Objectives: Several small studies have shown that exome sequencing, whole exome (WES) or using a clinically focused panel, increases the diagnostic yield in structurally abnormal fetuses with a normal microarray result. Series using a multidisciplinary team, including clinical geneticists, to identify fetuses with a high likelihood of a genetic aetiology, report diagnostic rates of up to 80%. In the PAGE study we are prospectively sequencing fetus-parent trios in unselected pregnancies complicated by any fetal abnormality to determine the diagnostic yield in different categories of clinical findings. Recruitment ends in March 2018 and we will aim to report the findings to date.

Methods: Parents choosing invasive testing following sonographic detection of any unexpected fetal abnormality and normal chromosomal analysis were consented for WES of excess fetal sample and parental DNA. Expert genetic or fetal medicine review was not performed. Anomalies are classified by system (Table) and, except for increased nuchal translucency, exclude minor sonographic markers. Trio WES is conducted after pregnancy and potential variants identified by analysis of targeted genes. Pathogenicity is assigned after consideration of fetal phenotypes by our clinical review panel comprising scientists, geneticists and fetal medicine specialists. Causative pathogenic variants are validated. Results explaining the phenotype are reported to parents.

Results: Currently we have recruited 1318 families, of whom 783 with normal chromosomal analysis were eligible. Sequencing is ongoing with results available in 506 (Table). Overall a diagnostic genetic abnormality was identified in 41/506 cases (8.1%) and a further 15 (3%) had
a variant of uncertain significance (VUS) with potential clinical utility. Highest diagnostic yields were found in fetuses with multisystem (16%), cardiac (13.6%), and skeletal anomalies (12.5%). Diagnostic variants were only identified in two (2.1%) fetuses with isolated increased nuchal translucency (>4.0 mm).

**Conclusions:** The PAGE study be will complete this spring, and results to date are showing that WES with variant calling using a focused clinical panel facilitates genetic diagnosis in abnormal fetuses. The overall detection rate of 8.1% in this unselected cohort is lower than that reported by previous smaller-scale studies of cases sequenced after genetic review. Trio sequencing is likely to be needed to deliver results in a timely fashion within pregnancy. Currently this remains expensive and our large study is enabling identification of the clinical subgroups most likely to benefit from WES, thereby aiding cost-effective implementation into routine clinical practice.

T-1 Table.

<table>
<thead>
<tr>
<th>System</th>
<th>Total</th>
<th>Diagnosed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal</td>
<td>48</td>
<td>6 (12.5%)</td>
</tr>
<tr>
<td>Cardiac</td>
<td>66</td>
<td>9 (13.6%)</td>
</tr>
<tr>
<td>Spinal</td>
<td>10</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Facial</td>
<td>28</td>
<td>1 (3.5%)</td>
</tr>
<tr>
<td>NT&gt;4.0mm</td>
<td>93</td>
<td>2 (2.1%)</td>
</tr>
<tr>
<td>Abdominal</td>
<td>39</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td>Hydrops</td>
<td>25</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Brain</td>
<td>53</td>
<td>1 (1.9%)</td>
</tr>
<tr>
<td>Renal</td>
<td>14</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Chest</td>
<td>18</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Multisystem</td>
<td>112</td>
<td>18 (16%)</td>
</tr>
<tr>
<td><strong>TOTAL:</strong></td>
<td>506</td>
<td>41 (8.1%)</td>
</tr>
</tbody>
</table>

T-2

**The impact of additional anomalies on head growth in fetuses with congenital heart defects**

Amber van Nisselrooij\(^1\), Fenna AR Jansen\(^2\), Nan van Geloven\(^1\), Ingeborg Linskens\(^3\), Eva Pajkrt\(^4\), Sally A.B. Clur\(^4\), Jarda Hruda\(^3\), Jan van Lith\(^1\), Nico A. Blom\(^1\), Monique C Haak\(^5\)

\(^1\)Leiden University Medical Center, Leiden, Netherlands
\(^2\)Leiden University Medical Center, Department of Obstetrics and Fetal Medicine, Leiden, Netherlands
\(^3\)VU University Medical Center, Amsterdam, Netherlands
\(^4\)Academic Medical Center, Amsterdam, Netherlands

*Unedited draft - unpublished*
Objectives: Neurodevelopmental delay is frequently encountered in children with congenital heart defects (CHD). Recent cohorts have shown lower head circumference (HC) z-scores in isolated CHD, irrespective of type of CHD, altered cerebral flow or brain oxygenation. This study compares HC in non-isolated and isolated cases with CHD to evaluate the effect of additional pathology on head size.

Methods: All prenatally diagnosed CHD cases were selected from our previously described regional CAHAL database (2002-present). Cases with multiple pregnancy, maternal diabetes, severe structural brain anomalies and functional CHD were excluded. If additional pathology was present, cases were allocated to the non-isolated group and according to the type of pathology assigned to one of the three subgroups: genetic diagnosis, extra-cardiac structural malformation or maternal pathology/placental insufficiency. We used mixed-linear regression model to compare HC z-scores between isolated and non-isolated subjects based on the mean in- or decrease over time and HC at 20 and 36 weeks gestational age (GA).

Results: We included 916 prenatally diagnosed CHD cases, of which 379 (41.4%) were non-isolated. Non-isolated cases had significantly lower HC z-scores at 20- (z = –1.03 vs -0.35; p<0.001) and 36-weeks GA (z= –1.51 / –0.60; p<0.001) compared to isolated cases. The placental pathology group (N=37) showed the fastest the decrease in the mean HC z-score throughout pregnancy (z= –2.02 / –3.12; p=0.004), as head growth decreased with an estimated –0.06 (SD per week). Abdominal circumference (AC) z-scores were also significantly lower in non-isolated fetuses.

Conclusions: The significantly smaller HC observed amongst CHD cases appears to be strongly associated with additional pathology, in particular, factors causing placental insufficiency. We hypothesize that developmental pathways involved in both cardiogenesis as overall growth, combined with other perinatal factors, determine the risk of neurodevelopmental delay (ND).

Evaluating individual laboratory performance for cffDNA NIPT for aneuploidies: Report of the first international external quality assessment

Zandra Deans¹, Farrah Khawaja¹, Ros Hastings², Katrina Rack², Simon Patton³, Weronika Gutowska-Ding³, Lucy Jenkins⁴, Stephanie Allen⁵, Lyn Chitty⁶, Erik Sistermans⁷

¹UK NEQAS for Molecular Genetics, Edinburgh, United Kingdom
²CEQAS, Oxford, United Kingdom
³EMQN, Manchester, United Kingdom
⁴NE Thames Regional Genetics Laboratory, London, United Kingdom
⁵Birmingham Women’s NHS Foundation Trust, Birmingham, United Kingdom

Unedited draft - unpublished
Objectives: To deliver and maintain high standards for laboratory testing independent external quality assessment (EQA) is required. This provides important information for clinicians, laboratories and patients, demonstrating that accurate testing is being performed and reported. Providing an EQA for cell free fetal DNA (cffDNA) testing is challenging as sample acquisition (availability and scalability) is a limiting factor. We have previously reported the EQA to assess standards of reporting non-invasive prenatal testing (NIPT) for fetal aneuploidy. Here we describe the delivery and results of a large international pilot EQA for laboratory NIPT for aneuploidy using maternal plasma samples.

Methods: Eighty-six maternal plasma samples (10ml – 16ml) from pregnancies with known outcomes (low risk/high risk for trisomy 13, 18 or 21) were obtained from the RAPID sample bank. All were double spun within 8 hours of blood draw, aliquotted and stored at -80oC. Three EQA providers (CEQAS, EMQN, and UK NEQAS for Molecular Genetics) delivered the pilot assessing the standard of testing and reporting of NIPT of maternal plasma for fetal aneuploidy. Maternal plasma (4ml) with clinical scenarios were distributed to participating laboratories to perform NIPT and submit reports. These submissions assessed and feedback provided for genotyping, interpretation and clerical accuracy.

Results: Ninety-five laboratories from 30 different countries participated. The use of maternal plasma allowed any testing methods to be applied. Case 1 was a referral following routine antenatal screening with a low risk for trisomy 13, 18 and 21. Case 2 was a confirmatory test for a high risk trisomy 21 result. Two critical errors were reported, one false positive for case 1 and an incorrect high risk trisomy 18 for case 2. Reports lacked details of methods performed and associated limitations, and many embedded important information within the body of the text making it difficult to identify key clinical recommendations.

Conclusions: The growing international demand for participation demonstrates the clinical need for an independent evaluation of NIPT practice. This pilot EQA has demonstrated that the use of real maternal samples distributed at ambient temperature has enabled global participation with very low sample failure rate (2%). The genotyping accuracy was good but review of the large number of reports submitted highlighted the need for further standardisation and guidance on NIPT reporting.
Simultaneous detection of fetal chromosome aneuploidy and monogenic diseases by a novel noninvasive prenatal testing method: Targeted And Genome-wide simultaneous sequencing (TAGs-seq)

Lin Yang¹, Jia Zhao², Haiping Zhang¹, Zhu Zhu¹, Yicong Wang³, Fang Chen⁴, Yanping Lu⁵, Ya Gao¹

¹BGI-Shenzhen, Shenzhen, China
²BGI Education Center, University of Chinese Academy of Sciences, Shenzhen, China
³BGI China, Shenzhen, China
⁴BGI Research, Shenzhen, China
⁵Department of Obstetrics and Gynecology, Chinese PLA General Hospital, Beijing, China

Objectives: Next generation sequencing (NGS)-based cell-free DNA (cfDNA) analysis has been widely adopted for non-invasive prenatal testing (NIPT) for fetal chromosomal aneuploidy and monogenic diseases. However, the detection of chromosome aneuploidy and monogenic diseases requires different experiment procedures due to distinct sequencing strategies, and thus cannot be carried out at the same time. We intended to develop a new sequencing method embracing both advantages of targeted and genome-wide sequencing, so that it can simultaneously screen for fetal chromosome aneuploidy and dominant monogenic diseases in a noninvasive manner.

Methods: The novel method, called Targeted And Genome-wide simultaneous sequencing (TAGs-seq), integrates a step of multiplex PCR into the genome-wide NIPS library construction of cfDNA. As a result, whole genome and target region can be simultaneously amplified in one tube (Figure 1). After NGS, the genome-wide region displays a low sequencing depth (0.1-0.5X) and the target regions an ultra-high depth (1000-10000X), which were sufficient for measuring chromosome aneuploidy and detecting single base mutations, respectively.

Results: We validated in-blind the TAGs-seq NIPT in 66 plasma samples with previously confirmed outcomes. The NGS data of all samples for TAGs-seq with a 0.2-0.8X genome coverage and a >1000X targeted coverage, the percentage of on-target reads are 0.22-2.63%. The TAGs-seq NIPT identified 7 cases of common aneuploidy (T21, n=3; T18, n=2; T13, n=2), 6 cases of de novo single base mutations (FGFR3 c.1138G>A, n=5; c.1118A>G, n=1), and 53 cases of normal controls. All results were concordant to the invasive diagnostic results.

Conclusions: We developed a novel TAGs-seq NIPT, which exploited low-coverage whole-genome sequencing data to analyze chromosome aneuploidy, and high-depth targeted sequencing data to analyze single base mutations. Compared to conventional NIPT method, TAGs-seq NIPT provides a convenient, low-cost and expandable solution to detect fetal chromosome aneuploidy and de novo mutations in a single experiment.
The combinative effect of testosterone treatment and the timing of diagnosis on neurocognitive abilities and ADHD in 47,XXY (Klinefelter Syndrome)

Carole Samango-Sprouse¹, Patricia Lasutschinkow², Selena Chea², Teresa Sadeghin³, Andrea Gropman⁴

¹George Washington University School of Medicine and Health Sciences, Washington, DC, United States
²The Focus Foundation, Crofton, MD, United States
³The Focus Foundation, Davidsonville, MD, United States
⁴Children’s National Medical Center, DC, United States

Objectives: 47,XXY (KS) is the most frequently occurring X & Y chromosomal disorder (1:660). These boys may exhibit motor planning deficits and delays, language-based learning disabilities (LLD), ADHD, and executive dysfunction. Testosterone replacement has been shown to mitigate some neurodevelopmental differences. Few studies have documented the possible beneficial combinative effect of prenatal diagnosis and testosterone treatment on intellectual capabilities and behavior.

Methods: 288 males with 47,XXY were evaluated using the Child Behavior Checklist (CBCL), Weschler Intelligence Scale for Children (WISC), and the Leiter International Performance Scale.
78.5% were prenatally diagnosed with the remainder diagnosed postnatally. 71.5% received some type of testosterone treatment and 28.5% received none. Treatment included early hormonal treatment (E) before five years, hormonal booster treatment (B) between 5 and 10 years, testosterone treatment after 10 years (T), and combinations of the three. Treatment was based on the patient’s pediatric endocrinologist’s assessment of the size of phallus in comparison to neurotypical boys of the same age.

**Results:** Boys who received both E+B had significantly reduced ADHD symptoms on the CBCL in comparison to boys with B (P=0.007). Treated, prenatally diagnosed boys performed better than treated postnatally diagnosed in PIQ (P=0.038)\&PSI (P=0.002). Boys with prenatal diagnosis and treated with any testosterone performed significantly better in VIQ (P=0.005), PRI (P=0.003), PSI (P=0.023), and WMI (P=0.001) than untreated boys. Prenatal boys who received E+B+T did significantly better than untreated boys with prenatal diagnosis (P=0.012) on VIQ\&PRI on the WISC. On the LIPS, boys who received E+B+T performed significantly better when compared to untreated boys (P=0.020) and boys with some but not all testosterone treatment (P=0.019).

**Conclusions:** This study has further expanded our knowledge of the positive impact of testosterone treatment on the neurodevelopmental outcome of boys with 47,XXY and suggests that receiving multiple testosterone treatments (E+B+T) results in the most positive outcome. For the first time, a combinative effect of hormonal treatment and diagnosis suggests that boys with 47,XXY may need treatment at several life stages to optimizes neurodevelopmental outcome. Further exploration of the most advantageous timing and dosage for boys with 47,XXY is warranted. Additional study is required to investigate the relationship between repeated treatments of testosterone during childhood and maximum neurodevelopmental outcome.

Neurodevelopmental effects of maternal uterine artery Ad.VEGF-A165 treatment for fetal growth restriction in fetal guinea pigs

Tara Krishnan, Mariya Hristova, Owen Vaughan, Carlo Rossi, Jan Nouza, Anna David

**Objectives:** Fetal growth restriction (FGR) is the failure of a fetus to achieve normal intrauterine growth. There is currently no treatment available for FGR, which can cause behavioural disorders, impaired motor function, and carries a high mortality rate. FGR fetuses have reduced brain size, altered brain-to-bodyweight ratio and increased inflammation and neural cell death when compared with normally grown fetuses. Previously we showed that maternal uterine artery administration of an adenovirus vector containing the VEGF-A165 isoform(Ad.VEGF-A165) to pregnant guinea pigs with FGR mid-gestation increases fetal weight at term. Here we studied the effects of treatment on neurodevelopment in FGR guinea pig fetuses.
Methods: To induce FGR, Dunkin Hartley guinea pigs were fed normally (control, n=7) or 70% calorie intake one month before conception and during pregnancy. FGR dams received Ad.VEGF-A165 (n=9, FGR+VEGF group) or a vehicle (pluronic gel, n=7, FGR group) externally to their uterine arteries via laparotomy on day ~30 of pregnancy (term=65=days). Control dams underwent sham laparotomy. Fetal body and brain weights were determined on day~65, at post-mortem. Brain samples were fixed and sectioned (40 mm). Regional brain volumes, microglial ramification, astrogliosis and apoptosis were analysed using cresyl violet, IBA-1, GFAP and TUNEL stained sections respectively. Groups were compared using one-way ANOVA.

Results: Brain weight was reduced in FGR fetuses (2.268g±0.064g) but was similar to control values (2.531g±0.093g) in the FGR+VEGF group fetuses (2.517g±0.063g,P=0.02). There was no significant difference in fetal weight between the three groups (P>0.05). Consequently, brain:body weight ratio was lower in the FGR group (2.693g±0.082g), compared to the FGR+VEGF group fetuses (3.040g±0.081g) that were no different to controls (2.976g±0.120g,P=0.017). Microglial ramification was increased in cortex, hippocampus, thalamus, and striatum in FGR fetuses compared with control and FGR+VEGF fetuses. Astrogliosis was increased in FGR+VEGF fetuses in all analysed brain regions when compared with control fetuses. TUNEL staining showed no difference in apoptosis across the groups.

Conclusions: Administration of maternal uterine artery Ad.VEGF-A165 gene-therapy to FGR guinea pig pregnancies in mid-gestation improves fetal brain growth, normalises microglial activation, and does not adversely affect neurological anatomy or apoptosis. These data support the safety profile of maternal uterine artery Ad.VEGF therapy for the treatment of FGR.

This research was funded by Action Medical Research.

Set up an amniotic fluid stem cell-derived induced pluripotent stem cells disease model and in utero therapy for spina bifida

S.W. Steven Shaw

Taipei Chang Gung Memorial Hospital, Taipei Chang Gung Memorial Hospital, Taipei, Please Select, Taiwan

Objectives: The amniotic fluid stem cells (AFS) is a potential source of induced pluripotent stem (iPS) cells. The advantages of AFS-iPS cells when a genetic disorder is detected and the use of cells differentiated from AFS-iPS cells as a source for autologous cell therapy pre- or postnatally. Spina bifida (SB) is the most common neural tube defect disease with huge burden for society health insurance. We have set up the animal model of spina bifida in pregnant rodent.

Unedited draft - unpublished
**Methods:** Human SB and normal control AF will be collected from amniocentesis. AFS cells were underwent selection for C-kit+ population and then were reprogrammed into iPS cells using lentiviral vector encoding Yamanaka factors. AFS-iPS cells derived from different diseases were studied for potential treatment, drug investigation and disease modeling. The animal model was induced via retinoid acid to achieve 60-80% of SB fetal mice.

**Results:** Hundred thousand human AFS or AFS-iPS cells were injected into the amniotic cavity of each pup of pregnant mouse at E14 locally around the SB defect. Engraftment and long-term motoneural function studies showed the evidence in major organs including liver, muscles, and spinal cord with improvement of ability of exercise. The differentiation into neural stem cells was successfully induced from AFS / AFS-iPS cells and demonstrated the local effect in SB mice.

**Conclusions:** The development of AFS/ AFS-iPS cell lines from SB could accelerate the development of existing targets for different diseases after prenatal diagnosis. Prenatal transplantation of stem cells could be the possible way to improve the clinical outcome of spina bifida.

1-4

**Preliminary results of a long-term follow-up study in spontaneous twin anemia-polycythemia sequence**

Lisanne Tollenaar, Enrico Lopriore, Femke Slaghekke, Annemieke Middeldorp, Dick Oepkes, Jeanine Van Klink

*Leiden University Medical Center, Leiden, Netherlands*

**Objectives:** To study the long-term neurodevelopmental outcome in a large cohort of twins with spontaneous twin anemia-polycythemia sequence (TAPS).

**Methods:** Neurological, motor and cognitive development will be assessed in a consecutive cohort of spontaneous TAPS survivors born between 2005 and 2016. The primary outcome is severe neurodevelopmental impairment (NDI), a composite outcome including: cerebral palsy, bilateral hearing loss, bilateral blindness and severe motor and/or cognitive development delay. We will evaluate the correlation between adverse outcome and potential risk factors, such as type of TAPS management, prematurity and donor versus recipient status.

**Results:** In total, 37 spontaneous TAPS twins were evaluated at the Leiden University Medical Center. Overall perinatal survival was 88%(65/74). In total, 29 cases were managed expectantly, 4 cases were treated with laser surgery, 3 cases with intrauterine transfusion and in 1 case selective feticide was performed. In total, 65 children were eligible for long-term follow-up. Up to February 2018, we evaluated long-term outcome in 50/65 children. Severe NDI was detected in 3/50(6%) of the TAPS twins. A large difference in mild-to-moderate NDI was seen between
donors and recipient: 10/24(42%) and 4/26(15%), respectively. We expect to complete the follow-up in all cases in June 2018.

Conclusions: We will present the first study on long-term neurodevelopmental outcome in a cohort of spontaneous TAPS twins. Our data can be used to counsel parents and design adequately powered, prospective studies to determine the best management option in spontaneous TAPS.

2-2

Fetal fraction (FF) characterization by zygosity in twin gestation using single nucleotide polymorphism (SNP)- based noninvasive prenatal testing (NIPT)

Herman Hedriana\textsuperscript{1}, Samantha Leonard\textsuperscript{2}, Allison Ryan\textsuperscript{2}, Kimberly Martin\textsuperscript{2}

\textsuperscript{1}University of California Davis, El Macero, CA, United States
\textsuperscript{2}Natera Inc, San Carlos, CA, United States

Objectives: Data regarding comparison of FF in twins to singletons is limited. SNP-based NIPT distinguishes monozygotic (MZ) and dizygotic (DZ) twins with > 99% accuracy and, using informatics-driven SNP interpretation, individual FF in DZ twins can be estimated. The objective of this study was to characterize the FF in DZ and MZ twin pregnancies in comparison to singleton (S) pregnancies using SNP-based NIPT. FF is the basic quality metric for a reliable NIPT, therefore knowledge of twins FF distribution is essential.

Methods: Retrospective FF data were collected for SNP-based NIPT performed between 10/1/2017 and 01/31/2018. For the purpose of this descriptive study, FF were collated for MZ, DZ and S pregnancies, including maternal age (MA), gestational age of blood draw (GA) and maternal weight (MW). Pregnancy outcome data were not available as most were ongoing. The individual DZ-FF were summed for comparison with MZ and S. Where appropriate, data evaluation included descriptive statistics, two-sided \textit{t}-test and correlation analyses at the 5\% significance level. The study falls under our IRB exemption protocol.

Results: 35,206 tests were performed: 2,580 twins and 32,626 singletons. GA at blood draw GA was not different between groups (13.5 weeks). However, MW and MA were significantly greater in twins (\textit{P}<0.001). The table below compares the FF between groups including FF<2.8\% as an estimate for no call. The mean individual DZ-FF was 6.46\%±2.63\% (median 6.00\%, range 0.9\%-19.60\%). The FF difference between DZ fetuses was 1.37\%±1.69\% (median 8.00\%, range 0\%-18.40\%). However, the percent difference between DZ fetuses was 17.80\%±16.09\% (median 13.11\%, range 0\%-94.85\%). Twin FF was 20.91\% higher than singletons (17.19\% higher in MZ and 24.30\% higher in DZ, \textit{P}<0.0001).

Conclusions: Twins FF was less than intuitively expected. This characterization offers insight for further outcome studies to elucidate the clinical significance of FF in twins.

Unedited draft - unpublished
2-2 Table. Comparison of Fetal Fractions

<table>
<thead>
<tr>
<th>Categories, (n)</th>
<th>Mean Fetal Fraction±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monozygotes (854) vs. Dizygotes (1,726)</td>
<td>11.81%±5.33% vs. 12.92%±4.79%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Monozygotes (854) vs. Singletons (3,2626)</td>
<td>11.81%±5.33% vs. 9.78%±4.42%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dizygotes (1,726) vs. Singletons (32,626)</td>
<td>12.92%±4.79% vs. 9.78%±4.42%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fetal Fraction &lt;2.8% as estimates for No Call</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monozygotes (7) vs. Per fetus in DZ pair (96)</td>
<td>2.35%±0.11% vs. 2.20%±0.49%</td>
<td>0.4433</td>
</tr>
<tr>
<td>Monozygotes (7) vs. Singletons (551)</td>
<td>2.35%±0.11% vs. 2.33%±0.39%</td>
<td>0.9210</td>
</tr>
<tr>
<td>Per fetus in DZ pair (96) vs. Singletons (551)</td>
<td>2.20%±0.49% vs. 2.33%±0.39%</td>
<td>0.0021</td>
</tr>
</tbody>
</table>

DZ, dizygot; SD, standard deviation; P value <0.05 significant

2-3

Implementing NIPT as part of a national prenatal screening program: The Dutch TRIDENT studies

Robert-Jan Galjaard¹, Lidewij Henneman², Merryn Macville³, Caroline Bax⁴, Mireille N. Bekker⁵, Christine de Die-Smulders³, Ilse Feenstra⁶, Mariette Hoffer⁷, Nicolette den Hollander⁷, Maarten F.C.M. Knapen⁸, Irene Van Langen⁹, Klaske Lichtenbelt¹⁰, Paola Lombardi¹¹, Merel Van Maarle¹², Karuna van der Meij¹³, Mijntje Pieters³, Heleen Schuring-Blom¹⁰, Esther Sikkel¹⁴, Servi Stevens³, Ron Suijkerbuijk¹⁵, Jeanine van der Ven¹⁶, Diane Van Opstal¹⁷, Janneke Weiss¹⁸, Erik Sistermans¹⁸, Dutch NIPT Consortium¹⁹

¹ Department of Clinical Genetics, Erasmus MC Rotterdam, Rotterdam, Netherlands
² Dept. of Clinical Genetics, VU University Medical Center Amsterdam, Amsterdam, Netherlands
³ Maastricht University Medical Center, Maastricht, Netherlands
⁴ Dept. of Obstetrics, Amsterdam, Netherlands
⁵ Division Woman and Baby, Department of Obstetrics, University Medical Center Utrecht, Utrecht, Netherlands
⁶ Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands
⁷ Leiden University Medical Center, Leiden, Netherlands
⁸ Department of Obstetrics and Gynecology, Erasmus MC and Foundation Prenatal Screening Southwest Region of the Netherlands, Rotterdam, Netherlands
⁹ University Medical Center Groningen, University of Groningen, Groningen, Netherlands
¹⁰ UMC Utrecht, Utrecht, Netherlands
¹¹ Department of Clinical Genetics, Amsterdam, Netherlands

Unedited draft - unpublished
Objectives: In most countries Non-Invasive Prenatal Testing (NIPT) has been introduced commercially without any governmental guidance. In the Netherlands, prenatal screening for fetal anomaly is subject to a governmental license. NIPT has been implemented as part of the TRIDENT studies (Trial by Dutch laboratories for Evaluation of NIPT). TRIDENT-2 aims at offering NIPT to all pregnant women (~174,000 women/year) within the national prenatal screening program. Since April 2017 women can choose NIPT as a contingent test after first-trimester combined testing (FCT), but may also choose NIPT as first-tier screening test. The TRIDENT studies evaluate implementation and women’s perspectives.

Methods: All pregnant women in the Netherlands are offered prenatal screening and are counselled by certified counselors, generally midwives. A first-tier NIPT costs women € 175, comparable to the costs of FCT (~€ 168). NIPT is performed by three Dutch university clinical genetic laboratories using an in-house validated test. Women can choose to have analysis of chromosomes 21, 18, and 13 without or with a report of incidental findings (findings other than trisomy 21, 13, 18) on the remaining autosomes, respectively using the ‘targeted’ or ‘whole genome’ WISECONDOR pipeline. Sex chromosomes are not analyzed.

Results: After eight months of study, 48,234 tests have been performed (nationwide uptake of prenatal screening by NIPT as first-tier test was 40%) and 98.3% reports successfully issued. Failure rate was less than 2%. Mean turnaround time was 7 working days. 80% of women chose to have all autosomes analyzed. A total of 152 cases of T21 (0.3%), 32 cases of T18 (0.1%), 41 cases of T13 (0.1%) and 158 (0.3%) other chromosomal aberrations were found. First year results (and available follow-up) will be presented at the meeting.

Conclusions: The Netherlands are the first country where NIPT is incorporated as a first-line test into a governmentally supported and health care funded prenatal aneuploidy screening program. The incorporation of the test in a university hospital laboratory and clinical service guarantees appropriate counselling and allows for proper follow-up. This 3-year study aims to provide all necessary information for a successful introduction of NIPT within the Dutch National prenatal screening program.
OnePGT: A single workflow for concurrent PGT-M, PGT-SR and PGT-A on blastomere and trophectoderm biopsies

Aimee Paulussen¹, Cindy Melotte², Edith Coonen³, Jos Dreesen⁴, John Dumoulin⁵, Chris Van Uum⁶, Marion Drüsedau⁶, John Engelen¹, Kasper Derks⁵, Ron Van Golde⁵, Eftychia Dimitriadou², Heleen Masset², Katrien François⁷, Joke Allemersch⁸, Rebecca Richards⁷, Sara Moeys⁷, Jessie Theuns⁹, Masoud Zamani Esteki¹⁰, Christine de Die-Smulders³, Joris Vermeesch¹¹

¹Maastricht University Medical Center, Maastricht, Netherlands
²Center for Human Genetics, University Hospital of Leuven, KU Leuven, Leuven, Belgium
³Department of Clinical Genetics and Center for Reproductive Medicine, GROW School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, Netherlands
⁴Dept of Clinical Genetics, Maastricht, Netherlands
⁵Center for Reproductive Medicine, GROW School for Oncology and Developmental Biology, Maastricht, Netherlands
⁶Department of Clinical Genetics, GROW School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, Netherlands
⁷Agilent Technologies, Diagnostics and Genomics Group, Leuven, Belgium
⁸Agilent Technologies, Leuven, Belgium
⁹Agilent Technologies, Niel, Belgium
¹⁰Department of Clinical Genetics, GROW School for Oncology and Developmental Biology, Maastricht University Medical Center; Center for Human Genetics, University Hospital of Leuven, KU Leuven, Maastricht, Netherlands
¹¹Center for Medical Genetics, Katholieke Universiteit Leuven, Leuven, Belgium

Objectives: OnePGT is a genome-wide next-generation sequencing haplArithmisis-based solution designed to reinforce ranking of IVF embryos. The single workflow solution, consisting of wet lab reagents and dedicated data analysis software, allows concurrent PGT-M (Pre-implantation genetic testing of monogenic disorders), PGT-SR (Pre implantation genetic testing of structural rearrangements) and PGT-A (Pre implantation genetic testing of aneuploidies) hence enables enhanced genetic profiling. In collaboration with MUMC and KULeuven, a study was set up to assess the level of concordance between OnePGT and Gold Standard analysis of both single and few cell embryo biopsies for PGT-M, PGT-SR and PGT-A.

Methods: A total of 227 embryo samples, 179 blastomere and 48 trophectoderm biopsies, were included in the study; 118 embryos were obtained from KULeuven, and 109 from MUMC. For the KULeuven-arm of the study, left-over whole genome amplified DNA from the embryo biopsies was processed, while in the MUMC-arm, a second biopsy was taken and amplified. NGS libraries were generated using proprietary OnePGT reagents for all WGA embryo samples and for gDNA samples from parents and reference family member(s) in case of PGT-M. Libraries

Unedited draft - unpublished
were sequenced on NextSeq500 (Illumina) and resulting data were analyzed with the dedicated proprietary OnePGT software tools.

**Results:** A OnePGT call was made in 90.5% of the embryos analyzed for PGT-M. Of these, 92% were included in concordance analysis, resulting in 100% concordance. The samples excluded from concordance analysis, are under further investigation.

For PGT-SR, 36 embryos from MUMC were included in the study and fully analyzed. Due to mitotic errors, 3 embryos had to be excluded from concordance analysis. Of the remaining 33, all embryos (100%) were concordant with the Gold Standard. On the embryos obtained via UZLeuven, a PGT-A analysis was performed and 100% concordance was obtained for chromosomal aberrations > 50 Mb (limit reference method).

**Conclusions:** The proprietary OnePGT solution, consisting of all-in-one library preparation reagents and dedicated software for concurrent analysis of PGT-M, PGT-SR and PGT-A on a single embryo biopsy, successfully identified the presence of 26 different SGDs, translocations and chromosomal aneuploidies in over 200 embryo biopsies, with a concordance of 100% for PGT-M, PGT-SR and PGT-A. Hence this innovative genome-wide solution is expected to be of added value to improve ranking of IVF embryos based on enhanced genetic profiling.

3-1

Assessment of patient understanding of non-invasive prenatal testing following introduction of three systematic patient counselling models

Kelly Chen¹, Thomas Lee², Liza Kunz¹, Maximilian Schmid¹

¹Ariosa Diagnostics, Inc. and Roche Sequencing Solutions, Inc., San Jose, CA, United States
²Northwest Perinatal Center, Portland, OR, United States

**Objectives:** Non-invasive prenatal testing/screening (NIPT) is now widely available for clinical use. NIPT has detection rates for fetal trisomy 21 of >99% and false positive rates of <0.1%, an improved performance compared to combined testing in all pregnant patients, which has increased its utilization and consequently the need for genetic counseling, a standard for pre- and post-test management. The main objective was to evaluate patient understanding of NIPT before and after their participation in three different systematic patient counseling programs as alternatives for one-on-one pre-test genetic counseling.

**Methods:** Quasi-experimental, multi-center design pilot study. Participants had a singleton pregnancy, provided informed consent and were >18 years of age. After receiving genetic prenatal screening education via one of four methods (group flip book –GFB-, one-on-one video –OOOV-, group video –GV- and a control site –C-), participants were administered a survey exploring pre- and post-training knowledge of NIPT and common aneuploidies. Four sites
participated with one intervention per site. All interventions included the same content. Quorum IRB approved the protocol.

**Results:** A total of 1026 women participated in the 4 sites/intervention groups (GFB, OOOV, GV, C) with 283, 104, 214 and 425 participants respectively. All groups had good recognition of trisomy 21 (>90% all sites, p 0.12) as opposed to other trisomies (18, 13 or sex chromosomes aneuploidies –SCA-), which ranged from 28% to 52%. Less than 25% of participants identified all four conditions. Nearly 60% of participants demonstrated recognition of amniocentesis, while only 21% recognized nuchal translucency (p 0.001). GFB were best change in knowledge (25 to 75 inter quartile range –IQR- p<0.001) followed by GV (50 to 75IQR p<0.01).

**Conclusions:** GFB was the most effective method, followed by GV. OOOVs do not provide good results in this study. Although limited by its quasi-experimental design, this pilot study suggests that alternative delivery methods of counseling may be considered useful in light of increasing patient volume expected to undergo NIPT in the near future.

3-2

**Use of a novel tablet-based decision aid for prenatal aneuploidy screening and testing: A randomized controlled trial**

Laura Carlson¹, Emily Hardisty², Sarah Harris², Neeta Vora²

¹University of North Carolina at Chapel Hill, Chapel Hill, NC, United States
²University of North Carolina School of Medicine, Chapel Hill, NC, United States

**Objectives:** Decision aids (DA) are known to improve knowledge and decisional conflict surrounding important screening and treatment decisions. Prior DAs for aneuploidy screening showed effectiveness but did not include cell-free DNA. Our DA was constructed with input from MFMs and genetic counselors in English and Spanish and explores all aneuploidy screening and testing options via a tablet-based platform. We aimed to evaluate our DA in a randomized controlled trial. We hypothesized that knowledge following DA use alone would not be inferior to knowledge following standard genetic counseling (GC), with a noninferiority margin of 1 point on a knowledge questionnaire.

**Methods:** English and Spanish-speaking women <22 weeks with a singleton pregnancy were eligible. Women with abnormal ultrasound findings or prior aneuploidy screening in the current pregnancy were excluded.

Women were randomized to routine GC (group 1) or DA use before GC (group 2). All patients completed an initial KQ. Those in group 1 repeated the KQ and a decisional conflict questionnaire (DCQ) following GC. Those in group 2 self-administered the decision aid, then repeated the KQ and DCQ following DA and again following GC. T-test, chi-square, Wilcoxon rank-sum, and ANOVA were used as appropriate, and analysis was by intent to treat.

*Unedited draft - unpublished*
Results: 365 women were eligible for enrollment; 197 women participated. 105 were randomized to group 1 and 92 to group 2. Demographics and mean knowledge scores at enrollment were similar between groups; 24 women were Spanish-speaking and distribution did not differ between groups. Mean knowledge score in group 2 following completion of DA was not inferior to mean knowledge score following completion of GC in group 1 (figure). Decisional conflict was similar in group 2 following completion of DA to group 1, but was significantly reduced in group 2 following DA+GC (1.74 vs. 0.22, p=0.003).

Conclusions: Knowledge and decisional conflict surrounding aneuploidy screening and testing options in women who used a DA only were not inferior to outcomes in women who underwent GC only. Additionally, decisional conflict was significantly reduced among women who both used the DA and underwent GC compared to those who underwent GC alone. Given the non-inferiority of DA to GC with regard to knowledge, the use of a DA can be considered in areas where GC is not available. Additionally, our findings support the use of DA in addition to GC to decrease decisional conflict.
Accessibility of conflict of interest disclosures for professional medical societies of relevance to the practice of prenatal diagnosis

Sara Arian, Andrea Harbison, Hadi Erfani, Alireza Shamshirsaz, Ignatia Van den Veyver

*Baylor College of Medicine, Houston, TX, United States*

**Objectives:** Although conflict of interest (COI) can influence professional judgement or decision making by secondary interest, the increasing collaboration of medical professionals with commercial entities accelerates discovery with important individual and public health benefits. Thus, transparent disclosure of financial and other COIs is important to mitigate concerns about their influence on scientific investigations and quality of patient care, and maintain the public’s trust in medicine. This is especially relevant for professional societies that issue opinions and
practice guidance. Our objective was to investigate how COIs are communicated on websites of a representative sample of professional societies relevant to prenatal diagnosis.

**Methods:** We reviewed COI disclosure listings on websites of a representative sample of twelve United States (US)-based professional societies (N=10) and international societies with a significant US-based membership (N=2) with expertise relevant to prenatal diagnosis and engaged in producing statements, opinions and guidelines related to prenatal screening, diagnostic testing and fetal care. We searched the societies’ websites to investigate if a COI disclosure policy is provided, if COIs of board and committee members are stated, easy to find, and how can they be accessed. We also examined if COIs for individuals are disclosed or if only a generalized statement is provided.

**Results:** For the 12 society websites examined, we found COI statement for 7/12 (58%). For 3 of the 7 (43%), the statement was relatively easy to find on their websites. Table 1 shows more details of our COI evaluation. The COI policy was acknowledged for the members of the executive board of only 2/12 (17%) and for members of practice or publication committees of 4/12 (33%) of the societies. Interestingly, the reported COIs for these 7 societies are declared as a general statement and none disclosed the COI individually for each of their board or practice/publication committee members.

**Conclusions:** In this descriptive review of conflict of interest policies for professional medical societies, we found that the majority of these societies disclose COIs, but not in detail. This raises the question whether a uniform international standard or central repository for COI disclosures for professional medical and scientific societies should be considered.
Objectives: Branched-chain organic acidurias/acidemias are a group of autosomal recessive conditions characterized by impaired catabolism of branched-chain amino acids (BCAA). Clinical presentation ranges from severe, neonatal-onset with metabolic distress to a chronic, progressive form characterized by failure to thrive, developmental delay, and hypotonia. Historically, carrier screening guidelines were ethnicity-based and did not include testing for BCAA disorders. New technologies and increasing ethnic diversity have enabled expanded carrier testing as an acceptable clinical practice. This study assesses how many individuals tested positive for four different BCAA disorders when a pan-ethnic expanded carrier screening panel was implemented.

Methods: A retrospective database analysis was performed on samples received for expanded carrier testing via a commercially-available genotyping platform. The number of patients tested ranged from 24,158 to 84,413, depending on the disorder. Patients screened were divided into nine self-reported ethnicity categories. The expected numbers of carriers for maple syrup urine disease (MSUD), isovaleric acidemia (IVA), methylmalonic aciduria (MMA), and propionic acidemia (PA) were calculated based on established carrier frequencies specific to each ethnicity and the assay’s detection rates. The number of observed-to-expected carriers for each
disease were compared. Chi-squared analysis was performed to assess for statistical significance (p<0.05).

**Results:** Concordance was seen in the number of observed-to-expected carriers across most ethnicities and branched chain amino acid disorders. There were, however, statistically greater numbers of carriers of MSUD type 1b observed vs. expected amongst Caucasians (24 vs. 14.8, p= 0.017) and amongst patients who designated themselves as other or mixed ethnicity (11 vs. 5.9, p=0.013). The number of observed carriers for PA, PCCB-related, amongst individuals reporting Asian ethnicity was also greater than expected (6 vs. 2.02, p=0.005).

**Conclusions:** The number of observed carriers for branched chain amino acid disorders amongst certain ethnicities were significantly greater than expected based on historical carrier rate data. With increased mixing of the population, self-reported ethnicities may no longer be an adequate way to determine which couples are at highest risk for having children affected by these morbid and incurable, but potentially treatable, disorders. Pan-ethnic expanded carrier testing will increase the detection of carriers for branched chain amino acid disorders when compared with ethnicity-based screening recommendations, and allows for improved reproductive counseling.

4-2

**Haplotyping and copy number profiling of single cells by using extended family members: Broadening the applicability of PGD**

Jia Ding¹, Aspasia Destouni², Joris Vermeesch³

¹UZ Leuven, Leuven, Belgium
²KUL, Leuven, Belgium
³Center for Medical Genetics, Katholieke Universiteit Leuven, Leuven, Belgium

**Objectives:** Genome-wide linkage-analysis by haplotyping the entire genome of preimplantation embryos is being implemented as a generic approach for genetic diagnosis of inherited mutations. To enable the phasing of the genotypes into haplotypes, genotyping direct family members of the prospective parent carrying the mutation is required. Currently, the algorithm uses either (1) both parents of the affected prospective parent or (2) an affected or unaffected child of the affected couple. However, in many cases DNA from offspring and grandparents might not be available to phase the parental genotypes. This limitation deprives couples of gaining access to the generic genome-wide haplotyping-based PGD.

**Methods:** To expand the dynamic range of the technology in clinical practice, we optimized the algorithmic workflow to phase the parental genotypes using parental siblings. Our method exploits allelic states between family members and is independent of pedigree structure. As the genetic mutation is embedded in a local haplotype, it is possible to deduce the affected haplotype and copy number profiling of the embryo.

*Unedited draft - unpublished*
Results: Retrospective phasing of the parental haplotypes in twelve concluded PGD cases involving the analysis of 67 embryos in the Centre for Human Genetics, UZ Leuven between 2015-2016. We compared the genome-wide haplotypes and copy number states of each embryo, which were obtained with the new phasing approach to those initially obtained with the validated phasing strategy using grandparents and offspring during clinical PGD. In accordance to the expected degree of genetic relatedness between full siblings, new phasing approach could establish a diagnostic result in six out of the twelve (50%) of PGD couples included in this study.

Conclusions: We developed a method to enable generic haplotyping and genome-wide copy number profiling without the need to genotype grandparents or affected/unaffected offspring. This service significantly reduces the emotional stress imposed to the couple higher costs and increased waiting times in case a “private” protocol has to be set-up. More families can benefit from this new option without the need for customized test development. By including more than one extended family member, the chance of diagnosis the embryo will get increased. A rapid Pre-PGD work-up defines whether the carrier couple can benefit from this technology.

Restricting NIPT for fetal fraction only, does it still make sense?

Vincenzo Cirigliano1, Elena Ordoñez1, Laura Rueda2, Sara Nicolas2, Isabel Castilla2, Manuel Grau2, Cristina Puertollano2, Mireia Lechuga2

1Synlab, Barcelona, Spain
2Synlab, Esplugues de Llobregat, Spain

Objectives: One common approach to improve NIPT performance is limiting analysis to samples above predefined FF (mostly 4%) to not compromise detection (DR) and false positive rates (FPR). However, being NIPT sensitivity mostly dependent on sequencing depth, detection limits should be evaluated at different depths instead of only being set to an arbitrary FF. Paired-end MPSS allows discriminating fetal cfDNA by fragment sizes distribution. This provides FF estimates and allows performing counting statistics on fetal cfDNA, thus delivering more evident differences in case of fetal aneuploidy. We evaluated the clinical utility of this approach in screening a large cohort of consecutive average-risk pregnancies.

Methods: A total of 23668 samples were collected above 10w of gestation (728 twins) regardless their risk category. Trisomies 13, 18 and 21 were screened using the NeoBona test, generating trisomy likelihood ratios (Tscore) for each chromosome of interest based on estimated FF, inter-chromosome statistics from fragments size distributions and the total sequencing counts on each chromosome. Chromosome specific cut-offs were applied at Tscores to classify normal and aneuploid cases. High-risk results were followed up by invasive diagnostic procedures in 98% of cases, low risk results were assumed to be concordant with pregnancy outcome unless otherwise advised by the referral centre.
Results: A total of 98.2% of cases were reported at first draw, 472 as high risk (2%). Ten FP were observed (cumulative FPR 0.04%), 1 T21 was classified as normal male with 7% FF (overall DR 99.8%). FF below 4% was observed in 1361 samples (5.7%), 84% yielded valid results, at high risk for trisomies in 34 cases, including 2 FP results. T18 and T13 were detected even below 1% FF, accounting for 20% of all cases in this study, the lower FF for T21 was 2%. Including low FF cases didn’t impact DR while only increasing overall FPR by 0.005%.

Conclusions: The new analysis algorithm exploiting paired-end MPSS output of NeoBona test, enabled additional counting statistics to be performed on cfDNA of fetal origin while also monitoring sequencing counts reached for the estimated FF in each sample. In the course of this study, this approach provided valid results in the vast majority of cases independently from the FF, allowing detecting all trisomies in samples reported with less than 4% fetal cfDNA at reduced FPR. The increased proportion of T18 and T13 observed in this subset of samples confirms the clinical utility of extending NIPT benefits to a wider population of pregnancies.

Predicting fetoplacental chromosomal mosaicism during non-invasive prenatal testing

Maria Neofytou1, Nathalie Brison2, Luc Dehaspe1, Baran Bayindir3, Kris Van Den Bogaert1, Hilde Peeters1, Van Esch Hilde1, Griet Van Buggenhout1, Annick Vogels1, Jeroen Breckpot1, Thomy de Ravel1, Eric Legius1, Koenraad Devriendt1, Joris Vermeesch1, Leila Dardour4

1Centre for Human Genetics - Katholieke Universiteit Leuven, Leuven, Belgium
2Center for Human Genetics, University Hospital Leuven, Leuven, Belgium
3Doctor, Leuven, Belgium
4Department of Human Genetics, Faculty of Medicine, Sousse, Tunisia

Objectives: Non-invasive prenatal detection of trisomies 21, 18 and 13 can be achieved with high accuracy through sequencing of maternal plasma-derived cell-free DNA (cfDNA). However, fetoplacental mosaicism is a main cause for false positive/negative NIPT results. We further improved the analytical power of genome-wide cfDNA screening by predicting the occurrence of fetoplacental mosaicism.

Methods: Trisomy Z-scores increase linearly with increasing fetal fractions. Z-scores that do not correlate with the actual detected fetal fraction are indicative of the presence of placental chromosomal mosaicism. This analysis pipeline was applied to whole genome sequencing data derived from ~20,000 maternal plasma samples. Following an abnormal NIPT, test results were validated by conventional invasive prenatal or postnatal genetic testing.

Results: The new analysis pipeline identified 134, 24 and 7 non-mosaic trisomies 21, 18 and 13 respectively. All for whom follow-up information was available were confirmed upon invasive testing. The incidence of other, rare autosomal trisomies (RATs) was ~0.3%, with trisomy 7 and 16 being the most prevalent. Three of these RATs, predicted as full trisomies in the placenta,
were found to be mosaic in the fetus; 25 other RATs were predicted to be mosaic, 8 of which have been confirmed in placental tissue. The new pipeline also correctly predicted twin pregnancies with discordant fetal sex.

**Conclusions:** This improved analysis pipeline permits the detection of autosomal aneuploidies and pinpoints pregnancies at risk of fetoplacental mosaicism. This knowledge can influence the estimation of the risk for miscarriage, aid in genetic counseling and improve prenatal management.

5-2

**Is cell-free DNA testing appropriate in fetuses with increased nuchal translucency?**

Jezid Miranda\(^1\), Virginia Borobio\(^2\), Celia Badenas\(^3\), Laia Rodriguez-Revenga\(^3\), Antoni Borrell\(^4\)

\(^1\)Fetal i+D Fetal Medicine Research, BCNatal – Barcelona Center for Maternal-Fetal and Neonatal Medicine, Barcelona, Spain
\(^2\)Department of Maternal-Fetal Medicine, Institute Gynecology, Obstetrics and Neonatology, Hospital Clinic Barcelona, Barcelona, Spain
\(^3\)Hospital Clinic – Centro de Diagnóstico Biomédico – Bioquímica y Genética Molecular, Barcelona, Spain
\(^4\)Department of Maternal-Fetal Medicine, Institute Gynecology, Obstetrics and Neonatology, Hospital Clinic of Barcelona, Barcelona, Spain

**Objectives:** Cell-free DNA is currently considered an advanced screening test, however, its utility in patients with an abnormal first-trimester ultrasound remains to be established. The objective of this study was to assess the frequency of atypical and submicroscopic chromosomal anomalies (not detectable using standard cell-free DNA testing), as well as fetal structural abnormalities observed at the first-trimester scan, in fetuses with an increased nuchal translucency (NT).

**Methods:** Prospective cohort study. From January 2013 to December 2017, 227 fetuses with an NT \(\geq 99^{th}\) centile at 11-13 weeks' gestation underwent genetic testing in chorionic villi by means of QF-PCR and chromosomal microarray analysis (CMA), in a clinical setting in which more than 95% of pregnant women receive first-trimester combined screening. The frequency of common, atypical and submicroscopic chromosomal anomalies (not detectable using standard cell-free DNA testing), as well as fetal structural abnormalities observed at the first-trimester scan, was determined in the study group.

**Results:** In 67 cases (30%) common aneuploidies (involving chromosomes 13, 18, 21 or X) were identified. Among the remaining 160 fetuses that would receive a theoretical negative cell-free DNA, five (3.1%) had a clinically relevant atypical chromosomal anomaly (two triploidies, two mosaicism, and one monosomy 5), and six (3.8%) had submicroscopic anomalies. Finally,
among the 149 fetuses with normal QF-PCR and CMA, a major fetal malformation was observed in 16 (11%) fetuses at the early anomaly scan, and in 18 (12%) in the second or third trimester.

**Conclusions:** Cell-free DNA does not appear to be the appropriate genetic test in fetuses with a NT above the 99th centile, given that 6.9% of them will have a chromosomal anomaly not detectable with the current tests. Additionally, 11% of those fetuses will have a major structural abnormality identifiable using first-trimester ultrasound.

5-2 Figure.

5-3

**Early delivery in fetal gastroschisis: A randomized controlled trial of elective 34 week delivery versus routine obstetrical care**

Alireza Shamshirsaz\(^1\), Timothy Lee\(^1\), Amy Hair\(^1\), Hadi Erfani\(^1\), Jimmy Espinoza\(^1\), Amir Shamshirsaz\(^1\), Karin Fox\(^1\), Manisha Gandhi\(^1\), Ahmed Nassr\(^1\), Steven Abrams\(^2\), Oluyinka Olutoye\(^1\), Michael Belfort\(^1\)

*Unedited draft - unpublished*
Objectives: Hypothesis: Elective late preterm delivery of the fetus with gastroschisis may help limit injury to extruded fetal gut and thus promote faster recovery of neonatal gut function and earlier hospital discharge. We therefore compared outcomes of elective late preterm delivery (ED) with routine obstetric care (RC) in babies with gastroschisis.

Methods: Between May 2013 and September 2015, all women with a sonographically diagnosed gastroschisis referred to a single tertiary center before 34 weeks' gestation were invited to participate in this randomized controlled trial. Eligible patients were randomized to elective delivery at 34 weeks (ED) versus routine obstetric care (RC). The mode of delivery was not stipulated. The primary outcome measure was duration on total parenteral nutrition (TPN). Outcome variables were reported as mean (SD), median [range] or rate (%) and compared using appropriate statistical methods. The analysis was done according to intention to treat principle.

Results: 21/25 (84%) eligible women were randomized; 10 to elective late preterm delivery and 11 to routine obstetric care (Fig). The trial was stopped at the first interim analysis after 21 of an expected 86 patients were enrolled (24%) for both patient safety concerns and for futility. The median gestational age at delivery was 34.3 weeks [34, 36] in the ED group and 36.7 [27, 38] in the RC group. There was significantly more neonatal sepsis in the ED group than in the RC group (40% vs 0%, P=0.03) (Table).

Conclusions: This study demonstrates no benefit of elective late preterm delivery of fetuses with gastroschisis when postnatal gastroschisis management is similar to that used in routine care.
### Baseline Characteristics of Recruited Cases

<table>
<thead>
<tr>
<th></th>
<th>Early Delivery Group, N=10</th>
<th>Routine Care Group, N=11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Age, mean(SD)</td>
<td>22.5 (7.1)</td>
<td>20.8 (3.2)</td>
</tr>
<tr>
<td>Gravidity, median[range]</td>
<td>1.5 [1, 11]</td>
<td>1 [1, 3]</td>
</tr>
<tr>
<td>Parity, median[range]</td>
<td>0.5 [0.2]</td>
<td>0 [0.1]</td>
</tr>
<tr>
<td>GA at Diagnosis median[range]</td>
<td>19.5 [13, 28]</td>
<td>19.7 [12, 29]</td>
</tr>
<tr>
<td>GA at Delivery median[range]</td>
<td>34.3 [34, 36]</td>
<td>36.7 [27, 38]</td>
</tr>
<tr>
<td>BMI, mean(SD)</td>
<td>25.3 (6.1)</td>
<td>23.5 (1.7)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>African American</td>
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<td>0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mode of Delivery, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>4 (induced:4)</td>
<td>7 (induced:5)</td>
</tr>
<tr>
<td>CS</td>
<td>6 (induced:2)</td>
<td>4 (induced:2)</td>
</tr>
<tr>
<td>Male Fetus, n (%)</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Tobacco Use, n (%)</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

### Primary and Secondary Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Early Delivery</th>
<th>Routine Care</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of TPN (day), median[range]</td>
<td>54 [17, 248]</td>
<td>21 [9, 465]</td>
</tr>
<tr>
<td>P. Value</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Time to Closure (day), median[range]</td>
<td>7 [2, 21]</td>
<td>5.5 [1, 8]</td>
</tr>
<tr>
<td>P. Value</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Hospital Stay (day), median[range]</td>
<td>70.5 [22, 137]</td>
<td>31 [19, 186]</td>
</tr>
<tr>
<td>P. Value</td>
<td>0.15</td>
<td></td>
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</table>

### Early Neonatal Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Early Delivery</th>
<th>Routine Care</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Weight (gr), mean(SD)</td>
<td>2110 (296)</td>
<td>2615 (469)</td>
</tr>
<tr>
<td>P. Value</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Primary Closure, n (%)</td>
<td>1 (10%)</td>
<td>1 (9.1%)</td>
</tr>
<tr>
<td>Total intubation Time (day), median[range]</td>
<td>4 [1, 14]</td>
<td>3 [1, 13]</td>
</tr>
<tr>
<td>P. Value</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Nasal O2 Time (day), median[range]</td>
<td>6 [2, 10]</td>
<td>1.2 [1, 7]</td>
</tr>
<tr>
<td>P. Value</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Surfactant, n (%)</td>
<td>1 (10%)</td>
<td>0</td>
</tr>
<tr>
<td>NEC, n (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BPD, n (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RDS, n (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sepsis, n (%)</td>
<td>4 (40%)</td>
<td>0</td>
</tr>
<tr>
<td>P. Value</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Apgar 5 &lt; 7, n (%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

GA = gestational age; BMI = body mass index; NEC = necrotizing enterocolitis; BPD = bronchopulmonary dysplasia; RDS = respiratory distress syndrome

Values are presented as mean (SD) (independent t-test), median (range) (Mann-Whitney U test) and n (%) (Chi square test).
ASPRE trial: incidence of preterm preeclampsia in patients fulfilling ACOG and NICE criteria according to risk by the FMF algorithm

Liona Poon

The Chinese University of Hong Kong, The Chinese University of Hong Kong, Shatin, Hong Kong SAR

Objectives: To report the incidence of preterm preeclampsia (PE) in women that fulfilled the screening criteria of the National Institute for Health and Clinical Excellence (NICE) and American Congress of Obstetricians and Gynecologists (ACOG) and compare the incidence in those that were screen positive and screen negative by the Fetal Medicine Foundation (FMF) algorithm.

Methods: This was a secondary analysis of data from the ASPRE study. The study population consisted of women with singleton pregnancies who had prospective screening for preterm-PE by means of the FMF algorithm that combines maternal factors and biomarkers at 11-13 weeks’ gestation. We estimated the incidence of preterm-PE in those fulfilling the NICE and ACOG criteria; in these patients we then calculated the incidence of preterm-PE in those that were screen negative relative to those that were screen positive by the FMF algorithm.

Results: 34,573 women with singleton pregnancies were included. There were 239 (0.7%) cases of preterm-PE. At least one ACOG-criteria was fulfilled in 22,287 (64.5%) of pregnancies and the incidence of preterm-PE was 0.97%; in those that were FMF-screen-positive the incidence was 4.80%, in those that were screen-negative it was 0.25% and the relative incidence was 0.051. In 1,392 (4.0%) pregnancies at least one NICE high-risk criteria was fulfilled and the incidence of preterm-PE was 5.17%; in the subgroups of screen-positive and screen-negative by the FMF algorithm the incidence of preterm-PE was 8.71% and 0.65%, respectively, and the relative incidence was 0.075.

Conclusions: In ACOG or NICE screen positive women that are screen negative by the FMF algorithm the risk of preterm-PE is reduced to within or below background levels. The results provide further evidence to support risk based screening using biomarkers.

Clinical application of targeted next-generation sequencing on fetuses with congenital heart defects

Fengchang Qiao¹, Ping Hu¹, Wang Yan², Zhengfeng Xu²

¹Department of Prenatal Diagnosis, the Affiliated Obstetrics and Gynecology Hospital of Nanjing

Unedited draft - unpublished
Objectives: This study aimed to determine the diagnostic yield of targeted next-generation sequencing (NGS) in prenatal diagnosis of congenital heart defects (CHDs) and for investigating the possible genetic etiology of prenatal CHD cases.

Methods: Forty-four fetuses with CHDs and normal molecular karyotypes underwent targeted NGS using DNA obtained via amniocentesis, were recruited in this study. Fetal genomic DNA was directly extracted from amniotic fluid cells in each prenatal case. A customized targeted NGS panel containing 77 CHD-associated genes was designed to detect variants in the coding regions and the splicing sites of these genes. The detected variants were then interpreted following the guidelines recommended by American College of Medical Genetics and Genomics.

Results: In the 44 fetuses, the detection rates of pathogenic and likely pathogenic variations were 13.6% (6/44) and 2.27% (1/44), respectively. The 6 pathogenic variations were identified on genes of CHD7 (associated with CHARGE syndrome), CITED2 (associated with Tetralogy of Fallot, Ventricular Septal Defect and Atrial Septal Defect), ZFPM2 (associated with Tetralogy of Fallot), MYH6 (associated with Atrial Septal Defect, Familial Isolated Dilated Cardiomyopathy), KMT2D (associated with Kabuki syndrome). One likely pathogenic variation was on JAG1, associated with Tetralogy of Fallot and Alagille syndrome.

Conclusions: Targeted NGS of fetuses with isolated and non-isolated CHDs achieved a high diagnostic yield in our cohort, with an acceptable turnaround time for the prenatal setting. Our results have important implications for clinical management and genetic counseling.

Implementing fast whole exome sequencing (WES) as diagnostic test for fetal multiple congenital anomalies on ultrasound

Nicole Corsten-Janssen1, K. Bouman1, Joke Verheij1, Julia El Mecky1, Helga Westers1, Rianne Kinds1, Ron Suijkerbuijk1, Beike Leegte1, Arjen Scheper1, Birgit Sikkema-Raddatz1, Richard Sinke1, Irene Van Langen2, Rolf Sijmons1, Cleo van Diemen1

1University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, Netherlands
2University Medical Center Groningen, University of Groningen, Groningen, Netherlands

Objectives: Identifying the cause of fetal anomalies seen on ultrasound is paramount to improve reproductive choice and/or perinatal management. The conventional test (chromosomal microarray) leads to a diagnosis in approximately 25% of fetuses with multiple congenital anomalies (MCA) on ultrasound. WES is a promising technique to improve diagnostic yield. However, implementing WES in prenatal setting is challenging due to uncertainties.
around fetal phenotyping, required short turnaround times, and technical and ethical handling of incidental findings and variant interpretation. Here, we discuss the implementation of WES as a routine fast test for fetuses with MCA on ultrasound in our center.

**Methods:** Phase 1 (concluded): Blind retrospective WES analysis of six fetuses with known postnatal genetic diagnosis to test if this diagnosis could be made on the fetal phenotype only. Variants were filtered using human phenotype ontology (HPO) or using our custom virtual prenatal gene panel (all known disease genes, excluding late-onset diseases). Phase 2 (Starting March 5th): Prospective study of rapid trio WES analysis in addition to conventional genetic tests for fetuses with two or more congenital malformations on ultrasound. Measures: diagnostic yield; turnaround times; clinical consequences; differences in prenatal and postnatal genotype interpretation; and impact on couples and caregivers.

**Results:** Phase 1: Five of six known diagnosis could be confirmed by WES using our prenatal gene panel. HPO was not helpful in filtering variants. One causal pathogenic *PTPN11* mutation was missed due to generally low capture efficiency and thus coverage in WES data. Modeling the WES pipeline shows a theoretical turn-around time of 8 working days after the invasive procedure, which is sufficient in most cases. Phase 2: The preliminary results of the prospective study will be available during the conference. Approximately 20 rapid trio WES analysis are expected.

**Conclusions:** Retrospective analysis and modeling of our pipeline show that implementing WES as a routine test in the prenatal setting is technically feasible in our center. With our implementation study we aim to investigate the feasibility, pro’s and con’s of prenatal WES in daily practice.

6-3

**The ethics of clinical applications of germline genome modification: A systematic review of reasons**

Ivy Van Dijke¹, Lance Bosch², Annelien Bredenoord³, Martina Cornel⁴, Sjoerd Repping², Saskia Hendriks²

¹VU University Medical Center Amsterdam (VUMC) and Academic Medical Center University of Amsterdam (AMC), Amsterdam, Netherlands
²Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands
³UMC Utrecht, Utrecht, Netherlands
⁴VU University Medical Center, Amsterdam, Netherlands

**Objectives:** Germline genome modification is still unsafe and insufficiently effective for clinical purposes. However, the recent progress made using CRISPR-Cas has led scientists to expect to overcome the technical hurdles in the foreseeable future. This has invited a fierce debate on the socio-ethical and legal implications of germline genome modification. A systematic
overview of the reasons presented in the literature in favour or against germline genome modification is missing. We aimed to identify all reasons that have been presented for or against the future clinical application of germline genome modification.

**Methods:** The database ‘Medline/Pubmed’ was systematically searched for articles published between January 2011 and June 2016. Articles were selected based on eligibility, and the reference lists of eligible studies were hand searched. All types of articles (e.g. reviews, opinion articles), except for original biological research were eligible. Articles covering reasons for or against clinical application of intentional modification of the nuclear DNA of the germline (i.e. embryo, zygote, gametes or precursor cells of gametes) were included.

**Results:** A total of 169 reasons were identified: 90 reasons for, and 79 reasons against clinical application of germline genome modification. None of the included articles mentioned more than 60/169 reasons. The reasons could be categorised into: (i) quality of life of affected individuals; (ii) safety; (iii) effectiveness; (iv) existence of a clinical need or alternative; (v) costs; (vi) effects on homo sapiens as a species; (vii) social justice; (viii) potential for misuse; (ix) special interests exercising influence; (x) parental rights and duties; (xi) comparability to acceptable processes; (xii) rights of the unborn child; (xiii) human life and dignity.

**Conclusions:** Clinical introduction of germline genome modification should only be considered based on reassuring outcomes of appropriate preclinical effectiveness and safety studies. In the meantime, there is an evident need for a proper pre-implementation process which should address all reasons provided. Such a pre-implantation process is essential for a responsible introduction of germline genome modification or any other new medical technique with potential large scale socio-ethical and legal implications. The provided overview of all reasons will aid in allowing for a systematic and thorough debate on the introduction of germline genome editing.

6-4

**Prenatal diagnosis for single gene disorders in Victoria, Australia, 1977-2015**

Alice Poulton, Jane Halliday, Sharon Lewis, David Amor, Lisa Hui

*Murdoch Childrens Research Institute, Parkville, VIC, Australia*

**Objectives:** This study aimed to examine the historical and contemporary use of prenatal diagnosis (PnDx) for single gene disorders in the Australian state of Victoria (~70,000 annual births), over a 39 year period. The type and scope of disorders for which PnDx has been done were described to reflect how such testing had changed with advances in genetic technology and knowledge of the human genome. The study also aimed to examine information on preimplantation genetic diagnosis (PGD) for single gene disorders.
Methods: This population-based study included data held in a register of all women in Victoria who had PnDx from 1977-2015. Single gene disorders were categorised using a systematic hierarchical approach designed to reflect potential distinctive aspects of the PnDx decision-making process e.g. type of potential disability (physical or neurodevelopmental), severity, and age of onset of the disorder. Data on PGD for single gene disorders from the two clinics undertaking these tests in Victoria were categorised in the same way for comparison. Trend data were analysed using chi squared tests.

Results: There was an initial increase in PnDx for single gene disorders ($\chi^2=19.18, p<0.001$), steadying at ≈115 each year since the late 1990s. The scope of disorders has doubled (n=22 in 1993, n=45 in 2015). Most tests (68%) were for disorders that primarily impair physical ability, while disorders impairing cognitive ability comprised 21%. Adult onset conditions (3%) and disorders lethal in infancy (2%) were proportionately low. 5% were unable to be categorised. PGD for single gene disorders has seen rapid growth with approximately 25% done for adult onset conditions.

Conclusions: PnDx for single gene disorders is performed in about 1 in 500 pregnancies, with no observable changes since PGD and carrier screening became available during the past decade. Testing for adult onset disorders is not common during pregnancy, but appears to be more acceptable in the assisted reproduction setting. Advancing technologies in fetal genomic sequencing will lead to further shifts in testing for single gene disorders, however the direction of change is unknown. Understanding and reporting trends and changes can contribute to planning future service delivery, providing an overview of interest in and scope of single gene testing to date.

E-1

The BElgian PREnatal MicroArray consortium: Towards relating prenatally detected CNVs, prenatal phenotype and postnatal clinical data

Joke Muys1, Bettina Blaumeiser2, Yves Jacquemyn1, Koenraad Devriendt3, Sandra Janssens4, Kathelijn Keymolen5, Sonia Rombout6, Jean-Stéphane Gatot7, Julie Desir6, Yves Sznajer8, Marije Meuwissen2, Joris Vermeesch3, Bjorn Menten4, Annelies Fieuw5, Benoît Parmentier6, Saskia Bulk7, Bruno Pichon9, Claude Bandelier8, Erik Fransen1, Kris Van Den Bogaert3, Annelies Dheedene4, Marjan De Rademaeker5, Anne Destree6, Winnie Courtens7, Anne Deleener10, Nathalie Brison11, Olivier Vanacker4, Ann Van Den Bogaert5, Mauricette Jamar7, Patrizia Chiarappa12, Damien Lederer6, The Belgian Microarray Prenatal Consortium (BEMAPRE), Katrien Janssens2

1University Hospital Antwerp, Edegem, Belgium
2Center for Medical Genetics, Universiteit Antwerpen, Antwerp, Belgium
3Center for Medical Genetics, Katholieke Universiteit Leuven, Leuven, Belgium
4Center for Medical Genetics, Universiteit Gent, Ghent, Belgium

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Objectives: The uncertainty concerning the significance and clinical implications of some prenatally identified CNVs detected using microarray (CMA) underscores the urgency and importance of the development of an appropriate database relating prenatal genetic and ultrasound findings with postnatal clinical and neurodevelopmental data. The unique BELgian PREnatal MicroArray (BEMAPRE) collection allows us to identify the most frequent pathogenic CNVs, susceptibility CNVs and VOUS in the Belgian prenatal population and to calculate added values for the use of CMA versus karyotyping. Moreover, this database is the basis for longitudinal studies on the developmental effect of CNVs.

Methods: The BEMAPRE consortium is a collaboration of all eight genetic centers in Belgium. We collected data from invasive prenatal procedures performed between May 2013 and July 2016. In this period, 13266 prenatal CMAs were performed in Belgium. For each invasive prenatal procedure, centers provided the indication for the invasive test and the obtained CMA results. Descriptive statistics were used to describe population, patient and CNV characteristics. SPSS 24 was applied to analyze data. Frequency tables describing the association between indication and mutation type were visualized using correspondence analysis.

Results: Invasive prenatal procedures were performed in on average 3.6% of pregnancies per year. There was a gradual decline of 61.3% since 2007. Added values for using CMA versus conventional karyotyping were 1.8% in the general invasive population; 2.7% in cases with an ultrasound anomaly. The 22q11.2 duplication and the 15q11.2 duplication are respectively the most frequently reported and unreported susceptibility CNVs. Correspondence analysis did not detect an association between indication of the test and finding a susceptibility CNV. Up to 22.1% of reported diagnoses would have remained undetected with ‘genome-wide NIPT’ as the first-tier test. Postnatal follow-up is currently ongoing.

Conclusions: Our prenatal strategy is unique, as Belgium is currently the only country with a nationwide uniform approach of prenatal CMA analysis, reporting and communal CNV data storage. Only a limited number of susceptibility CNVs and none of the VOUS are reported. No association between the indication of the test and finding a susceptibility CNV was detected. With the implementation of NIPT, invasive prenatal testing increasingly becomes restricted to pregnancies with ultrasound anomalies and those with a familial genetic disorder. Subchromosomal pathogenic CNVs will be missed.
Improving uptake of perinatal autopsy: Lessons learned from parents and key stakeholders

Celine Lewis¹, Megan Riddington², Melissa Hill³, Zahira Latif⁴, Monica Lakhanpaul⁵, Owen Arthurs⁶, John Hutchinson⁷, Neil Sebire⁸, Lyn Chitty⁹

¹North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom
²Genetics and Genomic Medicine, Great Ormond Street Hospital and UCL Institute of Child Health, London, United Kingdom
³NE Thames Regional Genetics Laboratory, Great Ormond Street Hospital NHS Foundation Trust & UCL Great Ormond Street Institute for Child Health, London, United Kingdom
⁴College of Medical and Dental Sciences, Birmingham, United Kingdom
⁵Faculty of Population Health Sciences, London, United Kingdom
⁶Department of Radiology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom
⁷Department of Histopathology, London, United Kingdom
⁸Department of Histopathology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom
⁹Genetics and Genomic Medicine, UCL Great Ormond Street Institute of Child Health and North-East Thames Regional Genetics Service, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom

Objectives: Autopsy examination is the single most useful investigation in providing information to parents about why their baby or child died. Nevertheless, uptake rates have declined globally due to dislike of the invasive procedure, poor communication and religious objections. A number of less invasive techniques have been developed in recent years including MRI based imaging techniques with/without laparoscopic examination and organ biopsy. Here we aim to: 1) understand current barriers to parental consent to autopsy, 2) explore parental views towards less invasive autopsy, 3) examine attitudes of Muslim and Jewish communities towards less invasive techniques.

Methods: This was a qualitative study involving thematic analysis of: (1) free-text comments from 856 questionnaires from bereaved parents; (2) interviews with 20 questionnaire responders; (3) interviews with 16 religious and community leaders (6 Muslim, 6 Jewish and 4 from other dominant faiths in the UK as a comparator); and (4) ten focus groups with Muslim (n=60) and Jewish (n=16) parents. Bereaved parents were recruited through seven hospitals in England and four support groups in the UK (Antenatal Results and Choices, Sands, The Lullaby Trust and Child Bereavement UK). Muslim and Jewish parents were recruited through key informants from those communities.

Unedited draft - unpublished
Results: Regarding standard autopsy, for some parents the need for answers outweighed invasiveness concerns, however for others the child having “been through enough” was a barrier to consent. Non-invasive autopsy (NIA) enabled the child to “rest in peace” and put parents “at ease”. Minimally invasive autopsy (MIA) was a “good compromise” as it overcame limitations of NIA but enabled tissues samples to be taken. Muslim and Jewish participants agreed that whilst NIA was religiously permissible, MIA was less acceptable but where required by law was more acceptable than standard autopsy. The need to bury the body swiftly was a key priority.

Conclusions: The majority of parents are undecided about autopsy in the initial period after loss. Availability of less invasive options is likely to have a significant impact on uptake rates and decrease the burden of decision-making for many parents. Clear guidance around timing, communication and support are also likely to have a positive impact. Our research suggests that less invasive autopsy offers a viable alternative to many Muslim and Jewish parents who currently decline. These findings are relevant to healthcare providers involved in offering or conducting fetal, perinatal or paediatric autopsy, especially in countries with significant Muslim and/or Jewish communities.

E-3

Sensitivity of non-invasive prenatal testing for cancer detection and treatment monitoring in pregnant women

Liesbeth Lenaerts¹, Joris Vermeesch², Nathalie Brison³, Maria Neofytou⁴, Magali Verheecke⁵, Luc Dehaspe⁶, Hans Wildiers⁷, Sigrid Hatse⁸, Frédéric Amant⁵

¹Catholic University Leuven, Leuven, Belgium
²Center for Medical Genetics, Katholieke Universiteit Leuven, Leuven, Belgium
³Center for Human Genetics, University Hospital Leuven, Leuven, Belgium
⁴The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus
⁵UZ/KU Leuven, Leuven, Belgium
⁶Center for Human Genetics, KU Leuven, Leuven, Belgium
⁷Medical Oncology, University Hospitals Leuven, Leuven, Belgium
⁸Department of Oncology, KU Leuven, Leuven, Belgium

Objectives: We developed and implemented a genomewide cell-free DNA analysis pipeline, coined GIPSeq (Genomic Imbalance Profiling from cell-free DNA SEQuencing) for non-invasive prenatal testing (NIPT), allowing genome-wide detection of foetal and maternal chromosomal imbalances. Analysis of about 40,000 asymptomatic pregnant women identified 7 profiles reminiscent of cancer-related copy number variations (CNVs). All seven were referred to the oncology unit and whole-body diffusion-weighted magnetic resonance imaging uncovered a malignant process in six of them. Based on these incidental findings, we set out to explore the sensitivity of GIPSeq for cancer detection and treatment monitoring in pregnant women.
Methods: Pregnant women diagnosed with invasive breast or cervical cancer (i.e. the most prevalent solid cancer types encountered during pregnancy) were recruited in University Hospitals Leuven or via the International Network on Cancer, Infertility and Pregnancy (INCIP). A plasma sample was taken for GIPSeq. Non-pregnant premenopausal breast or cervical cancer patients were included as controls. In case of an aberrant GIPSeq-profile, consecutive plasma samples were taken to assess treatment response. Tumour biopsy specimens were subjected to shallow sequencing to investigate the specificity of CNVs detected in cell-free DNA.

Results: Pregnant breast (n=17) and cervical (n=4) cancer patients, and non-pregnant breast (n=17) and cervical (n=11) cancer controls were enrolled. For breast cancer, detection of abnormal GIPSeq-profiles significantly correlated with tumour stage. Furthermore, sensitivity was 3.3 times higher in pregnant (47.1%) than in non-pregnant (14.3%) breast cancers; the underlying reasons being explored. No aberrant GIPSeq-profiles were detected in pregnant cervical cancers, whereas sensitivity was 18% in non-pregnant controls and not depending on cancer stage. For true positive cases, the CNV-profile of tumor biopsy DNA showed comparable genomic imbalances as seen in circulating DNA. Furthermore, GIPSeq-profiling was able to follow treatment response.

Conclusions: These preliminary results indicate that GIPSeq of circulating cell-free DNA is able to detect cancer-specific CNV-profiles in a proportion of cancer cases, and that detection might depend on the pregnancy status of the women. Further recruitment is ongoing and supplementary analyses are planned to improve the sensitivity of the pipeline for cancer detection. Optimizing a cancer detection and treatment monitoring in pregnant women may ultimately lead to improved prognosis.

LB-1

The potential diagnostic yield of Whole Exome Sequencing in 391 pregnancies complicated by fetal ultrasound anomalies and with normal chromosomal microarray results

Kathleen Romijn1, Malgorzata Srebniak1, Marike Polak2, Alice Brooks1, Yolande van Bever1, Grazia Verheijen-Mancini1, Marieke Joosten1, Lutgarde Govaerts1, Joan Kromosoeto1, Maarten F.C.M. Knapen3, Attie Go4, Marjon van Slegtenhorst1, Hennie Bruggenwirth1, Diane Van Opstal4, Lies Hoefsloot1, Robert-Jan Galjaard1, Karin Diderich1

1Department of Clinical Genetics, Erasmus MC, Rotterdam, Netherlands
2Institute of Psychology, Erasmus University Rotterdam, Rotterdam, Netherlands
3Department of Obstetrics and Gynecology, Erasmus MC and Foundation Prenatal Screening Southwest Region of the Netherlands, Rotterdam, Netherlands
4Erasmus Medical Center, Rotterdam, Netherlands

Objectives: The aim of this retrospective cohort study is to determine the potential diagnostic yield of prenatal whole exome sequencing (WES) in fetuses with ultrasound abnormalities and with normal chromosomal microarray results. In cases of fetal abnormalities, it is now standard
practice to perform chromosomal microarray, but whole genome testing for single-gene mutations is not yet routinely offered. Possibly, couples who have chosen for prenatal array testing would also choose for prenatal WES to discover the cause of the ultrasound anomaly found in their pregnancy.

**Methods:** In the period January 1st, 2013- January 1st, 2017 845 pregnant women with fetal ultrasound anomalies who received normal microarray results were referred for additional genetic counselling. 391 couples wanted additional molecular testing to be performed. The couples were not selected based on the severity of ultrasound anomalies. Most of the couples received only targeted molecular testing and in only 131 cases WES (large panels or open exome) was performed (after birth). The results of these molecular tests were evaluated retrospectively, regardless of the time of the genetic diagnosis (before or after birth).

**Results:** In 81 (20.7%) of 391 fetuses molecular testing provided a genetic diagnosis with identification of (likely) pathogenic variants in 58 different candidate genes. In 84% of the abnormal cases (68/81) the variant was clearly pathogenic and would be reported prenatally regardless of the fetal phenotype. In the remaining cases, additional phenotypic data were necessary for the interpretation of the molecular results and clinical geneticists could finally conclude that these findings were (very likely) causal. In one diagnosed case an additional unexpected/incidental finding was detected, which concerned an early onset syndromic disorder.

**Conclusions:** Our retrospective cohort study shows that WES, if routinely offered to patients in a prenatal setting, would significantly increase the diagnostic yield in fetuses with ultrasound abnormalities. However because not all fetuses were tested with WES and some of the investigations were not yet completed at the time of the study, we presume that this yield of 20.7% could be an underestimation. Concluding, WES if performed prenatally would lead to an early diagnosis of a genetic disorder in a significant percentage of cases irrespective of the (incomplete) fetal phenotype.

**LB-2**

**A new era in aneuploidy screening for multifetal gestations: Clinical laboratory experience screening >30,000 cell-free DNA samples**

Brittany Dyr¹, Theresa Boomer², Eyad Almasri², Vanessa Nitibhon², Jason Chibuk³

¹Integrated Genetics, Ponte Vedra, FL, United States  
²Sequenom Laboratories, San Diego, CA, United States  
³Sequenom Inc., Integrated Genetics, De Pere, WI, United States

**Objectives:** Traditional serum screening for twins demonstrates lower sensitivity and higher false positive rates, and pseudo aneuploidy risk calculations when compared to performance in singleton gestations.¹ Clinical validation studies have established high sensitivity and specificity
of cell-free DNA (cfDNA) screening for aneuploidy in singleton and multifetal pregnancy\textsuperscript{2-4}, especially when compared to performance of traditional serum screening. Since introducing cfDNA screening in 2011, Sequenom Laboratories has analyzed over 750,000 clinical samples. More than 30,000 of these samples are from multifetal gestations (including twins, triplets and higher order multiples). The clinical laboratory experience with the first 30,000 multifetal samples will be discussed.

**Methods:** More than 30,000 maternal plasma samples from multifetal gestations were subjected to DNA extraction and library preparation followed by massively parallel sequencing as described by Jensen et al.\textsuperscript{2} Sequencing data were analyzed to detect autosomal trisomies and other subchromosomal events as described by Zhao et al.\textsuperscript{3} Fetal fraction requirements were adjusted in proportion to fetal number. Outcome data were collected through provider solicitation.

**Results:** The predominant indication for testing in this large multifetal cohort was advanced maternal age (>60%). Compared with singletons, in which 6.1% of samples indicated abnormal serum screening, this was the indication for testing in multifetal gestations in 3.5% of samples. The positivity rate in multifetal samples for trisomy 21 was 1.50%, 0.47% for trisomy 18, and 0.21% for trisomy 13. The test had a total non-reportable rate of 5.95%. Average fetal fraction for all samples was 12.2%. Estimated performance based on ad hoc clinical outcome shows that sensitivity and specificity meet or exceed the original performance from clinical validation studies.

**Conclusions:** In over half a million samples submitted to one clinical laboratory, approximately 4% of samples are from multifetal gestations, which is greater than the rate of multiple births in the US\textsuperscript{5}, suggesting that providers are turning to cfDNA for aneuploidy screening for multifetal gestations. CfDNA screening overcomes some disadvantages of traditional serum screening in multifetal gestations, including providing a result for trisomies 18 or 13 and the availability of screening in higher order multiples. MaterniT\textsuperscript{®}21 PLUS offers patients with multifetal gestations accurate and reliable screening for fetal aneuploidy and has met or exceeded performance from the original clinical validation studies.