported in multiple gestations, especially in the bicorials.

**P2-131**

**Analysis of pregnancy outcome of monochorionic diamniotic (MD) twins ineligible for fetoscopic laser photocagulation of placental communicating vessels (FLP)**

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**Objectives**: FLP is effective for twin to twin transfusion syndrome (TTTS). In MD twins, however, poor pregnancy outcomes are also caused by similar situations such as twin anemia-polycythemia sequence (TAPS), twin amniotic fluid discordance (TAFD), and selective fetal growth restriction (sFGR), which are not eligible for FLP. We traced medical records and find the consequences of MD twins, elucidate the periods of TTTS occurrence and, tried to draw some suggestions to expand FLP indications.

**Methods**: Forty-three MD pregnancies from 2013 through 2017 at our hospital were analyzed retrospectively based on the medical records. Informed consent for data analysis were
obtained as Japan Society of Obstetrics and Gynecology registry protocols. 25 MD twins of normal amnion without FGR were assigned as control group; CG, and 18 cases of suspected of TTTS, TAPS, TAFD, and/or sFGR were assigned as suspected group; SG. The mean Apgar scores (MAP) of the first twin at 1min and at 5 min were analyzed. Statistical analysis was performed on R language.

Results: There were 25 MD twins CG), and 18 cases of SG. The MAP of the first twin at 1min were 7.7 vs 6.4 (CG vs SG) (p<0.01) and at 5min 8.8 vs 8.1 (CG vs SG) (not significant). The MAP of the second twin at 1min were 7.9 vs 6.7 (CG vs SG) (p<0.01) and 9.1 vs 8.4 (CG vs SG) (p<0.01). There were four cases of TTTS, one occurred at 28 weeks of gestation, one at 30 weeks deteriorated from sFGR, two at 31 weeks deteriorated from sFGR. All of them yielded low APSs without later neurological complications.

Conclusions: The fact that SG group showed lower APS without later neurological complications might suggests the current management at our center seemed effective to prevent poor outcomes. Some TTTS occurred after the current deadline of FLP indications, or 26 weeks. Some MD twins with particular findings such as TAPS or some MD twins with worsening temporal change might be indicated for FLP. Study to expand FLP indication is expected and closer management is essential in near term pregnancies.

P2-132

Cervical length in the second trimester for screening for risk of spontaneous preterm birth in uncomplicated twin pregnancies

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Objectives: The Guideline of Ultrasound in Twin Pregnancies of the International Society of Ultrasound in Obstetrics and Gynecology (2016) recommends screening for preterm birth in uncomplicated twins with one cervical length (CL) measurement around 20 weeks, and states that 25mm is the cut-off most commonly used. Our aim is to evaluate the performance of the CL around 20 weeks as a predictor of preterm birth (PTB) before 34 weeks.

Methods: Cohort study of uncomplicated twins followed at the Fetal Medicine Unit of the Hospital Italiano de Buenos Aires between 2013-2017, with a transvaginal CL measurement between 18-22 weeks. Short cervix was defined as a CL less than 25 mm and PTB as spontaneous onset of labor and subsequent delivery before 34 weeks. Exclusion criteria included iatrogenic delivery before 34 weeks, IUGR, fetal death, fetal anomaly, polihydranmios, twin-twin transfusion syndrome, twin anemia-polycythemia sequence, twin-reversed arterial perfusion sequence and monoamniotic twins. We reported the sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio and negative likelihood ratio.
Results: We included 226 patients, 169 (75%) were dichorionic and 57 (25%) monochorionic. There were 21/226 (9.3%) PTB (range of gestational age: 26+1-33+6 weeks). One of those 21 PTB (4.7%) had a short cervix (21 mm) and delivered at 26+1 weeks. Among the patients that delivered after 34 weeks, 2/205 (1%) had a short cervix. The sensitivity was 4.8% (95% CI 0.1-23.8%), the specificity 99% (95% CI 96.5-99.9%), the NPV 91% (95% CI 86.5-94.4%), the PPV 33.3% (95% CI 0.8-90.6%), the positive likelihood ratio 4.88 (95% CI 0.46-51.5) and the negative likelihood ratio 0.96 (95% CI 0.87-1.06).

Conclusions: In our series, one CL measurement around 20 weeks in uncomplicated twin pregnancies was a poor predictor for PTB. Further prospective studies are required to determine the best performance of CL in twins, including the best cut-off, the gestational age, and one vs. serial measurements

P2-133

Serial cervical length measurements for screening for spontaneous preterm birth in uncomplicated twin pregnancies

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Objectives: There is great heterogeneity in the guidelines of multiple pregnancies regarding screening for spontaneous preterm birth in uncomplicated twins with cervical length (CL). The RCOG does not include it, ISUOG recommends one measurement around 20 weeks, and the Argentinean Guideline of management of twins includes serial measurements every 4 weeks. Our aim is to evaluate the performance of serial CL measurements as a predictor of preterm birth (PTB) before 34 weeks.

Methods: Cohort study of uncomplicated twins followed at the Fetal Medicine Unit of the Hospital Italiano de Buenos Aires between 2013-2017, with CL measurements every 4 weeks between 18 to 33 weeks or delivery. Short cervix was defined as a CL less than 25mm, and PTB as spontaneous onset of labor and subsequent delivery before 34 weeks. Exclusion criteria included iatrogenic delivery before 34 weeks, IUGR, fetal death, fetal anomaly, polihydramnios, TTTS, TAPS, twin-reversed arterial perfusion sequence and monoamnionicity. We reported the sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio and negative likelihood ratio.

Results: We included 253 patients, 188 (74%) were dichorionic and 65 (26%) monochorionic. We observed short cervix in 14/23 patients with PTB and in 44/230 patients that delivered after 34 weeks. The sensitivity was 60.9% (95% CI 38.5-80.3%), the specificity 80.9% (95% CI 75-85.7%), the NPV 95.4% (95% CI 91.4-97.9%), the PPV 24.1% (95% CI 13.9-37.2%), the positive likelihood ratio 3.18 (95% CI 2.09-4.85) and the negative likelihood ratio 0.48 (95% CI 0.29-0.81).
Conclusions: The detection of a short cervix in at least one of the follow-up visits in uncomplicated twin pregnancies increased 3 times the risk of PTB and identified more than half of the patients that delivered before 34 weeks. Further prospective studies are required to determine its role in clinical practice.

P2-134

Perinatal complications: A comparison of dichorionic twins and monochorionic twins

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Objectives: To evaluate the incidences of the perinatal complications between dichorionic twins (DCT) and monochorionic twins (MCT).

Methods: This was a retrospective study of women with twin pregnancies in the Guangdong Women and Children Hospital from April 2013 to December 2015. All pregnancies were classified into two groups according to chorionicity. This study included 950 twin pregnancies (752 were DCT and 198 were MCT). The incidences of the perinatal complications in different groups were compared.

Results: Women with MCT were slightly younger (P < 0.01), less likely to use ART (P < 0.01), and less likely to require caesarean section (P < 0.01) than women with DCT. Moreover, the median gestational age was earlier in the MCT group than in the DCT group. The incidences of gestational diabetes mellitus, gestational hypertension disorders, intrahepatic cholestasis of pregnancy, preterm premature rupture of membranes, hyperthyroidism/hypothyroidism and anemia had no statistical differences ((P > 0.05), while the incidences of placenta previa was higher in the DCT group than in the MCT group (P< 0.05).

Conclusions: Perinatal complications between dichorionic twins and monochorionic twins have no significant differences except placenta previa. Dichorionic twins pregnancy is associated with increased risk of placenta previa.

P2-135

Predicting fetal gender of twins using non-invasive prenatal screening of cell-free DNA

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Unedited draft - unpublished


**Objectives:** Cell-free DNA (cfDNA) testing can determine the fetal gender in a singleton pregnancy. However, the complexity increases when a twin pregnancy occurs. Currently it is difficult to predict the gender of both individuals in dizygotic twins. In the current study, we investigated whether a laboratory-developed NIPT analysis method can be applied for the accurate gender determination in twin pregnancies.

**Methods:** A retrospective analysis was performed at the Centre for Human Genetics (KU Leuven, Leuven, Belgium) for all NIPT samples of twin pregnancies collected in 2016 and 2017. These samples were screened for the presence of fetal trisomy 21, 18 and 13. Library preparation, whole genome sequencing as well as genome-wide genomic representation profiling was performed as previously described (Bayindir et al., 2015; Brison et al., 2017). Data from 68 twin pregnancies for whom the sex is known was used for verification and validation of gender assessment of the twins.

**Results:** Of 68 twin pregnancies, primary NIPT generated data permitted us to study the amount of X chromosome and Y chromosome reads in function of the fetal cfDNA. Plotting the normalized frequency of X chromosome and Y chromosome reads allowed us to distinguish the gender of each individual in 67 twin pregnancies.

**Conclusions:** Despite that the contribution from each twin to fetal cfDNA fraction can differ by as much as two-fold, comparing the normalized frequency of both X and Y chromosome reads in function to the fetal cfDNA fraction, allowed us to determine the gender of each individual in 67 twin pregnancies. Accordingly, the current in-house employed analysis pipeline used for the detection of autosomal aneuploidies can be utilized to determine the gender in twin pregnancies.

P2-136-LB

**Can non-invasive prenatal testing (NIPT) be helpful in pregnancies with ultrasound anomalies?**

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**Objectives:** Originally, non-invasive prenatal testing (NIPT) was only reserved for cases interested in specific chromosomal abnormalities. Recently, the scope of testing has increased to include selected subchromosomal deletions-duplications as well as pathogenic rearrangements over 6 Mb. NIPT is classically prohibited in case of abnormal fetal ultrasound.

*Unedited draft - unpublished*
Since 2013 we have carried 8767 NIPT including 420 performed in an abnormal fetal scan situation. Our study aims to evaluate the application, after thorough genetic counseling, of genome-wide NIPT as an alternative to invasive prenatal testing in pregnancies with abnormal ultrasound findings.

Methods: Retrospective study analysis of 420 singleton and multiple pregnancies presenting abnormal signs at sonographic examination. NIPT was performed by massively parallel sequencing of cell-free DNA in maternal plasma, allowing genome-wide detection of whole-chromosome, as well as partial autosomal aneuploidy.

Results: NIPT was indicated by the presence of increased nuchal translucency (INT) < 3.5 mm or > 3.5 mm (n = 127; n = 7), absence of nasal bone (n = 33), soft markers (n = 136), IUGR (n = 40), long-bone shortness (n = 35), multiple congenital anomalies (n = 4), other isolated anomalies (n = 60). We found 15 positive NIPT: 8 Down Syndrome [INT (5), shortness of long-bones (2), single umbilical artery (1)]; trisomy 7 (1); trisomy 15 (1); trisomy 18 (1); Jacobsen Syndrome (1) [IUGR]; Di George Syndrome [hexadactyly, IUGR]; del2p25.1p23.3 (1) [INT]; tetrasomy 22q (1) [cardiac defect]. All anomalies were confirmed by invasive procedure except one. No false-negative was reported.

Conclusions: After thorough genetic counseling, genome-wide NIPT can be an interesting alternative to invasive testing in cases of fetal malformation because our study reports that it avoided invasive procedure in more than 95% of cases with abnormal fetal ultrasound findings.

P2-137-LB

Expedition of whole exome sequencing for diagnosis of prenatal phenotypes

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Objectives: i) Development, refinement and implementation of a rapid whole exome sequencing workflow from DNA to report for the diagnosis of fetal abnormalities detected on ultrasound; ii) Expansion of fetal phenotype in a single centre.

Methods: DNA (CVS, amniotic fluid, fetal blood or tissue, parental gDNA) was enriched using the Agilent Sureselect CREv2 and sequenced using Illumina NextSeq 500. Maternal cell contamination exclusion was performed where relevant. HPO terms and pedigree information were loaded into the NextSeq run folder at initiation of the sequencing run. Data from completed runs was automatically collected from the instrument daily, processed using DRAGEN BIO-IT processor and Sapientia™. Gene panels and variant filter settings were

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preconfigured in Sapientia™ to further expedite analysis. Analysis was stratified by candidate genes, phenotype relevant panels and DDG2P with Exomiser prioritisation as relevant for each case.

**Results:** To date 14 cases have been tested; 10 trios, 3 singletons and 1 NICU singleton (known antenatally). A molecular diagnosis was made in 5/14 (36%), including mutations in CHD7, PIEZO1, PTPN11, BICD2 and FGFR2. Using our workflow, DNA to primary result has currently been reduced to 7 working days. In three cases, the molecular diagnosis was not the primary suspected clinical diagnosis (CHD7, BICD2, FGFR2). The causative mutation was identified using DDG2P gene panel and Exomiser variant prioritisation in 3 cases (PIEZO1, CHD7, FGFR2) and phenotype specific panel in two (PTPN11, BICD2).

**Conclusions:** TAT for whole exome sequencing for prenatal diagnosis can be reduced using streamlined laboratory and data processing protocols. As has been previously described, the prenatal phenotype of many disorders is not well known; stratifying analysis on a per patient basis, using candidate genes, followed by extension to phenotype relevant panels and prioritisation by phenotype is highlighted in this setting. We are validating a parallel SNP barcoding method to help obviate the need for Sanger confirmation which adds significant time to that reported here. We continue to identify areas for improvement to facilitate translation to routine diagnostic use.

P2-138-LB

**Analysis of noninvasive prenatal testing (NIPT) outcomes in Down syndrome in all prenatal diagnosis centers in Beijing, China**

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**Objectives:** To understand the effect of analysis of noninvasive prenatal testing (NIPT) in Beijing.

**Methods:** Descriptive statistics method was used to analyze the noninvasive prenatal testing of pregnant women in all prenatal diagnostic centers in Beijing in 2016. Data of all women received NIPT in 6 prenatal diagnostic centers were abstracted in this study.

**Results:** In 2016, 15,699 women received noninvasive prenatal testing in 6 prenatal diagnostic centers in Beijing, for Down’s syndrome 59 cases were positive in NIPT and 55 cases were diagnosed by amniocentesis, positive predictive value is 93.22%. For the indications of NIPT, 15699 cases (23.83%) is serological screening borderline risk or interventional surgical contraindications, the positive predictive value is 100% in this population. 11945 cases (76.09%) is advanced maternal age, high risk of serological screening or no clinical indications, the
positive predictive value is 95.96% in this population. 380 cases (2.42%) is fetal structural abnormality or soft index in ultrasound test,

**Conclusions:** NIPT has become one of the most important prenatal screening methods for Down’s syndrome, under current clinical practice circumstance, obstetricians should improve their knowledge of clinical indications and importance of genetic counseling before and after testing should be emphasized to improve screening quality.

P2-139-LB

**Association of adverse pregnancy outcomes with magnesium transporter genes expression in the first trimester**

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**Objectives:** Magnesium (Mg^{2+}) is involved in the regulation of numerous biochemical and physiological functions during pregnancy for fetal development. However, most of the pregnant women are in a status of magnesium deficiency due to inadequate dietary intake. In order to maintain the various functions of Mg^{2+}, both extracellular and intracellular Mg^{2+} concentrations are regulated by complex control mechanisms. Magnesium deficiency may still occur in pregnant women despite normal plasma concentration, because intracellular levels may decrease to maintain the extracellular concentration. The aim of this study is to determine whether or not magnesium transporter genes expression is associated with adverse pregnancy outcomes.

**Methods:** From November 2015 to April 2018, we completed the magnesium transporter gene expression analysis in 181 pregnant women. In order to establish a platform to determine Mg^2+ status and the expression levels of Mg^2+ transporter genes from maternal blood, total RNA is extracted from blood samples and reverse transcribed to cDNA. Expression levels of five Mg^2+ transporter genes, SLC41A1, CNNM2, MagT1, TRPM6, and TRPM7, are determined by quantitative real-time PCR. Relationships between different variables were evaluated using Spearman’s test. Gene expression levels are determined for relative quantification with non-pregnant women as control. Differences between groups were evaluated using the Mann–Whitney U test.

**Results:** Our data showed that the expressions of SLC41A1, CNNM2, and TRPM7 in the first trimester are significantly decreased, and that the expression of TRPM6 is significantly increased (p< 0.05). We observed that the gene expression level of MagT1 in the first trimester is negatively correlated with both systolic and diastolic blood pressure at the time of delivery. In addition, both the genes expression of MagT1 and TRPM7 is negatively correlated with the 1-hour blood glucose level (p< 0.05). Furthermore, we demonstrated that MagT1 and TRPM7
genes expression in the first trimester is significantly decreased in pregnancies complicated with PIH or preeclampsia.

Conclusions: We demonstrated that magnesium transporter genes expression is indeed associated with adverse pregnancy outcomes. Whether or not magnesium transporter genes expression in the first trimester can be used as a valuable marker to predict pregnancy associated hypertension disorders or GDM is an interesting field to be explored in the future.