The utility of whole exome sequencing in prenatal diagnosis

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OBJECTIVES: During the past several years, clinical whole-exome sequencing (WES) has been utilized for patients with complex clinical presentations. With a molecular diagnosis rate of about 30%, clinical WES is an effective way of ending the expensive and time-consuming diagnostic odyssey. With the continuous improvement of WES methodology including updated sequencing instrumentation, shorter turnaround time (TAT) and improved analysis, clinical exome is now considered for prenatal diagnosis. Here, we report the first 52 consecutive prenatal cases. Prenatal cases are defined as a fetal sample obtained through either an invasive procedure or a product of conception (POC). METHODS: This cohort includes 37 cases with WES performed for the proband only followed by Sanger sequencing studies of parental samples and 15 trio WES. For the final report, all contributing changes are confirmed by Sanger sequencing for trio cases. The exome sequencing was performed on the Illumina HiSeq2500 with an average of ~11.4 Gb data per exome, and 97% of the targeted exome regions are sequenced at a depth of 20X. Exome sequencing data were analysed for small nucleotide changes and large CNVs. Individual DNA samples were analysed by the Illumina HumanExome-12v1 array for quality control and large CNVs detection. RESULTS: The proband WES yielded a diagnosis of 32% (12/37) cases, whereas trio WES provided diagnosis in 40% (6/15) cases. For the five patients with a brain abnormality on ultrasound, we were provided diagnosis for three individuals (60%), for the 21 patients with a brain anomaly and other organ system involved, we provided diagnosis for seven patients (33%) and for the 26 patients with an anomaly not including the brain, we provided a molecular diagnosis for eight patients (31%). For individuals with cardiac anomaly and other organ system anomaly (six patients), we were able to provide diagnosis for three individual (50%). CONCLUSIONS: With rapid turnaround time and comprehensive analysis, the prenatal trio WES is a valuable tool in prenatal diagnosis. The next step in improving the current test will include analysis of DNA from direct chorionic villus sampling or amniotic fluid to further reduce the TAT.

Validation of non-invasive prenatal diagnosis (NIPD) of multiple single gene disorders for clinical implementation

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OBJECTIVES: Through the NIPSIGEN project (Non-Invasive Prenatal diagnosis for Single Gene disorders; funded by the Health Innovation Challenge Fund; HICF-R6-381), we have recently developed a clinical test for the non-invasive prenatal diagnosis (NIPD) of Duchenne and Becker muscular dystrophies (DMD/BMD) in at-risk pregnancies. Building on this experience, we are now validating an improved NIPD test for multiple single gene disorders, namely, DMD/BMD, spinal muscular atrophy (SMA), congenital adrenal hyperplasia (CAH) and cystic fibrosis (CF), which we intend to implement into clinical practice within the UK National Health Service (NHS). METHODS: Cell-free DNA (cfDNA) was extracted from maternal plasma; maternal, paternal and proband DNA was extracted from leukocytes. DNA sample libraries were prepared for massively parallel sequencing on an Illumina MiSeq using a custom built capture probe library. Over 12 000 SNPs with >40% probability of being heterozygous were targeted across multiple genomic regions containing the genes of interest on chromosomes X, 5, 6 and 7. Sequencing data were analysed by relative haplotype dosage (RHIDO) on informative SNPs to determine fetal inheritance of the maternal and paternal mutant alleles using the proband haplotypes as reference. RESULTS: The test achieved 100% sensitivity and specificity on patients tested so far, which included four pregnancies at risk of SMA, DMD/BMD and at-risk of SMA. All results concurred with expected outcomes apart from one DMD patient for whom an inconclusive result was observed. Test validation was also performed on 11 healthy control pregnancies, where the fetal DNA obtained from the CVS was used to identify the reference haplotypes. As a result, test simulation of nine DMD/BMD, four SMA, two CAH and two CF cases was successfully achieved. CONCLUSIONS: To date, the implementation of NIPD testing for single gene disorders into clinical practice has been hindered by the elevated costs incurred. In this respect, our method allows for a multiplexing capacity of two to three patients per MiSeq sequencing run, while maintaining a high testing accuracy and allowing for multiple disorders to be tested simultaneously. Together, these characteristics render the test feasible from a clinical perspective, potentially opening the path for implementation of NIPD for single gene disorder testing into clinical practice in the UK and elsewhere.
1-4
Non-invasive prenatal diagnosis (NIPD) for cystic fibrosis – beyond paternal mutation exclusion
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OBJECTIVES: Cystic fibrosis (CF) is a relatively common, serious genetic disorder with an incidence of 1/2500 in North European Caucasians. In our Regional Genetics Laboratory, we offer NIPD for the detection or exclusion of the paternal allele in pregnancies at risk of CF where parents are heterozygous for different mutations. However, definitive prenatal diagnosis in high-risk pregnancies where parents carry the same mutation or where we detect the paternal allele using NIPD is currently only available by invasive prenatal testing. Our objective was to develop a definitive non-invasive prenatal diagnostic assay for CF, regardless of the genotype.

METHOD: Parental and proband DNA was prepared using the Agilent SureSelect XT protocol and cfDNA extracted from 4 mL of maternal plasma using the NEBNext® Ultra II kit. Libraries were enriched using an Agilent SureSelect Custom Enrichment Assay targeting CFTR and flanking highly heterozygous SNPs. Libraries were sequenced on an Illumina NextSeq. Parental and proband samples were used to link SNPs with the mutant allele. In cfDNA, SNP analysis identified the presence of a paternal mutant allele and relative haplotype dosage analysis allowed us to determine if the maternal mutant allele had been inherited. RESULTS: Of four retrospective cases tested to date, our approach could be used to correctly genotype the fetus in all cases as summarized in the table below. Further cases are being tested and will be reported. CONCLUSIONS: We have successfully developed NIPD for definitive diagnosis of CF. This will extend our NIPD service to allow maternal allele detection including families where parents carry the same mutation; due to the high (4%) carrier frequency of the CFTR p.Phe508del mutation, this will occur in an estimated 47% of carrier parents. In this retrospective, cohort 4/4 correct diagnoses were made. Prospective samples will now be tested. While DNA from an affected proband is currently required to assign SNP haplotypes, advances in technology such as synthetic long reads will mean that in the future proband, DNA may not be needed.

1-5
Haplotype-assisted noninvasive prenatal diagnosis of common monogenic diseases using massively parallel sequencing of plasma cell-free DNA
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OBJECTIVES: Since 2012, haplotype-assisted noninvasive prenatal diagnosis (NIPD) has been developed in our laboratory, and validated in a number of monogenic diseases using clinical samples from trio families. The purpose of this study was to summarize the performance of this method in different monogenic diseases. METHOD: Customized panels were designed to capture the coding region and flanking area of disease-associated genes. Genomic DNA from each trio family (father, pregnant mother and proband child) and plasma cell-free DNA from each pregnant mother were target-sequenced using the customized panels. Fetal fraction was calculated with the differently homozygous SNPs in both parents. Parental and fetal haplotype were deduced based on genetic information from the trio family and hidden Markov model. Amniocentesis was performed to confirm NIPD results. RESULTS: A total of 97 trio families covering 12 monogenic diseases have been tested before receiving prenatal diagnosis (Table). The mutations consisted of point mutations and copy number variants. Mean gestational age of pregnant women while receiving NIPD was 18 weeks. Fetal fraction was between 2.8% and 22.6%, with the mean of 9.1%. Sixty pregnancies were identified as disease-affected. So far, amniotic fluid was tested for prenatal diagnosis in 70 pregnancies, all consistent to NIPD results. CONCLUSIONS: Haplotype-assisted NIPD showed high accuracy in predicting the existence of fetal monogenic disease and can be used in different diseases and mutation types.

1-5 Table. Summary of monogenic diseases that have been tested by NIPD

| Family ID | Paternal mutation | Maternal mutation | Fetal fraction (%) | NIPD result (Pat/Mat) | Matched invasive result |
|-----------|-------------------|-------------------|--------------------|-----------------------|------------------------|
| 9         | p.Phe508del/N     | p.Phe508del/N     | 11                 | p.Phe508del/p.Phe508del | Affected               |
| 10        | p.Phe508del/N     | p.Phe508del/N     | 10                 | N/p.Phe508del          | Unaffected Carrier     |
| 11        | p.Phe508del/N     | c.3528delC/N      | 13                 | p.Phe508del/c.3528delC | Affected               |
| 13        | p.Phe508del/N     | p.Phe508del/N     | 23                 | N/p.Phe508del          | Unaffected Carrier     |

1-4 Table.
### 2-1

**Expansion of assessment methods to measure effects of fetal spina bifida induction and repair in the fetal lamb**

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OBJECTIVES: The Management of Myelomeningocele Study (MOMS) provided level-I evidence that fetal spina bifida aperta (SBA) repair by open approach, as opposed to postnatal repair, reduces the need for ventriculo-peritoneal shunting and improves motor outcomes at 30 months. Since less invasive techniques are contemplated, we aimed to add sensitive and more comprehensive assessment tools for evaluating outcomes in the fetal lamb model. METHODS: Spina bifida aperta was surgically induced without or with myelotomy at 75 days of gestation (term = 145 days). Myelotomy-SBA was repaired at 100 days using a standard open approach. Lambs were delivered by cesarean section just prior to term (median: 142 days). At 1 to 2 days postnatally, they underwent gross examination, neurologic assessment, whole-body magnetic resonance neuroimaging (MRI), somatosensory and motor evoked potentials (SEP and MEP) and histology of the central nervous system. RESULTS: Spina bifida aperta was successfully induced in 35 fetuses (nine without and 26 with myelotomy), without intra-operative losses. In seven fetuses, it was repaired. Currently 13 ewes have been delivered of 31 lambs: eight were dead prenatally (4/13 SBA, 4/18 controls), two SBA had lumbar evisceration. The seven SBA survivors were compared to the 14 controls: 4/7 had lumbar leakage, 1/7 had spontaneous complete skin closure; 7/7 had hindlimb somatosensory deficits; MRI confirmed 4/7 lumbar cerebral-spinal fluid leakage and 2/7 hindbrain herniation; 4/4 and 2/2 had respectively absent hindlimb SEP and MEP; 5/5 had disrupted spinal cord covered with fibrotic tissue. CONCLUSIONS: Comprehensive structural and functional assessment of the SBA fetal lamb model is feasible and reproducible, which can be used to measure effects of utero induction and repair.

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### 2-2

**Connexin 43 is overexpressed in human fetal membrane defects after fetoscopic surgery**

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OBJECTIVES: Preterm premature rupture of the fetal membrane (PPROM) remains a major complication after fetoscopic interventions because the defect in the amniotic membrane (AM) does not heal spontaneously. We examined whether surgically induced membrane defects elevate connexin 43 (Cx43) expression in the AM wound edge and drives structural changes in collagen architecture that affects healing after fetoscopic surgery. METHODS: Fetal membranes were collected at cesarean delivery from women who underwent fetal surgery (fetoscopic surgery n = 12; hysterotomy n = 2) between 18 and 29 weeks before birth. Collagen microstructure in the amniochorion was investigated by scanning electron microscopy (SEM). Collagen alignment in the wound edge of the AM was examined by Second Harmonic generation (SHG). Immunofluorescence was used to examine cell morphology and compare Cx43 expression in the epithelial and fibroblast layer of the AM. Cx43 gene expression was quantified by RT-qPCR in wound edge and intact control AM collected from the same patient. RESULTS: Scanning electron microscopy showed densely packed collagen in the wound edge of the amniochorion. This collagen arrangement changes in the fibroblast layer with evidence of highly aligned collagen fibers along the wound edge AM but not in control membranes. Cx43 expression was increased by 112.9% in wound edge AM compared to control membranes (p < 0.001), with preferential distribution in the fibroblast layer compared to the epithelial layer (p < 0.01). Mesenchymal cells had a flattened morphology, and there was...
Evidence of poor epithelial cell migration across the fetal defect. Cx43 gene expression was significantly increased in wound edge AM compared to patient matched controls \((p < 0.001)\).

CONCLUSIONS: We propose that overexpression of Cx43 in the fetal AM after fetal surgery induces morphological and structural changes in the collagenous matrix that interferes with normal remodeling mechanisms. These processes may help to explain the increased tissue weakening and incidence of preterm birth post fetoscopic surgery.

BOOSTB4 – A clinical study on pre- and/or postnatal stem cells transplantation for treatment of osteogenesis imperfecta

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OBJECTIVES: Osteogenesis imperfecta (OI) is a heterogeneous inherited condition, severe forms of which present in-utero. There is no cure or effective treatment. Severely affected individuals suffer lifetime multiple fractures giving pain, short stature and orthopedic problems. Preliminary clinical experience in three cases indicates that transplantation of fetal liver mesenchymal stem cells (MSC) before and after birth may ameliorate symptoms. The main objective of the Boost Brittle Bones Before Birth (BOOSTB4) phase I/II multicentre study is to evaluate the safety and efficacy of prenatal and/or postnatal MSC transplantation in the severest viable forms of OI (type III and severe type IV). METHODS: Rapid exome sequencing using a panel targeted for skeletal disorders is underway, and the clinical trial protocol for prenatal and postnatal therapy and follow-up is being finalized. To inform further development of rapid in-utero diagnosis and NIPD, we seek referrals for rapid exome sequencing and development of NIPD. Recruitment to the main treatment study will soon commence. Over 2 years, the patients will receive multiple infusions of same-donor MSC. CONCLUSIONS: Prenatal stem cell transplantation shows promise for the treatment of inherited single gene disorders. Demonstration that transplantation before birth improves early outcome in patients with severe OI would represent a major step forward in their management. If successful, such treatment could be relevant for the management of a range of other inherited birth defects. The BOOSTB4 consortium welcomes clinical cases for diagnosis of OI using rapid exome sequencing or NIPD and, for the first time, inclusion of European patients in the clinical trial on treatment of OI with fetal MSC prenatally and/or postnataally. Contact Cecilia Götherström for more information: Cecilia.Gotherstrom@ki.se

External quality assessment of testing cell free DNA for fetal sex determination

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OBJECTIVES: Participation in external quality assessment (EQA) enables a laboratory to measure the standard of testing compared to other laboratories providing a similar service. The introduction of cell free fetal DNA testing provided EQA providers with a challenge to be able to deliver assessment for a technique with such low levels of DNA. The issues arising around sourcing, validating and transporting suitable EQA material to participating laboratories are discussed. METHODS: UK National External Quality Assessment Service (UK NEQAS) for Molecular Genetics and European Molecular Quality Testing Network (EMQN) provided three pilot EQAs between 2013 and 2015, to up to 36
laboratories from 18 countries. Three plasma samples, with corresponding clinical cases, were distributed for each EQA run. Testing to determine fetal sex using cell free DNA (cfDNA) techniques was requested. The 2013 EQA run required the sexing results to be reported as stand-alone clinical reports. Subsequent runs were developed to provide full clinical details and therefore required the sexing results to be reported in the context of the clinical scenario. RESULTS: Results were scored for the accurate reporting of the presence/absence of Y chromosome specific DNA sequences and a conclusion that the result indicated a male/female fetus. Sexing accuracy was high with only two sexing errors reported in the 2014 EQA and one error reported in 2015. Differences were observed when reporting important limitations of the tests performed and the subsequent impact of the result to the patient. Report formats were variable, and often the sex of the fetus was not clearly stated. Feedback comments were provided to all participants for each EQA run. CONCLUSIONS: External quality assessment is possible for cfDNA sex determination, and continued participation has shown an improvement in the reporting of these results. However, as the diversity of applications and methodologies for cfDNA analysis increases, the pooling of EQA samples is not feasible, and other EQA approaches need to be explored.

3-2 Realization of the Belgian Prenatal Microarray (BEMAPRE) database and update of the Belgian reporting approach

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OBJECTIVES: In 2013, the eight Belgian genetic centers established national guidelines for prenatal copy number variant (CNV) classification and reporting. Following this uniform reporting policy, the idea arose to create a national prenatal CNV database, the BEMAPRE database. We report on the establishment and current status of this database. Because of the uncertain outcome of so-called susceptibility CNVs – risk factors for neurodevelopmental disorders with variable expression – a limited list of seven CNVs for which the risk for a severe phenotype was deemed sufficiently large, was composed. However, this list needs to be re-evaluated on a regular basis. METHODS: Belgian genetic centers agreed on using a database provided by Cartagenia NV (Agilent Technologies). It was decided to include all prenatal cases with a non-benign CNV with a minimal size of 400 kb. A simple, unambiguous, uniform labeling system for all CNVs was implemented. Criteria for minimal genotypic and phenotypic information were drafted. A small study group, consisting of geneticists, prenatal laboratory staff and ultrasound specialists, was established. Based on the most recent literature (both case reports and reviews), the original list of reported susceptibility CNVs was re-evaluated and adjusted where necessary. RESULTS: All centers have imported their data into the database or are in the process of doing so. Approximately, one third of all CNVs in the database are pathogenic in nature (55.4% de novo, 25.4% maternally inherited, 19.2% paternally inherited). Two thirds of cases in the database are variants of unknown significance (11.4% de novo, 45.7% maternally inherited, 42.9% paternally inherited). The first results will be presented. A few selected difficult cases that were discussed nationally will be presented. CONCLUSIONS: The BEMAPRE database is almost fully established; nearly all prenatal data are imported. The resulting database constitutes an elaborate source of data, which we will now start mining for genotype–phenotype correlations.

3-3 Maternal cell contamination may significantly confound the interpretation of prenatal NGS testing

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OBJECTIVES: Maternal cell contamination (MCC) poses a significant risk for prenatal misdiagnosis in molecular diagnostics. Previous studies using microarrays have demonstrated that 20% MCC can confound interpretation of clinical testing results. As next generation sequencing (NGS) is more sensitive than microarrays, even lower levels of MCC may compromise prenatal NGS results, affecting pregnancy management. This study examines the sensitivity and specificity of NGS variant calling from prenatal samples with varying levels of MCC. Such characterization is extremely important, as NGS is rapidly becoming the standard of care in prenatal molecular diagnostics for high-risk pregnancies. METHOD: Maternal cell contamination was simulated at levels of 1%, 5%, 7.5%, 10%, 20% and 50% by spiking maternal DNA into prenatal samples. The prenatal samples were previously confirmed to be negative for MCC by DNA typing assays. NGS was performed after capturing 78 genes via a targeted capture kit,
and variants were called using GATK. In addition to previously identified disease-causing variants, all discordant variants between the maternal and prenatal samples were compared to determine both sensitivity and specificity. RESULTS: All samples had an average coverage of 600X across the 78 genes. NGS sensitivity was compromised by as little as 5% MCC when the prenatal sample was homozygous for a variant, with 47% (8/17) of variants being miscalled, and at 50% MCC when the prenatal sample was heterozygous for a variant, with 49% (19/39) of variants being miscalled. NGS specificity was seriously compromised at 20% MCC with as high as 68% (36/53) of variants being miscalled when the prenatal sample was homozygous for the reference allele.

CONCLUSIONS: Maternal cell contamination may confound NGS testing, causing erroneous interpretation of clinical results and affecting pregnancy management. In particular, MCC affects NGS sensitivity at much lower contamination levels than observed for microarray analysis. NGS specificity is compromised at levels similar to what is observed for microarray analysis, but future work will determine if even lower levels of MCC affect specificity. Potential MCC must be considered when interpreting results from prenatal samples as levels as low as 5% may result in prenatal misdiagnosis.

4-1 Chromosomal microarray analysis in fetuses with aberrant right subclavian artery

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OBJECTIVES: The objective of this study was to evaluate the correlation between aberrant right subclavian artery (ARSA), with or without other ultrasound (US) abnormality, and risk factors for aneuploidies and chromosomal microarray analysis (CMA) results. METHOD: This was a multicentre study, and the genetic analysis was done in the same laboratory. Fetuses diagnosed with ARSA were evaluated by CMA. The clinical investigation included nuchal translucency, first and second trimester biochemical screening, early and late second trimester fetal anatomic scans and fetal echocardiogram. Comparative Genomic Hybridization (CGH) Microarray analysis or Single Nucleotide Polymorphism (SNP) Array technology was used for CMA. RESULTS: Chromosomal microarray analysis results were available for 63 fetuses. No pathogenic variants were found among 36 fetuses with isolated ARSA. Additional findings and/or risk for aneuploidies were present in 27 fetuses, of which five had pathogenic CMA results. Trisomy 21 (T21) – fetus with echogenic intracardiac focus (EIF); 22q11 deletion – fetus with EIF and 1:230 biochemical screening increased risk for T21; 22q11 duplication – fetus with hypoplastic right kidney and choroid plexus cysts; 22q11 deletion – fetus with right aortic arch and clubfoot; and both 22q11 deletion and 1q21 duplication – fetus with abnormal nuchal translucency (4 mm) and ventricular septal defect. CONCLUSIONS: The results suggest that in fetuses with ARSA as an isolated finding, an invasive procedure for CMA testing is not indicated. However, CMA is recommended when additional US abnormalities or risk factors for aneuploidies are detected. The chromosomal findings in four of the five abnormal cases were not DS.

Table 1. Indications for CMA in fetuses with ARSA and the results according to additional findings

4-2 Early sonographic evaluation of facial integrity in dystrophic fetuses via 3D-ultrasound

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OBJECTIVES: Evaluation of the impact of visualization of the retronasal triangle view (RNTV) on the diagnosis of cranio-facial anomalies in dystrophic viable and non-viable fetuses in the first and early second trimester via 3D multiplanar reconstruction using complete volume data sets. METHODS: A total of 121 complete three-dimensional volume data sets of fetuses with genetic and/or structural anomalies were analysed. After standardized acquisition of complete fetal head volume with the fetus facing the transducer the volume was adjusted to visualize the correct cranio-facial symmetry, presence of facial clefts and midfacial hypoplasia. RESULTS: In 112/121 cases, the multiplanar reconstruction of all anomalies in dystrophic viable and non-viable fetuses in the retronasal triangle view (RNTV) on the diagnosis of cranio-facial anomalies was feasible. Correct configuration of the RNTV was verified via navigating in the corresponding coronal plane and could be achieved in 29/48 non-viable fetuses and 56/64 viable fetuses. Using the same plane, an absent mandibular gap indicating a micrognathia was detected in 28 fetuses (seven non-viable and 21 viable fetuses). In 16 fetuses (4 vs 12), facial clefts were diagnosed. An absent nasal bone was identified in 27 volumes (4 vs 23). Chromosomal anomalies or single gene disorders could be confirmed in 55/112 cases. CONCLUSIONS: Anomalies of the maxilla-mandible complex may be frequently found in gestically altered and syndromic fetuses. 3D multiplanar evaluation of affected fetuses offers the opportunity of a reliable and reproducible evaluation of the cranio-facial integrity using the RNTV via standardized planes at an early gestational age. It can add valuable information to the prenatal assessment of dystrophic fetuses even in the non-viable ones where image clarity is often reduced.
OBJECTIVES: To evaluate males with XXY from the Netherlands (NL) and the United States (US) to compare and contrast their neurodevelopmental profile. METHODS: The NL (n = 44) and US cohort (n = 35) averaged 11.8 years of age. IQ profiles were measured using the Wechsler Intelligence Scales. The Amsterdam Neuropsychological Tasks (ANT) and the Child Behavior Checklist (CBCL) assessed neurocognitive profile. ANOVA was used to compare group differences. Multivariate regressions were used to assess contribution of independent variables. RESULTS: The US cohort had a higher SES (p<0.001), PIQ (p<0.001), and VIQ (p<0.001). A predictive effect was found for nationality, predicting reaction time (p = 0.001), VIQ (p<0.001), and PIQ (p<0.001). Pre/postnatal diagnosis predicted PIQ (p = 0.023), reaction time (p = 0.022), stability of reaction time (p = 0.01), and face memory (p = 0.01). SES was a significant predictor of VIQ (p = 0.002) and PIQ (p = 0.006) in the combined cohort and the US cohort, though not in the NL cohort. Testosterone was a (borderline) predictor for PIQ (p = 0.051). In the NL group testosterone was predictive of decreased total (p = 0.008) and internalizing problems (p = 0.044). CONCLUSIONS: This is the first international collaborative investigation of the neurocognitive capabilities of boys with XXY. This study further supports the need for early detection and hormonal treatment. The fact that nationality was a strong predictor of VIQ and PIQ implicates other unidentified variables, such as timing and frequency of services, which needs further investigation. SES and testosterone were associated with stronger cognitive profiles. Prenatal diagnosis was also shown to be highly predictive of improved neurodevelopmental outcome.

5-2

A large prospective study of the neurodevelopmental outcome in prenatally diagnosed males with 47, XXY

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OBJECTIVES: 47, XXY is the most commonly occurring X and Y chromosomal variation, affecting 1 : 660 men (Chang et al., 2015). When left untreated the phenotype is associated with androgen deficiency, language-based learning disorders, and developmental dyspraxia (Samango-Sprouse et al., 2013). Our previous research has shown that boys with 47, XXY who receive biologic treatment perform better on measures of neurocognition, neuromotor, and neurobehavior than untreated boys (Samango-Sprouse et al., 2015). We prospectively examined a large cohort of prenatally identified boys with 47, XXY and provide the first detailed characterization in more than 30 years. METHODS: A total of 158 prenatally identified males with 47, XXY were administered the Bayley Scales of Infant Development (BSID), the Wechsler Scales of Intelligence and Preschool Language Scales as part of a comprehensive neurodevelopmental examination. Thirty-nine of the boys had received Early Hormonal Treatment (EHT) in infancy, and 119 were untreated. Results were analysed across motor,
Randomized controlled trial comparing preimplantation genetic screening utilizing next generation sequencing to standard morphologic assessment for embryo selection and single embryo transfer after vitrification

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OBJECTIVES: The objective of this trial was to evaluate the efficacy of PGS in improving pregnancy outcomes by comparing ongoing pregnancy rates at 20 weeks of gestation in women undergoing in vitro fertilization (IVF) and single embryo transfer (SET) after vitrification in two groups. In the intervention group, a single embryo is selected for transfer using preimplantation genetic screening (PGS) with next generation sequencing (NGS), and in the control group, a single embryo is selected by standard morphologic assessment. METHOD: This is a single blinded, multicenter, randomized, controlled trial in women undergoing elective in vitro fertilization (IVF) cycles. Trophectoderm (TE) biopsy on day 5 or 6 of embryo development followed by PGS with NGS for embryo selection in the intervention group is compared to embryo selection based on standard morphology in the control group. Randomization is one to one stratified by three maternal age classes. All embryos groups undergo vitrification followed by SET in a subsequent unstimulated cycle. Primary outcome is ongoing pregnancy rates at 20 weeks of gestation between the two groups. RESULTS: Our recruitment strategy, including data based site selection, aggressive study start up with focused and ongoing collaborative face to face site visits, and appropriate resource allocation, has resulted in enrollment rates exceeding our timelines with full enrollment accomplished in 18 months. Enrollment commenced September 2014 and an adequate number of subjects have been enrolled to ensure 600 embryo transfers with at least 300 transfers in each arm. CONCLUSIONS: The regionally diverse settings and broad eligibility criteria included in this trial are applicable to the general IVF population. Large-scale trials such as this can support the use of PGS with SET to improve IVF outcomes, thus reducing the need for multi-embryo transfer and its higher incidence of multiple gestations. While this study was designed to address scalability and performance of PGS in a variety of patient populations and settings utilizing individualized practices, all sites met strict qualification criteria. The results of this study cannot be extrapolated to clinics that do not perform at this level with or without PGS.

Clinical experience with multigene carrier panels

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OBJECTIVES: The goal of carrier screening is to identify individuals at-risk for having a child with genetic disease. Professional guidelines recommend screening for select genetic conditions based on ethnicity. Expanded carrier screening includes diseases beyond what is currently recommended. A recent joint statement from professional societies acknowledges the potential benefits of this testing. However, limited data have investigated the positivity rates of expanded carrier screens. These data are critical to providers when deciding what testing to offer, and assessing implications for their patients and practices. We describe our experience with three screening panels varying in size from 3 to >200 disorders. METHODS: We reviewed outcomes for three multigene carrier screening panels: Trio (three diseases), Standard (23 diseases), and Global (>200 diseases). All panels utilized targeted genotype analysis of pre-selected mutations via next-generation sequencing (NGS). Standard and Global panels also included hemoglobinopathy evaluation (MCV and electrophoresis) and hexosaminidase-A enzyme analysis for Tay-Sachs disease screening. We calculated the frequency of positive results for each panel. RESULTS: Positivity rates were 5.37% for the Trio panel, 12.10% for the Standard panel, and 34.14% for the Global panel. The most frequent positive results in the Global panel were for (in descending order) hemoglobinopathies, glucose-6-phosphate dehydrogenase deficiency, cystic fibrosis, familial Mediterranean fever, pseudocholinesterase deficiency, spinal muscular atrophy, and primary congenital glaucoma. CONCLUSIONS: Two hundred diseases, the likelihood of identifying a carrier can be as high as 34%. Understanding panel positivity rates is important for providers to choose the right test for their practice, set appropriate expectations for patients, and plan for follow-up counseling and partner testing.
5-5

Aneuploidy screening in polarbodies is superior to trophectoderm analysis as it detects the meiotic errors and circumvents decision making of mosaic embryo results

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OBJECTIVES: Lately mosaic embryos have been transferred and resulted in life births. Mosaicism in early embryos develops through postzygotic mitotic errors to different degrees and varying persistence. Therefore, decisions which embryos to transfer – which degree of mosaicism in which affected chromosomes – are notoriously difficult, and there are no criteria which would assist. Alternatively, there are polar bodies as products of the two meiotic divisions providing clear information of the chromosome content of the maternal part (~90% of all meiotic errors) of the zygote. Therefore, aneuploidy screening in eggs gives relevant and robust information about which embryos to transfer. METHODS: As both polar bodies have to be analysed, a fast and cost-efficient method is needed. We have developed a simple PCR method (Molecular Copy number Counting – MCC) to count chromatids directly with DNA at limiting dilution and digital readout. After dispensation of the polar body DNA into eight PCR reaction units, two rounds of specific PCR amplification are required – a first round multiplex PCR for all markers on all chromosomes and a second specific marker monoplex PCR. The read out is digital – the number of PCR products equals the number of chromatids. RESULTS: We analysed the oocytes of 375 cycles; the largest demand for MCC was in the age group of above 38 years with 234 cycles (68%). In the younger groups, almost all cycles ended in an embryo transfer, but 35% (81 cycles) of the highest age group had only aneuploid embryos, and hence, no transfer was made. As expected, pregnancy rates decreased with age across the groups. However, the age effect is almost extinguished if pregnancy rates are evaluated after embryo transfer. CONCLUSIONS: Polar body analysis with MCC has proven a direct and robust method with beneficial biological outcome, especially in couples with poor prognosis. The method allows a strategy that is fast and reduces costs to the minimum necessary to identify euploid oocytes, exclude aneuploid oocytes, and avoid futile cycles with aneuploid embryos.

5-6

The timing of reproductive genetic counseling is the most important factor influencing acceptance of expanded carrier screening

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OBJECTIVES: Expanded carrier screening (ECS) for more than 100 genetic diseases is now available to women who are pregnant or considering pregnancy and their reproductive partners. The factors that influence uptake of ECS have not been extensively studied. Our objective was to retrospectively examine which factors influence a woman’s decision to undergo ECS. METHODS: We reviewed 500 consecutive medical records from women with documented prenatal or preconception genetic counseling for various indications at a large prenatal genetic counseling service between April 2012 and June 2013. No patients were excluded, and they were offered different preconception or prenatal screening and/or diagnostic tests. We tabulated acceptance rates for ECS by women and their reproductive partners along with various factors, including demographic information, personal and family history of genetic disease, timing, and indication for counseling. Data were analysed using descriptive statistics, t-tests, and chi-square tests. RESULTS: Expanded carrier screening was offered to 483 of 500 women and 192 (39.8%) total accepted. Of the subset (n = 67) seen preconceptionally 46 (68.7%) accepted ECS. This was significantly more than those seen during pregnancy (n = 416), of whom 146 (35.1%) accepted screening (p ≤ 0.001). For pregnant patients, the mean gestational age of those accepting carrier screening (12 weeks 3 days; n = 146) was significantly lower than for those declining (13 weeks 4 days; n = 270; p ≤ 0.001). Maternal and paternal ethnicity did not significantly affect ECS acceptance, although some trends were identified. CONCLUSIONS: These results suggest that women who receive genetic counseling prior to or earlier in the first trimester of pregnancy are more likely to accept ECS. This supports the importance of presenting reproductive age patients and their partners early with options for genetic carrier screening. Study of additional medical records is ongoing, and overall results may help delineate how other factors such as maternal ethnicity and family history influence acceptance of ECS. Other areas of further research may include the effects of socio-economic status and insurance coverage on ECS acceptance rates. These data can guide individualized counseling about carrier screening options.
OBJECTIVES: An association between increased nuchal translucency (NT) and confined placental mosaicism (CPM) of trisomy 16 has recently been reported. Very low levels of serum pregnancy-associated plasma protein A (PAPP-A) have also been demonstrated for CPM of trisomy 16. Furthermore, the influence of CPM subtypes on fetal growth, whatever the trisomy confined to placenta, still remains controversial. We wanted to re-evaluate the link between NT, PAPP-A levels and CPM, as well as the influence of CPM subtypes (types 2 and 3) on fetal growth and adverse pregnancy outcomes. METHODS: From July 2009 to December 2015, 5512 chorionic villus samplings were performed in our Center. Conventional karyotype after long-term cultured villi (LTC-villi) was systematically established. In case of suspicion of CPM, karyotype after short-term cultured villi was performed to define type 2 CPM (chromosomal abnormality limited to the mesenchymal core) or type 3 CPM (chromosomal abnormality found both in the cytotrophoblast and the mesenchymal core). Amniocentesis was performed to exclude fetal mosaicism, and uniparental disomy testing was carried out, if appropriate. Results were compared to a control population of 93 patients in whom karyotype was strictly normal after LTC-villi. RESULTS: Thirty-six CPM were observed (0.65%, 13 type 2, 23 type 3). The mean NT was not increased for types 2 and 3 CPM. For type 3 CPM, the median serum PAPP-A was statistically lower than for the control population. Incidence of intrauterine growth restriction (IUGR), neonatal hypotrophy, and stillbirth was comparable between type 2 CPM and the control population. In type 3 CPM, IUGR was noticed in 77.3%, neonatal hypotrophy in 72.2%, and intrauterine fetal death or stillbirth was deplored in 17.4% of cases (Table 1). The percentage of abnormal cells after LTC was negatively associated with birth weight. CONCLUSIONS: Increased NT did not appear to be associated with type 2 or type 3 CPM. Interestingly, median serum PAPP-A level was decreased in type 3 CPM, both for trisomy 16 and other trisomies. Regarding fetal growth, our study confirmed that when a CPM is suspected, CPM subtypes need to be carefully established. Although type 2 CPM has no effect on fetal development, type 3 CPM is associated with IUGR, intrauterine fetal death, neonatal hypotrophy, and stillbirth. When a type 3 CPM is diagnosed, we recommend therefore a close ultrasonographic monitoring in order to manage the fetal growth restriction.

Table 1. Confined placental mosaicism: re-evaluation of pregnancy characteristics and influence on fetal growth

### 6-2 The development of a placental murine xenograft model

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#### 6-1 Confined placental mosaicism: Re-evaluation of pregnancy characteristics and influence on fetal growth

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**Pregnancy characteristics**

|                      | Control population (n = 93) | Type 2 and 3 CPM (n = 36) | P-value | Type 2 CPM (n = 13) | P-value | Type 3 CPM (n = 23) | P-value |
|----------------------|-----------------------------|---------------------------|---------|----------------------|---------|---------------------|---------|
| Maternal age (years) (mean ± sd) | 34 ± 6                      | 34 ± 6                    | NS      | 33 ± 6               | NS      | 34 ± 6              | NS      |
| Pregnancy obtained after ART (%) | 5.38                        | 8.33                      | NS      | 7.69                 | NS      | 8.70                | NS      |
| Nuchal translucency (mm) (mean ± sd) | 1.7 ± 0.9                   | 1.3 ± 0.6                 | <0.01   | 1.5 ± 0.7            | NS      | 1.2 ± 0.5           | <0.01   |
| PAPP-A (MoM) [median (Q1-Q3)] | 0.76 (0.48–1.20)            | 0.26 (0.16–0.63)          | <0.001  | 0.63 (0.32–0.84)     | NS      | 0.19 (0.11–0.39)    | <0.001  |

**Prenatal diagnosis indication (%)**

- Maternal age: 12.9
- Antecedent: 16.1
- First trimester maternal serum screening: 35.5
- Second trimester maternal serum screening: 8.6
- Abnormal findings on ultrasound scan: 26.9
- Intrauterine growth restriction (%): 18.3
- Gestational age (WA) (mean ± sd): 39.4 ± 2
- Neonatal hypotrophy (%): 17.1
- Severe neonatal hypotrophy (%): 6.10
- Intrauterine fetal death, stillbirth (%): 3.57

**ART, assisted reproductive technologies; CPM, confined placental mosaicism; MoM, multiple of the median; NS, non significant; PAPP-A, pregnancy-associated plasma protein A; Q1, quartile 1; Q3, quartile 3; sd, standard deviation; WA, week of amenorrhea.**
OBJECTIVES: Children intrauterine exposed to chemotherapy are more prone to be born with a birth weight below the 10th percentile, defined as ‘Small for Gestational Age (SGA)’. Pathways for angiogenesis, proliferation, and inflammation are known to be related to SGA. The mechanism underlying SGA following in utero exposure to cancer treatment is so far unexplored, and models to explore the effect of chemotherapy on the placental growth and function are not available. This study investigates the development of a suitable murine xenograft model to examine the possible effect of chemotherapeutic agents or other drugs on the placental tissue. METHODS: The test population consisted of five examination groups of six immunodeficient nude mice. After ovariectomy and hormonal stimulation with 17b-estradiol and/or progesterone pellets, placental tissue derived from first or third trimester pregnancies was engrafted subcutaneously bilateral in the flanks. General morphology was evaluated by H&E and immunohistochemistry (hCG, CD31). hCG secretion was measured in serum and urine by ELISA. The expression level of selected genes (IGF-1, PIGF, IGF-2, eNOS, Flk-1, and Flt-1) was compared by using RT-qPCR. WTSS and IPA (Ingenuity Pathway Analysis) are ongoing to investigate the stability of selected pathways relevant for the angiogenesis, inflammation, and proliferation. RESULTS: Comparing the placental tissue before and after engraftment, we found preserved structure and histological features. hCG secretion, evaluated by ELISA in the urine and blood, was present for 3 weeks. (Fig. 1) RT-qPCR analysis showed preserved genetic characteristics of selected genes relevant for angiogenesis, proliferation, and inflammation of the primary engrafted placental tissue. Results on WTSS and IPA are expected to be ready for presentation in July 2016. CONCLUSIONS: We created a murine xenograft model with proven stability and preserved structure of the engrafted placental tissue for 3 weeks. This established murine xenograft model can be used to examine the effect of cancer treatment on the placental growth and function, in order to identify risk factors related to SGA and to develop preventive measures. Moreover, it could serve as a model for other fetal toxicity studies.

6-5
Incremental yield of genomic microarray in early growth restricted fetuses over karyotyping

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OBJECTIVES: Fetal genetic disease is a well recognized cause of early and severe intra-uterine growth restriction. However, scarce data are available about the association between fetal growth restriction with microdeletion and microduplication syndromes. The aim of this multicenter study was to assess the incremental yield of genomic microarray over karyotyping in early and severe growth restriction. METHOD: This multicenter study included 137 consecutive early (<25 weeks) and severe (<3rd percentile on estimated fetal weight) growth restricted fetuses with a normal karyotype or quantitative fluorescent polymerase chain reaction (QF-PCR), studied during a 3-year period (January 2013–December 2015) in three centers of Barcelona. BAC (Bacterial Artificial Chromosome) array-CGH (CytoChip Focus Constitutional, BlueGnome, Illumina) was performed in DNA extracted from amniotic fluid. The incremental yield was defined by the rate of fetuses presenting with a pathogenic copy number variants below 10 Mb in normal karyotype/QF-PCR results. RESULTS: In our series, the incremental yield of genomic microarray over karyotyping was 7.3% (95%CI: 2.9 to 11.7) (10/137). Among malformed fetuses, this rate was 15.0% (3/20); in non-malformed fetuses with other findings (soft markers, abnormal amniotic fluid…) was 2.6% (1/38); and in fetuses with IUGR as isolated finding 7.6% (6/79). CONCLUSIONS: Our findings support the use of genomic microarray after a normal QF-PCR/karyotype given that provides a 7.6% incremental yield in growth restricted fetuses as isolated finding, increasing up to 15.0% in malformed fetuses.

6-6 Discordant hypoxic condition and oxidative stress in placental shares of monochorionic twins with selective intra-uterine growth restriction

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OBJECTIVES: A physiological redox state is essential for placental and fetal development. Hypoxia and imbalanced oxidative stress are key factors in the pathogenesis of intra-uterine growth restriction (IUGR). However, their roles in selective intra-uterine growth restriction (sIUGR) in monochorionic twins (MCT) are still unknown. This study explored the characteristics of hypoxia/oxidative stress in the placenta shares of MCT and their possible connections with sIUGR. METHODS: The expression levels of hypoxia inducible factor-1α (HIF-1α, hypoxia marker), malondialdehyde (MDA, lipid oxidation marker), and 8-hydroxydeoxyguanosine (8-OHdG, oxidative DNA damage marker) were evaluated in the placental shares of normal MCT (Group A) and sIUGR MCT (Group B). Expression was evaluated in the larger twins (A1/B1) and smaller twins (A2/B2). The relationship between birth weight and each marker was analysed. RESULTS: HIF-1α expression was significantly higher in the placenta shares of sIUGR MCT, indicating more severe hypoxia. HIF-1α expression was also significantly higher in the placenta share of the growth-restricted fetus (B2) than in the co-twin (B1) (P < 0.05). There were also larger inter-twin differences in HIF-1α expression in sIUGR MCT than normal MCT. The oxidative stress markers MDA and 8-OHdG were significantly higher in the placenta share of growth restricted fetus (B2) than the co-twin (B1) in sIUGR MCT (P < 0.05). The inter-twin differences in MDA and 8-OHdG were also significantly larger in sIUGR MCT than normal MCT (P < 0.05). CONCLUSIONS: The distinctive pattern of hypoxia and oxidative stress observed in sIUGR MCT pregnancies suggests that hypoxia related oxidative stress plays an important role in the pathogenesis of sIUGR.

7-1 Clinical implementation of NIPT: Understanding the role of non-specialist provider education

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OBJECTIVES: As cell-free DNA (cfDNA) prenatal screening expands to low risk pregnancies and for microdeletions non-specialist providers (general Ob/Gyns, certified nurse midwives (CNM) may increasingly be required to counsel women/families before and after testing. Yet these providers have few opportunities to achieve and maintain competence in medical genetics or genetic counseling leaving. The objectives of this research are to (1) determine stakeholders’ perspectives on gaps in physician education about cfDNA screening, especially for non-specialists and (2) to systematically assess suitability of currently available online courses for educating non-specialist providers about technical, clinical, and ethical aspects of cfDNA screening. METHODS: Thematic content analysis was performed on 75 semi-structured interviews conducted with representatives of eight stakeholder groups to identify barriers to appropriate clinical implementation of cfDNA in the US. Online continuing medical education (CME) courses were identified by keyword searches. We conducted content analysis of ten online CME courses on prenatal genomic screening along the following broad themes: (1) types and uses of prenatal genetic screening and testing in general; (2) information about cfDNA screening, specifically types of tests available, their uses and performance; and (3) ethical legal and social issues (ELS) surrounding cfDNA screening. RESULTS: Stakeholders concerns surrounded the (1) expanding responsibilities for general Ob/Gyns versus limited time and capacity they have for providing pre-test and post-test genetic counseling for cfDNA screening; (2) gaps in Ob/Gyn’s knowledge about cfDNA screening and genetic conditions tested; and (3) potential lack of accurate and unbiased educational resources due
OBJECTIVES: Many laboratories plan to provide non-invasive cfDNA screening for conditions other than Trisomy 21, PPV of microdeletion testing, incidental findings, reasons for false positives and their implications for informed-decision making. CME courses were high variable in their content and topics covered. Variable and poor coverage of technical, clinical and ELS issues in currently available online CME courses raise need for more systematic development and assessment of educational resources targeted to such non-specialists. These approaches can better prepare non-specialist Ob/Gyn providers to facilitate patient informed decision-making and foster more ethical and effective use of cfDNA screening.

7-2

Ensuring high quality NIPD and NIPT for the patient

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OBJECTIVES: Many laboratories plan to provide non-invasive prenatal testing (NIPT) by analysing cell free DNA (cfDNA) in maternal plasma. When new testing approaches are introduced, all stakeholders must be assured that high quality testing is performed. This can be achieved through participation in external quality assessment (EQA) schemes; however, EQA for cfDNA is challenging. Initial schemes using spiked DNA plasma samples for fetal sex determination were sub-optimal. Here, we describe the development of EQA for aneuploidy NIPT and a scouring exercise for the need for EQA for non-invasive prenatal diagnosis (NIPD) for fetal sexing and single gene disorders. METHODS: Collaboration between EQA providers (CEQAS, EMQN, and UK NEQAS for Molecular Genetics) has been established to provide a pilot EQA to assess the standard of testing for NIPD for fetal sexing and single gene disorders. OBJECTIVES: Results for cfDNA aneuploidy screening generally do not include a PPV. Therefore, some individuals use published sensitivity and specificity information to determine the PPV for cfDNA testing in their patients. This information has been helpful in the counseling of patients with positive cfDNA screening tests. However, as calculations of PPV are strongly dependent on the sensitivity and specificity of the test, as well as the prevalence of the disorder in the tested population, small inaccuracies, especially in the specificity, have a strong effect on PPV. Therefore, calculated PPVs do not necessarily reflect what’s seen in the clinical laboratory and in real-world observations. OBJECTIVES: Results for cfDNA aneuploidy screening generally do not include a PPV. Therefore, some individuals use published sensitivity and specificity information to determine the PPV for cfDNA testing in their patients. This information has been helpful in the counseling of patients with positive cfDNA screening tests. However, as calculations of PPV are strongly dependent on the sensitivity and specificity of the test, as well as the prevalence of the disorder in the tested population, small inaccuracies, especially in the specificity, have a strong effect on PPV. Therefore, calculated PPVs do not necessarily reflect what’s seen in the clinical laboratory and in real-world observations. OBJECTIVES: Participants from all stakeholders groups perceive educating non-specialist providers as one of the most important barriers for appropriate clinical implementation of cfDNA screening. Further empirical data are needed to understand non-specialist provider’s educational experiences and specific needs surrounding cfDNA – screening education. Variable and poor coverage of technical, clinical and ELS issues in currently available online CME courses raise need for more systematic development and assessment of educational resources targeted to such non-specialists. These approaches can better prepare non-specialist Ob/Gyn providers to facilitate patient informed decision-making and foster more ethical and effective use of cfDNA screening.

7-3

Odds of being affected given a positive result (OAPR) in cell-free DNA screening for fetal autosomal and sex chromosome aneuploidy: Data from four cytogenetic laboratories

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OBJECTIVES: Results for cfDNA aneuploidy screening generally do not include a PPV. Therefore, some individuals use published sensitivity and specificity information to determine the PPV for cfDNA testing in their patients. This information has been helpful in the counseling of patients with positive cfDNA screening tests. However, as calculations of PPV are strongly dependent on the sensitivity and specificity of the test, as well as the prevalence of the disorder in the tested population, small inaccuracies, especially in the specificity, have a strong effect on PPV. Therefore, calculated PPVs do not necessarily reflect what’s seen in the clinical laboratory and in real-world observations. METHODS: Four reference laboratories in the United States and Australia queried their internal laboratory information systems to identify patients who underwent cytogenetic testing (sample types: products of conception, chorionic villi, amniotic fluid, or blood) after abnormal cfDNA testing and determined if the results were concordant with the screening results. When the result was screen positive for autosomal aneuploidy, and maternal age was available, a PPV for each case was calculated using the PPV calculator developed in conjunction with NSGC (https://www.pernatalquality.org/Vendors/NSGC/NIPT/) which is based on sensitivity and specificity information derived from a meta-analysis of cfDNA studies, as opposed to individual laboratory validation data. RESULTS: A total of 538 cfDNA-positive cases were identified with 384 results confirming the cfDNA results (OAPR
71.4%). The OAPRs for each chromosomal aneuploidy was as follows: T13-48.1%, T18-73.2%, T21-92.1%. Monosomy X-18.7%, XXX-54.6%, XXY-63.3%. And XYY-88.9%. A PPV was calculated for each autosomal aneuploidy case with a maternal age. The average calculated PPV for each disorder was significantly lower than the observed TPR. T21- 82.4% v. 91.9% (p=0.003); T18- 46.7% v. 75.6% (p=0.0001); T13- 29.2% v. 48.9% (p=0.0001). CONCLUSIONS: We compared our observed TPRs to those of previously published observational studies and determined that our OAPRs were not statistically different from those seen in previous studies. This indicates that the OAPRs are relatively consistent over time. Additionally, the disparity between the calculated PPV and observed TPR demonstrates that the PPV calculator significantly underestimated the performance of cfDNA screening for all three autosomal aneuploidies. This underestimation, along with the general consistency of OAPR across multiple studies, supports the use of the OAPR as a useful tool in the counseling of patients with positive cfDNA screening results.

7-4
Fetal aneuploidy screening results in maternal plasma samples redrawn due to insufficient fetal cfDNA in the initial sample

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OBJECTIVES: The objective of this study was to determine the redraw success rate of samples initially receiving no cfDNA result due to low fetal fraction (FF) and to characterize the results of redrawn samples. METHODS: A review of initial cfDNA results of 229,454 samples submitted to Ariosa Diagnostics for HarmonyTM Prenatal Test was conducted. cfDNA samples were reviewed and categorized as having an increased probability for trisomy 21, 18 and 13, decreased probability for trisomy and no reportable results due to low FF. Samples that received no reportable results due to low FF (<4%) were matched to repeat cfDNA samples. The results of redrawn samples were categorized and compared to the results of samples receiving a result on their initial cfDNA sample. The overall and redraw success rates were evaluated. Pregnancy outcome data was not obtained. RESULTS: Of the 229,454 samples submitted, 97.4% (223,649) received a result on their initial cfDNA sample, with 1.49% (3,287) receiving results indicating an increased probability for trisomy 21, 18, or 13. Non-reportable results due to low fetal fraction were seen in 1.8% (4,100) of the initial samples. Of this group, 74.2% (3,041) elected to have a repeat cfDNA sample collected, with 68% (2,058) receiving a reportable result on their second sample. While the mean fetal fraction was lower in the redrawn samples (6.58% compared to 11.49% in the initial samples), reportable results indicating a low probability for trisomy 21, 18. CONCLUSIONS: Of cfDNA samples redrawn after an initial sample failure due to low fetal fraction, 68% received a result with 98% of those results indicating a low probability for the common autosomal trisomies. It has been recommended that providers refer women receiving ‘no call’ cfDNA screen results for diagnostic testing because of an increased aneuploidy risk. Reflexing to invasive testing would result in an increased number of invasive prenatal diagnostic tests and concomitant increase in fetal loss. This study shows that submitting a second sample after an initial non-reportable cfDNA result due to low fetal fraction will yield a reportable result.

Table 1. Results comparison

7-5
Decisional regret in women receiving high-risk or inconclusive results from non-invasive prenatal genetic screening

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OBJECTIVES: Prenatal genetic screening has changed drastically over the last 5 years, with non-invasive cell-free DNA screening causing much of this change. Non-invasive prenatal testing (NIPT) panels have expanded rapidly from limited aneuploidy screening for trisomy 21 to screens for multiple aneuploidies and abnormalities, including sex chromosome aneuploidies and, recently, microdeletions. Expanded panels have been minimally, inconsistently validated, and providers report low levels of informed consent and difficulties assisting patients in interpreting results. This study sought to understand the experiences of women receiving NIPT results and the effect of high-risk, false, or inconclusive results on anxiety and pursuit of additional testing. METHODS: Forty semi-structured telephone interviews were conducted with women who were currently or had recently been pregnant and received high risk (n=15), false positive/negative (n=19), or inconclusive (n=5) results from NIPT. Among the study population, three countries and, among those in the United States, nineteen states were represented. RESULTS: Many women felt deceived by the advertised ‘99% accuracy’ NIPT statistic and asserted that improved pre-test counseling could have prevented much of their post-test anxiety. For example, all women who received high-risk microdeletion results said they were unaware of the risk, and their understanding was not improved by the information provided.

7-4 Table.

| T21 Increased probability | T18 Increased probability | T13 Increased probability | Total Increased probability | Median FF |
|---------------------------|---------------------------|---------------------------|-----------------------------|-----------|
| Redraw due to low FF     |                          | Total                       |                             |           |
| Reported                  | 0.83% (17)               | 0.53% (11)                | 0.63% (13)                  | 1.99% (41) | 6.58%     |
| First draw - adequate FF |                          | Total                       |                             |           |
| Reported                  | 1.09% (2411)             | 0.28% (610)                | 0.12% (266)                 | 1.49% (3287) | 11.49%   |

p=0.24, p=0.03, p=0.01, p=0.06, p=0.01
that NIPT included microdeletion screening. Most women felt pressured to pursue invasive testing; several reported feeling pressure from providers to terminate. False NIPT results had significant lasting effects on the women, their families, their pregnancies, and their willingness to have more children. Most women reported they would not do NIPT in future pregnancies or would only do it with a limited panel. CONCLUSIONS: Findings suggest significant concerns about how clinical practices are integrating NIPT and how they are maintaining ethical care of their patients. Study participants indicate problems with the dialog when NIPT is offered, the return of results, and the positive predictive value of expanded screenings. Given the confusion about NIPT accuracy, the prevalence of follow-up invasive tests, and the large number of women who regret doing NIPT, NIPT offering and the delivery of results need to be carefully approached. As NIPT continues to grow in scope and uptake, it is increasingly important to devote ample time for counseling and informed consent.

7-6 Plasma DNA tissue mapping to pinpoint site of occult maternal malignancy identified by NIPT

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OBJECTIVES: Non-invasive prenatal screening for fetal chromosomal aneuploidies by maternal plasma DNA analysis has become part of routine practice in many parts of the world. The non-invasive prenatal tests (NIPTs) are based on the detection of elevated or reduced amounts of DNA from the potentially aneuploid chromosomes as a result of the presence of DNA released by an affected fetus into the maternal circulation. However, malignancies also release DNA into the circulation. There have been reports of incidental detection of maternal occult malignancies as a result of NIPT.

METHODS: When the manifestations of the suspected malignancy is not obvious, these cases present as a dilemma in terms of clinical management. We have recently developed an approach that would allow one to determine the tissue of origin of plasma DNA molecules. The approach is based on genome-wide methylome analysis and interrogates the presence of DNA signatures from multiple organs simultaneously. We termed this as plasma DNA tissue mapping. RESULTS: We showed that the approach could pinpoint the anatomical locations of cancers. We have further applied plasma DNA tissue mapping to NIPT samples with suspected chromosomal aneuploidies. The approach could distinguish if the chromosomal aneuploidy originated from the placenta or otherwise. In a case of suspected maternal malignancy, plasma DNA tissue mapping identified the site of malignancy. CONCLUSIONS: With plasma DNA tissue mapping, the detection of chromosomal copy number aberrations and the identification of the site of pathology could be achieved in the same plasma sample. (Supported by the University Grants Committee (Areas of Excellence Scheme AoE/M-04/06) and Research Grants Council (Theme-based research scheme (T12-404/11) and (T12-403/15-N)) of the Hong Kong SAR Government).

8-1 Correlation between fetal autopsy and prenatal diagnosis: a systematic review of literature

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OBJECTIVES: The objective of this study was to review literature about the correlation between fetal autopsy and prenatal diagnosis of fetal malformations. METHODS: Search in PubMed, Medline, EMBASE, reference list. Inclusion criteria: fetal autopsy after TOP for fetal anomalies, prenatal diagnosis (PD) of malformations. Exclusion criteria: case-reports, non-English language, data in graphs or percentage. Sample size, prenatal diagnosis, indication for TOP, autopsy findings were abstracted. Malformations were grouped in central nervous system (CNS), genitourinary (GU), heart defects (CHD), gastrointestinal (GI), thorax, limbs, skeleton, genetics (abnormal karyotype), multiples (multiple malformations for which a single indication for TOP was not identified). Correspondence between autopsy and ultrasound was defined as agreement (same diagnosis), additional (additional findings undetected by ultrasound), unconfirmed (false positive/false negative ultrasound). PRISMA guidelines. RESULTS: A total of 4191 fetuses were pooled. Agreement was 70%, additional 21%, unconfirmed 9%. False positive and false negative rates were 3% and 4%, respectively. Malformation was described in 3153 (75%) fetuses, in which TOP occurred frequently for CNS (34%) and rarely for thorax (1%) malformations. The highest agreement between autopsy and PD was observed in GU anomalies (81%), followed by CNS (80%), genetics (79%), skeleton (77%), CHD (75%), thorax (70%); GI (63%), multiple (37%), limbs (23%). False positive rates were highest for thorax and lowest for CNS malformations. False negative rates were highest for limb and lowest for CNS malformations. CONCLUSIONS: Fetal autopsy is indicated in cases of TOP for malformations, since it provides additional information in 21% of cases. In only 3% of cases, ultrasound findings lead to unjustified TOP and this is mainly true for thorax anomalies.

8-2 Head circumference of fetuses with congenital heart disease decreases in the second half of pregnancy

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OBJECTIVES: Congenital heart disease (CHD) occurs in 6 to 8 per 1000 livebirths and is associated with poor perinatal outcome and risk of neurodevelopmental impairment. Brain injury, altered brain development and smaller brain volumes have been reported in children with CHD prior to cardiac surgery. Smaller fetal head circumference (HC) and microcephaly at birth, considered markers of fetal brain development, are reported in CHD cases. The purpose of this study is to evaluate the fetal HC growth pattern in isolated CHD. METHODS: This retrospective cohort study was performed at University Medical Center Utrecht and University Medical Center Groningen. Pregnancies with an estimated due date (EDD) between 2008 and 2013 and isolated CHD were included and classified into seven groups: aortic arch obstruction, conotruncal anomaly, hypoplastic left heart, hypoplastic right heart, septal defect, complete transposition of the great arteries and others. Fetal biometry was assessed at four variable time points between 18 and 35 weeks’ gestation (GA). Values were transformed into Z-scores. Linear mixed modeling was performed to enable analysis of repeated measurements and construction of growth models. RESULTS: A total of 246 livebirths with isolated CHD (chromosomal abnormalities were excluded) were eligible for analysis. The linear growth model predicted a smaller HC at 36 weeks’ GA in fetuses with a conotruncal anomaly compared to a reference group: mean Z-score 0.57, SD 0.78 and mean Z-score 0, SD 1, respectively (p < 0.0005) (Fig. 1). HC growth pattern showed a trend for a linear decrease starting from the 2nd trimester in all CHD groups, whereas abdominal circumference (AC) and femur length (FL) showed a constant growth pattern. Mean difference in HC Z-score between the first and last measurements was 0.64 SD (p < 0.0001). CONCLUSIONS: Fetuses with a conotruncal heart anomaly have smaller HC at 36 weeks’ GA. Furthermore, all CHD groups showed a slight deflection in HC growth from the 2nd trimester onwards. Data on neurological outcome were not available and therefore a possible relation between HC and neurological impairment could not be analysed. However, taking into account the modest reduction seen in our study we hypothesize that HC alone may not be a reliable marker for neurodevelopmental delay in fetuses with CHD.

8-3 Image.

8-3 Fetal neuronal migration disorder between 15 and 26 weeks of gestation
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OBJECTIVES: Migration takes place in the first and early-second trimesters and phenotype of migration in the cortex appears after 28 weeks of gestation. It has been believed that it is quite hard to detect migration disorder before 28 weeks. This study shows our experience of cases with neuronal migration disorder between 15 and 26 weeks. METHODS: Twenty-eight cases with diagnoses of migration disorder between 2011 and 2015 were enrolled in this study. Cases were divided into two groups by date of sonographic detection before and after 22 + 0 weeks. In terminated case, autopsy was done with parental consent. The rest of cases, the outcome and prognosis were investigated. RESULTS: All cases with migration disorders were complicated with other CNS and/or extracranial abnormalities. Among 28 cases, all 12 cases diagnosed as migration disorder between 15 and 21 weeks of gestation, were terminated before 22 weeks. In seven out of 12 cases, autopsy was done and brain histology was carefully examined and neuronal maldevelopment was confirmed. Among the rest of 16 cases diagnosed as migration disorder between 22 and 26 weeks of gestation, IUFD (1), infant death (1) and 12 fetuses were delivered and followed up, and two cases are still ongoing. There were no intact infants among 12 delivered infants. CONCLUSIONS: In most cases with neuronal migration disorder, neurological prognosis is not favorable. Early diagnosis of migration disorder has been possible and is essential for proper prenatal counseling, genetic investigation and management.

8-3 Image.

8-4 Diagnostic accuracy of congenital heart defects after referral from routine ultrasound screening
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OBJECTIVES: Accurate prenatal detection of fetal heart anomalies (CHD) within the legal limit for termination of pregnancy enables parental choices regarding continuation of the pregnancy and optimal neonatal care, including planning timing and location of delivery. Before 2007, the majority of CHD were diagnosed in the Netherlands either late or after birth with a negative impact on perinatal mortality. Since 2007, all women are offered a routine 20 weeks scan. The aim of this study was to evaluate screening and the accuracy of prenatal diagnosis of CHD in the Fetal Medicine Units (FMU) of two congenital cardiac surgery centers after 2007.

METHODS: A retrospective cohort study was performed at the University Medical Centers of Utrecht and Groningen. Cases with a prenatal or postnatal diagnosis of CHD between 2008 and 2013 were included. Prenatal information was matched with postnatal data obtained from the pediatric cardiology departments. Chromosomal anomalies (trisomy 13, 18 and 21) were excluded from the analysis. CHD was classified in 12 categories of anomalies: aortic arch, conotruncal, hypoplastic left heart complex, hypoplastic left heart syndrome, hypoplastic right heart syndrome, other univentricular defects, isomerism, septal defects, complete transposition of the great arteries, valvar anomaly, venous return anomaly and miscellaneous anomalies.

RESULTS: In total, 631 CHD cases were analysed. Overall 82.6% of prenatal diagnoses of CHD were accurate. A false-positive diagnosis occurred in 5.2% of cases. The percentage of missed cases decreased steadily from 42.3% in 2008 to 20.8% in 2013. CHD with an abnormal four chamber view (4CV) was more often prenatally detected compared to CHD with a normal 4CV: 89.4% versus 54.1%; \( p<0.01 \). After referral to a FMU accuracy of the exact prenatal classification of a specific defect was not significantly influenced by the (ab)normality of the 4CV (86 vs 80%; \( p=0.19 \)).

CONCLUSIONS: From 2008 to 2013, the percentage of CHD that were prenatally diagnosed increased to more than 80%. CHD with a normal 4CV remain the most challenging to detect in utero. After refinement of the diagnosis at a fetal medicine unit of a specialized center, the majority of parents were adequately informed and counseled on expected course of events after birth. Our results suggest that to further improve prenatal detection in our national screening program, more emphasis is needed to detect CHD with a normal 4CV.

8-5 From fetal dysmorphology to exome sequencing: The diagnostic performance for making a perinatal genetic diagnosis

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OBJECTIVES: The aim of this study was to study the serial diagnostic yield of a post-mortem dysmorphological examination, histopathological and radiological evaluation, basic cytogenetic, biochemical and molecular genetic testing and Exome sequencing for establishing a genetic diagnosis in fetuses with abnormal ultrasound findings or with intrauterine fetal demise. METHODS: Post-mortem evaluation was performed for a total of 90 fetuses. This included a dysmorphologic examination, radiographs and histopathology of the fetal tissues in all cases. Additionally, fetal karyotype was done in 26 cases, and specific biochemical or molecular genetic testing was done as indicated. Exome sequencing was performed in three cases on the fetal DNA, which either had history of similarly affected sibs or specific post-mortem findings indicating high risk for a monogenic etiology. The results of the post-mortem evaluations were compared with the prenatal findings, and the serial diagnostic contribution of each evaluation to the genetic diagnosis was ascertained. RESULTS: Post-mortem dysmorphologic evaluation indicated or strongly suggested a specific genetic disorder in 16 cases, was suggestive of possible genetic disorder/syndrome in 31 cases and was inconclusive or indicative of an acquired etiology in 43 cases. Further, histopathological and radiological evaluation confirmed or independently indicated a specific diagnosis in 8, 9 and 7, 4 cases, respectively. G-band karyotype had a diagnostic yield of four (26) and biochemical and/or molecular testing confirmed a genetic metabolic disorder in three cases. In at least three cases, exome sequencing was performed on the fetal DNA, leading to establishment of final genetic diagnosis. CONCLUSIONS: Genetic disorders are found in a significant number of fetuses.
undergoing post-mortem evaluation following pregnancy termination due to abnormal ultrasound findings or unexplained fetal demise. There is a progressive increase in diagnostic performance as additional investigations are performed, some universally, and others as indicated. Exome sequencing is contributory and should be performed in cases with high clinical suspicion of a single gene disorder.

TA-1
The effect of a decision aid on informed decision making in the era of non-invasive prenatal testing: A randomized controlled trial

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OBJECTIVES: Early in pregnancy, women and partners face the complex decision on whether or not to participate in prenatal testing for fetal chromosomal abnormalities. The choice to accept or decline prenatal testing should be made informed, that is, based on adequate decision-relevant knowledge and consistent with values and attitudes towards having testing performed. As the range of prenatal tests is expanding due to the development of new techniques such as non-invasive prenatal testing (NIPT), informed decision making is increasingly challenging. A randomized controlled trial was conducted to evaluate the effect of a web-based multimedia decision aid on decision making regarding prenatal testing. METHODS: Pregnant women were allocated to the control group, receiving standard prenatal care, or intervention group, additionally granted access to a web-based multimedia decision aid. The decision aid was developed to provide both written and audiovisual information on prenatal tests currently available, that is, prenatal screening by first-trimester combined testing, NIPT and invasive diagnostic testing. Furthermore, it contained self tests encouraging women to reflect on the potential benefits and harms of having prenatal tests performed. The primary outcome was informed decision making regarding prenatal testing. Secondary outcomes included knowledge, attitudes, test utilization, value-consistency, decisional conflict, decisional regret, and anxiety. RESULTS: The use of the decision aid improved informed decision making regarding prenatal testing. Of pregnant women allocated to the intervention group (n = 130), 82.3% made an informed choice compared to 66.4% of women in the control group (n = 131), p = 0.004. As the vast majority of pregnant women made decisions consistent with their attitudes towards having prenatal testing performed (92.7 vs 94.2%, p = 0.641), this improvement in informed decision making could be attributed mainly to an increase in the proportion of women having sufficient decision-relevant knowledge (88.5 vs 70.8%, p < 0.001). No significant differences were found in test utilization, decisional conflict, decisional regret, or anxiety. CONCLUSIONS: This study shows that the implementation of a web-based multimedia decision aid directly facilitates the ultimate goal of prenatal testing for fetal chromosomal abnormalities, which is enabling informed, and autonomous, reproductive choice.

TA-2
An integrated human/murine transcriptome and pathway approach to identify prenatal treatments for Down syndrome

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OBJECTIVES: The intellectual disability in Down syndrome (DS) originates with delays in neurogenesis and brain growth during fetal brain development. We hypothesize that novel prenatal treatments can be identified by targeting specific signaling pathways that are consistently perturbed in fetal tissues from humans with DS and in several mouse models. METHODS: We generated new gene expression datasets from amniocytes from seven mid-trimester fetuses with trisomy 21, age and sex-matched euploid fetuses, and embryonic day 15.5 forebrain from Ts1Cje, Ts65Dn, and Dp16 mouse models of DS, along with littermate controls. Functional pathway analyses were performed using the GSEA, DAVID and IPA databases. The new datasets (n = 4) were compared to other publicly available datasets from fetal cerebrum and cerebellum, amniotic fluid, iPSCs and neurons derived from humans with DS (total = 9). We used the Connectivity Map database and developed a murine adaptation to identify FDA-approved drugs that can rescue consistently dysregulated pathway abnormalities. RESULTS: USP16 and TTC3 were FDA-approved drugs that can rescue consistently dysregulated pathway abnormalities. RESULTS: USP16 and TTC3 were dysregulated in all affected human cells and two mouse models. Functional pathway analyses revealed similarities and differences between humans with DS and mouse models. DS-associated pathway abnormalities were either the result of gene dosage specific effects (increased transcriptional activity, abnormal neurogenesis and neuronal differentiation, mitochondrial dysfunction, oxidative stress and inflammation) or the consequence of a global cell stress response with activation of compensatory mechanisms (abnormal cell cycle and kinetochore regulation, increased proteolysis and activation of anti-apoptotic gens). Using a high stringency cut-off, CMap analyses identified seventeen molecules with high predictive scores to rescue abnormal gene expression. CONCLUSIONS:
Our novel integrated human/murine systems biology approach identified commonly dysregulated genes and pathways in both humans with DS and mouse models that can help to prioritize therapeutic molecules for further study. Studies testing the safety and efficacy of the top candidate molecules on human cells are ongoing prior to pre-clinical prenatal treatment in mice.

TA-3

Amniotic fluid transcriptomic changes in fetuses with myelomeningocele

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OBJECTIVES: Cell-free (cf) RNA in amniotic fluid supernatant (AFS) reflects developmental changes in gene expression in the living fetus, including genes specific to the central nervous system (CNS). Although it has been previously shown that CNS-specific transcripts are present in AFS (Hui et al. 2012), it is not known whether changes in the AFS transcriptome reflect the specific pathophysiology of fetal CNS disorders. In myelomeningocele (MMC), there is open communication between the CNS and amniotic fluid. We hypothesized that analysing AFS cfRNA in fetuses with open MMC would identify molecular pathophysiologic changes and novel disease mechanisms specific to this disease. METHODS: Ten pregnant women carrying fetuses with MMC were enrolled. Amniotic fluid was collected at the time of the open MMC repair. Archived AFS from sex and gestational age-matched euploid fetuses (n = 10) without MMC were used as controls. AFS cell-free RNA was isolated, processed and hybridized to Affymetrix GeneChip® Human Genome U133 Plus 2.0 arrays. Differentially-regulated genes were identified using paired t-tests (p < 0.05). Significantly altered expression in genes, pathways and networks was identified using Ingenuity Pathway Analysis® using a right-tailed Fisher Exact Test (p < 0.05). Multiple testing was performed with corrected p-values (<0.05) based on the Benjamini–Hochberg method. RESULTS: Control samples were from fetuses at slightly younger gestational ages (20.9+/−0.9 wks) than MMC samples (24.5+/−1.0 wks). Fetuses with MMC had characteristic gene expression patterns. 284 differentially-regulated genes were identified (176 up-regulated and 108 down-regulated) in AFS. Known genes associated with MMC (PRICKLE2, GLI3, RAB23, HES1, FOLR1) were differentially regulated. In addition, novel dysregulated genes were identified in association with neurodevelopment and neuronal regeneration (up-regulated GAP43 and ZEB1) or axonal growth and guidance (down-regulated ACAF1). Pathway analysis demonstrated a significant contribution of inflammation (Z-score 2.45) to pathology and a broad influence of Wnt signaling pathways (Wnt1, Wnt5A, TPR1, Z-score -1.3). CONCLUSIONS: Transcriptomic analyses of living fetuses with MMC using AFS cfRNA demonstrated differential regulation of specific genes and molecular pathways relevant to this CNS disorder, resulting in a new understanding of pathophysiologic changes. The data also suggested the importance of pathways involving secondary pathology, such as inflammation, in MMC. These newly identified pathways may lead to hypotheses that can test novel therapeutic targets as adjuncts to fetal surgical repair.

TA-4

A bespoke non-invasive prenatal diagnostic (NIPD) Service for paternal Mutation and de novo Recurrence Exclusion

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OBJECTIVES: Analysis of cell free DNA (cfDNA) in maternal plasma for the detection of fetal aneuploidy is available worldwide, but there is very limited availability of NIPD for women with pregnancies at increased risk of monogenic disorders. We offer a NIPD service for detection of paternal or de-novo alleles for the diagnosis of FGFR3 and FGFR2-related conditions and for cystic fibrosis in pregnancies where parents are heterozygous for different mutations. Our objective here is to expand the range of NIPD available to families at risk of a range of rare monogenic conditions as a safe, earlier alternative to invasive testing. METHOD: Primers were designed to target relevant mutations and included P5 and P7 Illumina sequencing adapters along with incorporation of a 6bp unique index. Bespoke primers were tested on paternal and/or proband DNA to verify the design before application in cfDNA. For cfDNA, in addition to the mutation specific primer, primers for HLA and ZFX/ZFY were included for each patient enabling us to confirm the presence...
OBJECTIVES: The aim of this study was to offer pregnant women requesting prenatal diagnosis (CVS or amniocentesis) the choice of receiving ‘targeted’ or ‘extended’ genomic results generated by chromosome microarray analysis. The ‘targeted’ analysis included only chromosome abnormalities of certain pathogenicity, whereas the ‘extended’ analysis also included abnormalities with incomplete penetrance and uncertain pathogenicity. Objectives were to (1) assess uptake rates of targeted versus extended analysis and the sociodemographic predictors of women’s choice; and (2) compare women in terms of the impact of their decision-making and response to receipt of fetal genomic results.

METHODS: Participants, recruited from public maternity hospital clinics and private obstetric practices, were provided with a study-specific decision aid before their diagnostic procedure and asked to make a choice about receiving targeted or extended fetal genomic information. Women were excluded if a fetal abnormality was suspected or the procedure was occurring within 48 h. Two surveys were completed. The first was completed before the procedure, and examined decisional conflict, state/trait anxiety, intolerance of uncertainty and health literacy. The second survey, sent approximately ten days after receiving the result, assessed decisional regret, satisfaction with decision and state anxiety.

RESULTS: We recruited 111 pregnant women, 40.5% of whom chose targeted analysis and 59.5% extended analysis. None of the socio-demographic variables examined were found to be associated with women’s choices, including maternal age, parity, religion, ethnicity, education, and financial situation; however, women who had been trying to conceive for 12 or more months and who had used fertility treatments were significantly more likely to choose extended analysis, with 78% and 92% respectively doing so. Also, women choosing extended analysis had significantly higher trait anxiety (mean score of 39.5 (SD9.3), compared to 36.2 (SD6.8) in women who chose targeted analysis (p < 0.05)).

CONCLUSIONS: This ground breaking research was possible because the laboratory at the Victorian Clinical Genetic Service was prepared to offer the two-tier analysis in an effort to determine if all women want all fetal genomic information. While current clinical practice typically involves extended analysis, GaP has demonstrated that this does not reflect the true preference of 40% of those having prenatal testing. Provision of a decision aid is an excellent way to allow women to determine satisfactorily the depth of information they wish to receive, while minimizing negative psychological impacts.