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Hui, L;Hutchinson, B;Poulton, A;Halliday, J

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Poster Abstracts of the ISPD 21st International Conference on Prenatal Diagnosis and Therapy, San Diego, California, USA, 9-12 July 2017

P-1

Description of the clinical case of a child with a structural anomaly (duplication of chromosome 16)

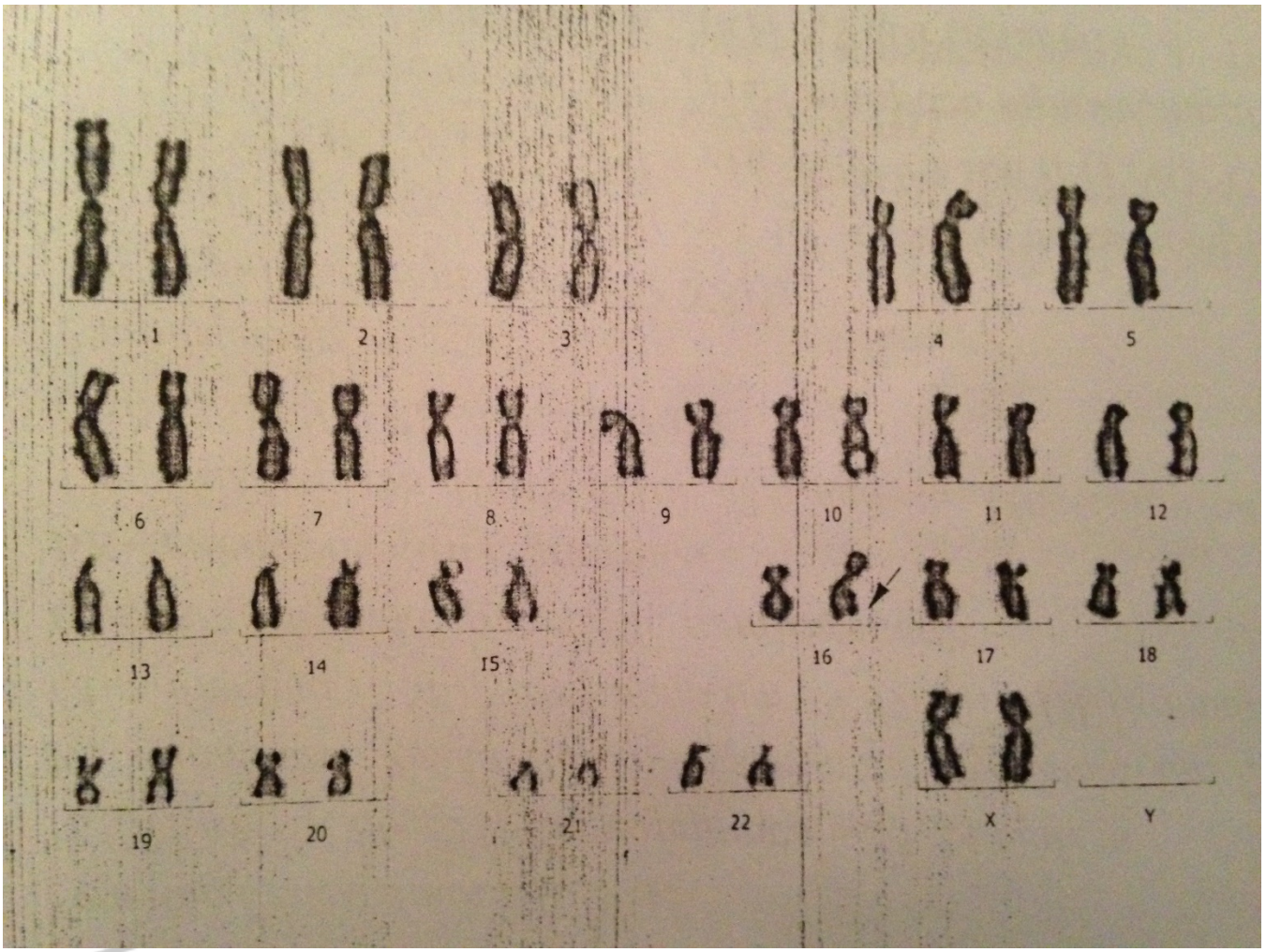
Assel Arginbekova

Association of Kazakhstan MD Genetics, Astana, Akmola Region, Kazakhstan

OBJECTIVES: Structural chromosome aberrations occurring in chromosome damage can't be restoring to the original structure. Due to the chromosome breaks occur structural changes. Further, they are connected in an anomalous combination. Chromosomal rearrangements occur spontaneously at low frequency and can be caused by damaging agents such as ionizing radiation, some viral infections and many chemicals. Changes that disrupt the normal balance of functional genes usually end up with abnormal manifestations. METHODS: The child was born of 4 pregnancy, childbirth 2 is by cesarean section, at term 39 weeks. Birth weight-3744,0 g, height 41 cm. Ultrasound at 33.4 weeks pregnant revealed multiple malformations of the central nervous system, spine cord, kidneys and limbs. During pregnancy, a woman prenatally not surveyed (began to be observed at 30 weeks). Phenotype: head shape hydrocephalic, flat noseband, high palate, low-set and dysmorphic auricle, a short neck, a barrel chest, in the lumbar region is determined by the formation of the size of 5 x 4 cm- rachischisis, spinal deformity, shortening lower limbs, bilateral clubfoot, foot deformity. RESULTS: From history the father of the baby before pregnancy began working with chemicals. The woman before the pregnancy had a miscarriage. The family has 2 healthy children.The result of karyotype (was carried out which graded G color): 46,XX, dup(16)(q22-24qter). The child has lived 101 days. The final diagnosis: Chromosomal structural abnormality. Duplication of the long arm of 16th chromosome. Multiple defects. Malformation of the Central nervous system: congenital bilateral hydrocephalus. Rachischisis of thoracic spine Th III-VIII. Malformation of the skeletal system: scoliosis Th III-VIII spines. Bilateral clubfoot. Malformation of the kidney: bilateral hypoplasia.Disseminated intravascular coagulation. Lower flaccid paraparesis. CONCLUSIONS: During the inspection noted multiple stigmas of dysembryogenesis in combination with multiple defects that suggested chromosomal pathology. Variations in gamete led to chromosomal imbalance. As a result of duplication in the chromosome caused multiple defects not compatible with life. The result was the death of a child. The reason was teratogenic effects on the reproductive quality of the father. In the case of the family it is considered a mutation de novo. It is important to emphasize the prenatal differential diagnosis of various diseases with developmental disabilities, to terminate such a pregnancy at an early stage.

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P-2

Prenatal diagnosis of interstitial duplication and triplication of chromosome 15q11-q13 in a fetus with cerebral ventricular dilatation

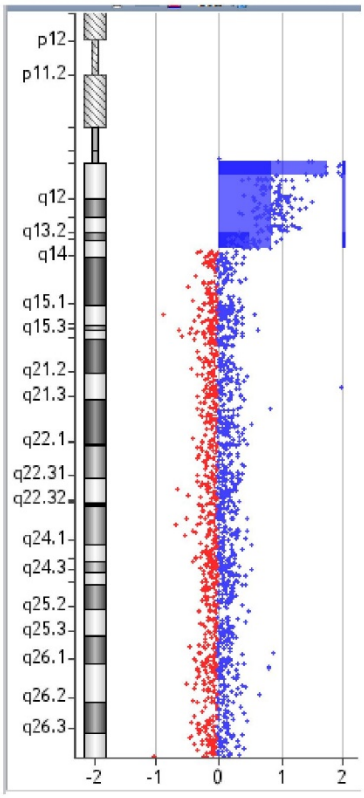
Sihem Darouich[1], Maryse Bonnière[2], Geneviève Quenum[2], Tania Attié Bitach[2], Michel Vekemans[2], Valerie Malan[2], Ferechte Razavi[3]

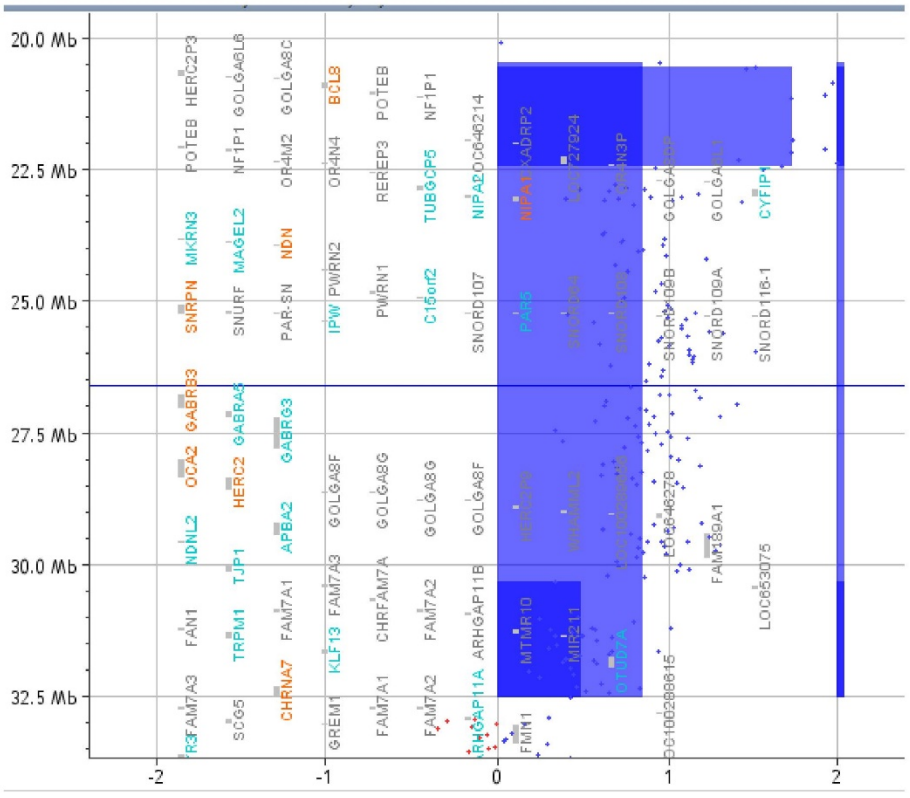
[1]*Fetopathology Unit, Bizerte, Tunisia*
[2]*Department of Genetics, Paris, France*
[3]*Division of Histology, Embryology and Cytogenetics, Necker Hospital for Sick Children, Paris, France*

OBJECTIVES: The proximal long arm of chromosome 15 is prone to cytogenetic abnormalities such as deletions, duplications, triplications, translocations and inversions. As the 15q11-q13 region is imprinted, theses rearrangements are associated with a variety of phenotypic abnormalities. Published data regarding the fetal phenotype are totally missing. Here, we report de novo duplication and triplication of chromosome 15q11-q13 in a fetus with cerebral ventricular dilatation (CVD). METHODS: We used karyotyping, FISH and array CGH to investigate the de novo complex rearrangements of proximal q15. Prenatal ultrasound and postmortem findings were described. RESULTS: A healthy 38-year-old primigravida woman was referred at 24 weeks' gestation for sonographically isolated severe CVD. Amniocentesis was performed and the karyotype revealed proximal duplication of q15. The array CGH detected a complex chromosomal rearrangement of 15q11-q13, consisting of a 7.8 Mb triplication of 15q11.2q13.1 and a 2.2 Mb duplication of 15q13.3, which were confirmed by FISH using BAC clones. The parental karyotypes were normal. The parents elected to interrupt the pregnancy. The fetus presented craniofacial dysmorphism and a triventricular dilatation associated with aqueductal stenosis. CONCLUSIONS: This report suggests that the interstitial duplication and triplication of the 15q11-q13 region may be a cause of fetal CVD and it provides additional support that these rearrangements are pathogenetic.

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P-3

Prenatal diagnosis of an unbalanced translocation 4q/6q in a fetus: Phenotype, cytogenetics and molecular characterization by array CGH

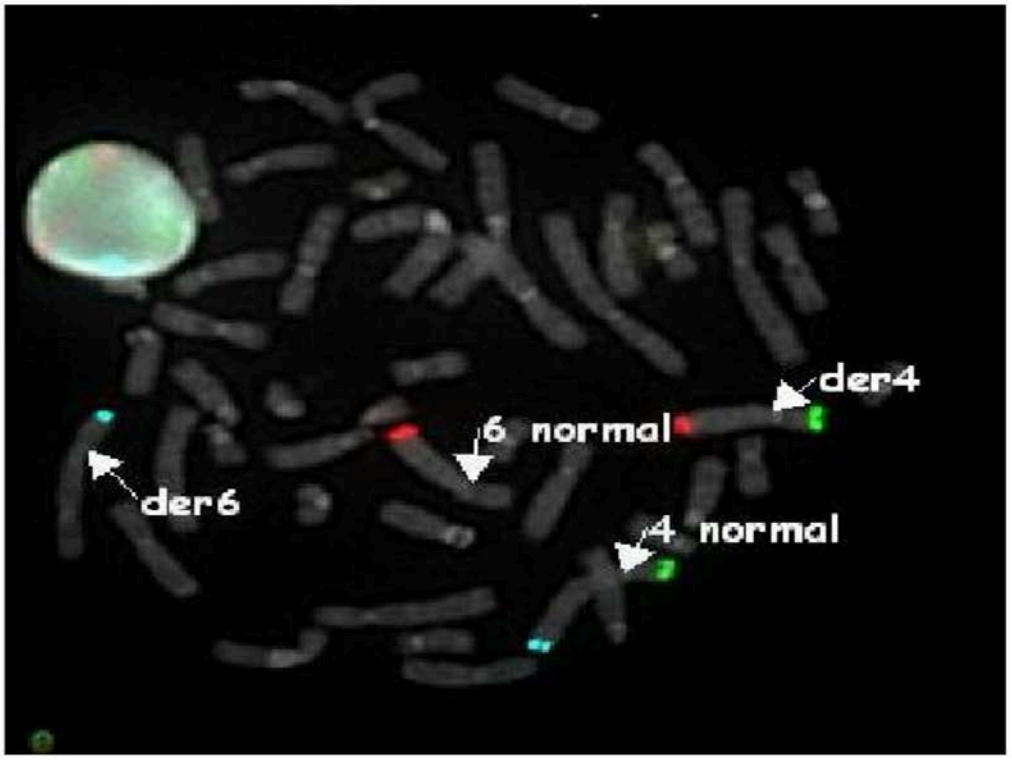
Sihem Darouich[1], Bettina Bessières[2], Tania Attié Bitach[2], Michel Vekemans[2], Valerie Malan[2], Ferechte Razavi[3]

[1]*Fetopathology Unit, Bizerte, Tunisia*
[2]*Department of Genetics, Paris, France*
[3]*Division of Histology, Embryology and Cytogenetics, Necker Hospital for Sick Children, Paris, France*

OBJECTIVES: Prenatal diagnosis of unbalanced translocation with normal conventional karyotype has been rarely reported in literature. We aim to highlight the importance of the use of array CGH in the detection of genomic unexpected imbalances. METHODS: We describe the prenatal ultrasound and autopsy findings of unbalanced translocation t(4;6)(q31.3;q25.3) in a fetus with an apparently normal karyotype. RESULTS: A healthy 33-year-old G5P3 woman was referred for prenatal diagnosis of spinal muscular atrophy. The genetic screening was negative. The second trimester ultrasound revealed facial dysmorphism, ventriculomegaly, corpus callosum agenesis and single umbilical artery. Amniocentesis was performed and karyotype was normal. The array CGH detected a duplication of 4q32.1q35.2 and a deletion of 6q26q27, consistent with FISH results. This defect resulted from the unbalanced segregation of a maternal translocation, t(4;6)(q31.3;q25.3). The pregnancy was interrupted at 25 weeks. The fetus was confirmed to have the sonographic abnormalities with the additional anomalies of third ventricle atresia and mild left heart hypoplasia. CONCLUSIONS: This case illustrates the usefulness of molecular characterization of fetus malformations for prenatal diagnosis and counseling. It also demonstrates the utility of the use of array CGH which provides a sensitive genome wide screen for unexpected imbalances.

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P-4

Implementation of a protocol for genetic testing on fetal cord blood

Lori Dobson, Nancy Herrig, Marie Discenza, Hayley Green-Morfesi, Rosemary Reiss, Kathryn Gray, Louise Wilkins-Haug

Brigham and Women's Hospital, Boston, Massachusetts, United States

OBJECTIVES: To develop a protocol for cord blood testing (CBT) obtained at the time of delivery for infants at high-risk for genetic conditions. METHODS: From November 2015 -March 2017, women with fetuses at risk for genetic conditions who declined invasive testing were offered CBT by their obstetrician or during prenatal neonatology consult. Genetic counselors reviewed genetic testing including diagnostic implications, informed consent and testing plan. Genetic testing may have included karyotype with preliminary FISH, chromosomal microarray (CMA) or single gene/panel testing, as well as DNA storage. Patients were provided cord blood packets to bring to delivery that included testing orders, appropriate blood tubes, and instructions for labor and delivery staff. Completed genetic testing results were reported to the appropriate provider teams. RESULTS: Twenty-eight patients elected CBT for fetal anomalies (n=19), positive cell-free DNA (cfDNA) (n=5), and family history (n=4). Completed testing (23/28; 82%) included 15 CMA, 3 FISH/karyotypes and 5 gene tests. Fifteen fetal anomaly cases completed CBT (13 CMA (no pathogenic variants detected) and two negative gene tests (Beckwith-Wiedemann, Noonan). Three positive cfDNA were confirmed (+21, XXY, and 5p deletion); one 45,X was not confirmed (46,XY). For familial conditions, CBT revealed one infant with a *TERC* mutation and three unaffected infants. CBT was unsuccessful in 4 (14%); all had postnatal or postmortem genetic testing. One subsequently declined testing. CONCLUSIONS: Fetal CBT completed indicated genetic testing for patients who decline prenatal invasive testing. Establishing a testing plan prenatally and completing necessary consents and paperwork, as well as providing patients with the appropriate tubes can help streamline testing and minimize errors at the time of delivery. Even when cord blood cannot be obtained, the testing plan can guide postnatal and postmortem testing. This type of protocol can expedite neonatal genetic diagnosis following delivery, and optimize care of the affected newborn. Further studies will be required to assess patients' desire for early postnatal diagnoses and their impact on neonatal care.

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P-5

Discordant phenotypes in monozygotic twins with 16p11.2 microdeletions including the SH2B1 gene

Lin Li, Linhuan Huang, Shaobin Lin, Yan-min Luo, Qun Fang

Fetal Medicine Center, Department of Gynaecology and Obstetrics, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China,

OBJECTIVES: Although monozygotic twins originate from a single zygote and share the same genetic material and a similar intrauterine environment, discordant phenotypes can be observed occasionally due to epigenetics, environment factors, asymmetric split of the embryo, discordant cell differentiation, abnormally placental blood flow and other mechanisms. A 200~240-kb *SH2B1*-containing deletion region on 16p11.2 is associated with early-onset obesity and developmental delay. Here, we describe a pair of monozygotic twin brothers with discordant clinical presentations. The *de novo* 244-kb 16p11.2 microdeletions sharing the same breakpoints were identified in both twins. METHODS: Intrauterine fetal growth restriction was revealed by ultrasound in both twins. Additionally, twin A exhibited coarctation of aorta, left ventricular noncompaction, atrial septal defect, pericardial effusion, left hydronephrosis and moderate developmental delay, whereas twin B exhibited single umbilical artery. Karyotype and chromosome microarray analysis were performed on both twins at 18 weeks of gestation. Peripheral blood was extracted from the two twins after birth and fluorescence *in situ* hybridization (FISH) experiments were performed. RESULTS: The karyotype in the twins showed a pair of normal males (46, XY). CMA results revealed the 244-kb deletions in 16p11.2 [arr 16p11.2 (28,807,417-29,051,191)×1] covering the *SH2B1* gene in both twins. No other imbalance was identified. SNP array confirmed that the twins were monozygotic. FISH analysis confirmed the presence of the heterozygous 16p11.2 deletion. Parental CMA results were normal, indicating that the fetal 16p11.2 microdeletion occurred *de novo*. CONCLUSIONS: The novel findings in monozygotic twins may expand the phenotypic spectrum of 16p11.2 microdeletion. Further studies are needed to strengthen the correlation between genotypes and abnormal clinical features.

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P-6

The importance of genetic diagnostic testing for prenatally detected skeletal anomalies

Jennifer Goldkamp[1], Emanuel Vlastos[2], Rachael Bradshaw[3]

[1]*St. Louis University, St. Louis, Missouri, United States*
[2]*St. Louis Fetal Care Institute, St. Louis, Missouri, United States*
[3]*St. Louis University School of Medicine, St. Louis, Missouri, United States*

OBJECTIVES: We present two cases of skeletal dysplasias that had suspected diagnoses based upon pre- and postnatal imaging; however, after genetic analyses were completed, were found to have an alternate diagnosis with different recurrence risks. METHODS: Introduction Skeletal dysplasias are a heterogeneous collection of conditions that can result from de novo mutations or be due to autosomal inheritance. Obtaining a specific diagnosis is imperative, as recurrence risks vary based on the condition. RESULTS: 1. 24yo G1 @ 20w whose fetus' long bones were <5th%. The patient delivered at 26w via cesarean due to malpresentation and preterm labor. Pre- and postnatal imaging suggested a diagnosis of chondrodysplasia punctata, rhizomelic type. Postnatal genetic testing identified spondylophiphyseal dysplasia. 2. 31yo G3P2 @ 21w whose fetus was found to have <5th% humeri, radii, and ulnas, increased cardiothoracic ratio, and frontal bossing. Suspected diagnosis of thanatophoric dysplasia. Delivery was accomplished vaginally at 34 weeks. Genetic test results received postnatally was consistent with a diagnosis of cranioectodermal dysplasia. CONCLUSIONS: It is important to pursue accurate diagnosis for patients who have a fetus affected by a skeletal dysplasia to allow for accurate recurrence risk assessment. Case 1 was suspected to have an autosomal recessive condition, however was diagnosed with a de novo autosomal dominant condition (*COL2A1* alteration, c.1340G>T). Case 2 was suspected to have a de novo autosomal dominant condition, however was diagnosed with an autosomal recessive condition (compound heterozygosity for alterations in *WDR35,* c.324_325dup and c.3203A>G). While antenatal ultrasound diagnosis is improving, genetic analysis is the gold standard for diagnosis and should be completed.

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P-7

Prenatal diagnosis of complete trisomy 9 in the second trimester

Caitlin Grabarits, Jill Slamon, Cynthia Fata, Melissa Straub, Kelly Bennett

Vanderbilt University Medical Center, Nashville, Tennessee, United States

OBJECTIVES: Trisomy 9 is a rare chromosome abnormality that constitutes 2.7% of all trisomy cases in pregnancy (Tonni, et al. 2012). Trisomy 9 typically results in a first trimester miscarriage, thus there is limited information regarding the incidence and presentation of trisomy 9 in the second trimester. Reported cases of complete trisomy 9 in the second trimester demonstrate a lack of unique ultrasound findings, often mirroring the ultrasound findings of more common aneuploidies such as trisomy 13 and 18. Trisomy 9 is often not detected by the most widely used aneuploidy screening techniques. However, previous literature reviews hypothesize that cases of trisomy 9 could be detected in the first trimester with an increased nuchal translucency measurement. This report shares the clinical course and presentation of complete trisomy 9 detected in the second trimester that had normal nuchal translucency and biochemistry screening. METHODS: The patient is a 32 year-old primagravid female of Caucasian descent with no significant family history who was referred to university-based fetal center for evaluation due to multiple fetal anomalies. Fetal ultrasonography and echocardiography identified a fetus with growth at less than the third percentile, scalp edema, ascites, bilateral pleural effusions, bilateral diaphragmatic eventrations, mesocardia, muscular VSD, small SVC, bilateral echogenic kidneys, duplicated left kidney, sacral ONTD, bilateral ventriculomegaly, low-set ears, and hypotelorism. Amniocentesis was performed at 21 weeks and 6 days gestation. FISH for chromosomes 21, 18, 13, and X was normal. Termination of pregnancy was elected at 23 weeks via induction of labor. RESULTS: Karyotype analysis revealed 47,XX, +9, and SNP microarray indicated non-mosaic trisomy 9. A complete autopsy was performed. On external examination, the fetus had low-set ears, telecanthus, micrognathia, a short, webbed neck, absent intergluteal fold, and a sacral open neural tube defect. Internal abnormalities included mesocardia, ventricular septal defect, pulmonary hypoplasia with abnormal lobation, right renal atrophy, left ureter duplication, hypoplastic bladder, intestinal malrotation, adrenal hypoplasia, and an enlarged thyroid. Neuropathologic examination revealed mildly dilated ventricles and agenesis of the corpus callosum. CONCLUSIONS: This report illustrates a second trimester prenatal diagnosis of complete trisomy 9 in a pregnancy with normal nuchal translucency, first trimester biochemistry, and msAFP screening. This pregnancy demonstrated a myriad of fetal anomalies that were not detected until 20 weeks gestation. This case is an example of trisomy 9 not detected by conventional aneuploidy screening, and demonstrates the need for adequate counseling regarding the limitations of these testing modalities in pregnancy.

Author Abstract

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P-8

Prenatal diagnosis for a case with Williams-Beuren syndrome associated with atypical phenotypes by single nucleotide polymorphisms array

Linhuan Huang[1], Lin Li[1], Shaobin Lin[1], Zhi-ming He[1], Xuan Huang[1], Yanmin Luo[2], Yi Zhou[1], Qun Fang[1]

[1]*Fetal Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*
[2]*The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*

OBJECTIVES: Williams-Beuren syndrome (WBS) is a common chromosome microdeletion syndrome, manifests as supravalvular aortic stenosis, intellectual disability, developmental delay and characteristic facial features. Early diagnosis and treatment are very helpful for patients and their families. The paper is to describe the prenatal phenotype of WBS and present the molecular characterization of the deletion in the case presented. METHODS: We retrospectively reviewed a fetus diagnosed with 7q11.23 microdeletion using single nucleotide polymorphisms array (SNP-array). Routine G-banding was performed to analyze the karyotype of the patient and her parents. In addition, SNP-array was used to determine the copy number variations, which was confirmed by fluorescence in situ hybridization. RESULTS: The fetus showed atypical phenotypes, including duodenal atresia and a single umbilical artery on the left side, and had no any congenital heart disease. Typical WBS deletions deletion in 7q11.23 region (1.44 Mb) were discovered by SNP-array for the fetus. The ELN gene was contained in the deletions region. Parental results showed that the deletion inherited from the mother with normal phenotype. CONCLUSIONS: Since the limitation of prenatal ultrasound and less pronounced phenotypes, it is not easy to diagnose WBS in fetus. Williams-Beuren syndrome may have phenotypic variability during the fetus period, our findings provide some information on the genotype-phenotype correlations of WBS.

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P-9

Application of chromosomal microarray analysis in prenatal diagnosis of fetal congenital cardiac defects

Linhuan Huang[1], Zhi-ming He[1], Xuan Huang[1], Shaobin Lin[1], Yanmin Luo[2], Yi Zhou[1], Qun Fang[1]

[1]*Fetal Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*
[2]*The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*

OBJECTIVES: Congenital heart disease (CHD) is the most frequent birth defect, genomic copy number variations (CNVs) are important in the etiology of CHD. To investigate the clinical value of chromosomal microarray analysis (CMA) in the prenatal diagnosis of chromosomal abnormalities in fetal CHD cases. METHODS: A total of 279 patients at 12 weeks ~ 37 weeks of gestation were identified CHD with prenatal ultrasound diagnosis. The cases were divided into isolated or non-isolated CHD. Karyotyping was performed in all cases, and CMA (CytoScan HD arrays, Affymetrix, California, USA) was performed in 90 cases. RESULTS: In our study, karyotype analysis identified chromosomal aberrations in 21.5% (60/219) of the cases, while CMA detected abnormalities (pathogenic CNVs and variant of uncertain clinical significance) in 27.8% (25/80) of the cases. CMA achieved a 7.6% detection rate of pathogenic CNVs among CHD cases with a normal karyotype. Among the 90 CHD fetuses, 58 (46.3%) were isolated CHD, 32 (24.1%) were cases with extracardiac anomalies. The detection rate of pathogenic CNVs in CHD with extracardiac anomalies was lower than but not statistically different from prenatally isolated CHD (9.4%, 3/32 vs. 12.1%, 7/58, P>0.05). CONCLUSIONS: Our study highlights the added value of CMA compared with karyotyping in evaluation of CHD cases. It should become an integral aspect in clinically molecular genetics diagnosis. The complexity of the extracardiac anomalies was not enhanced the frequency of pathogenic CNVs but not statistically different.

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P-10

22q12.3 microduplication overlapping the gene LARGE with male abnormal phenotype suggestive sex-differential risk loci for autism spectrum disorder

Xuan Huang[1], Yingjun Xie[2], Qun Fang[1]

[1]*Fetal Medicine Center, Department of Obstetrics and Gynecology, First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China*
[2]*Key Laboratory for Major Obstetric Diseases of Guangdong Province, Key Laboratory of Reproduction and Genetics of Guangdong Higher Education Institutes, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China*

OBJECTIVES: We report a three-generation Chinese family with only affected males diagnosed as autism disorder spectrum disease. METHODS: The proband boy was examined by brain MRI, Whole-genome single nucleotide polymorphism (SNP)-based microarray and fluorescence in situ hybridization(FISH). RESULTS: He presented features of autism spectrum disorder. Brain MRI image showed an abnormal high-intensity zone in the frontal white matter in the boy. Whole-genome SNP-microarray demonstrated an interstitial 575 Kb duplication of chromosome 22p12.3 that involved the *LARGE* gene among six family members including three presented normal female carriers and three males including a affected boy and two male fetuses. Fluorescence in situ hybridization analysis with special probes and LARGE gene sequencing were performed, which showed a submicroscopic 22q13 duplication that involved the LARGE gene. CONCLUSIONS: Combined with the review of the literatures, our finding support that the 22q12.3 microduplication overlaping the LARGE may be a male-only affected loci that is responsible for increasing autism spectrum disorder risk.

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P-13

Failure of NIPT to detect a novel ring 21 chromosomal rearrangement in a case of fetal hydrops

Melissa MacPherson[1], Kathleen Bone[2], Judy Chernos[3]

[1]*University of Calgary, Calgary, Alberta, Canada*
[2]*Western University, Cytogenetics, Department of Pathology and Laboratory Medicine, London Health Sciences Centre, London, Ontario, Canada*
[3]*Department of Medical Genetics, University of Calgary, Genetic Laboratory Services, Cytogenetics Laboratory, Alberta Children's Hospital, Calgary, Alberta, Canada*

OBJECTIVES: We present a case of fetal hydrops where NIPT failed to detect a novel ring 21 complex chromosomal rearrangement. Our patient, a 38 yo, G4,T2,P0,SA1,L2, had a positive first trimester screen with a 1/60 risk for trisomy 21. She subsequently underwent Harmony NIPT testing with a low risk screening result. At her detailed anatomy ultrasound, the fetus was found to have hydrops with bilateral atelectasis and large pleural effusions. METHODS: She underwent amniocentesis and fetal pleurocentesis. Rapid aneuploidy screening (RAD) by QF-PCR for common aneuploidies was performed on direct sample of amniocytes. Subsequent karyotype analysis was performed on cultured amniocytes. The chromosomal imbalance was further characterized using array CGH. RESULTS: RAD testing showed two copies each of chromosome 13 and 18 and one copy of X and Y. RAD testing was uninformative for chromosome 21. One marker on chromosome 21 was disomic, 2 markers were trisomic and 4 markers were uninformative. Karyotype analysis showed the following: 46,XY,r(21)(p11.2q22). The fetus had one normal copy of chromosome 21 and one ring 21 chromosomal rearrangement. Array CGH testing showed multiple areas of imbalance on chromosome 21 including four independent areas of trisomy and one area of monosomy. The Down Syndrome Critical Region was partially duplicated. Analysis of microsatellites showed r(21) was maternally derived. CONCLUSIONS: These findings give evidence for a chromothripsis event involving one copy of chromosome 21. Chromothripsis is a phenomenon by which a single step catastrophic genomic event shatters a chromosome into many pieces and reassembles it back into a functional structure. These rearrangements are more commonly seen in malignancies and are less frequently associated with constitutional chromosomal imbalances. This case illustrates a failure of NIPT testing to recognize a constitutional chromosomal rearrangement involving chromosome 21. The case highlights the importance of knowing the limitations of the various NIPT technologies in detecting chromosome imbalances when counselling patients both pre-and post testing.

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P-14

Two novel likely pathogenic variants in DYNC2H1 in a fetus with multiple skeletal abnormalities and ambiguous genitalia

Ludmila Matyakhina[1], Elizabeth Kramer Dugan[1], Melissa Gillette[2], Tracy Brandt[1], Lisa Vincent[1], Jeanne Meck[1]

[1]GeneDx, Gaithersburg, Maryland, United States
[3]St. Joseph Regional Medical Center, Mishawaka, Indiana, United States

OBJECTIVES: To describe our genetic test results on a fetus with abnormal ultrasound findings including bilateral shortening and polydactyly of the upper and lower extremities, small chest, femur length-to-abdominal circumference ratio of 0.12 (consistent with lethality), left cardiac axis deviation, and ambiguous genitalia. METHODS: This fetal sample was sent to our laboratory for testing due to multiple congenital anomalies consistent with a lethal skeletal dysplasia. Fetal DNA isolated from amniotic fluid was tested by a targeted 60K custom oligonucleotide microarray (Agilent) designed to detect pathogenic copy number variants (CNVs) associated with known microdeletion/duplication syndromes and UPD of several clinically relevant chromosomes. Concurrent testing for the coding regions of 22 genes associated with skeletal dysplasias and one known pathogenic variant in IFITM5 was performed by next generation sequencing and results were confirmed by capillary sequencing. RESULTS: Array testing yielded no clinically significant CNVs, but was consistent with an XY sex chromosome complement which was discordant with the provided clinical information indicating a female fetus. Additional review of ultrasound findings with the physician revealed that the fetus had "prominent genitalia," but was suspected to be female. Subsequently, the skeletal dysplasia panel identified two novel likely pathogenic variants in *DYNC2H1*: c.9841-2 A>G and p.Ala1542Val. Parental testing of these variants confirmed that they were inherited on opposite alleles (in trans). Postnatal evaluation of the deceased fetus confirmed prenatal findings and identified a scrotum with descended testes and aphallia. CONCLUSIONS: The finding of two compound heterozygous likely pathogenic variants in *DYNC2H1* is consistent with an autosomal recessive skeletal ciliopathy known as short-rib thoracic dysplasia 3 with or without polydactyly (SRTD3) in this fetus. The additional clinical observation of ambiguous genitalia aided variant interpretation in this fetus. While sex reversal has been reported in SRTD3, the data on its causal association with *DYNC2H1* variants is limited. Our results support a role for *DYNC2H1* in gonadal development and suggests that pathogenic variants in this gene can result in sex reversal.

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P-15

SNP microarray in cases with first trimester ultrasound abnormalities

Hiroyasu Ohashi[1], Ritsuko Pooh[2], Masayoshi Takeda[1], Osamu Shimokawa[3]

[1]*Osaka, Japan*
[2]*CRIFM Clinical Research Institute of Fetal Medicine, Osaka, Japan*
[3]*Ritz Medical Co., Ltd, Osaka, Japan*

OBJECTIVES: SNP microarray has often been utilized after karyotype analysis in a field of prenatal diagnosis. In this study, SNP microarray analysis has been utilized for further investigation in cases with ultrasound abnormalities which were not explained by karyotype. METHODS: Between 2016 and 2017, SNP microarray has been performed in specific case with ultrasound abnormalities but normal karyotype etc. SNP microarray is performed by CytoScan® 750K (Affymetrix) and data analysis by Chromosome Analysis Suite (ChAS) (Affymetrix). The data are compared with 3 databases, DGV, ISCA and DECIPHER. RESULTS: 3 cases had been diagnosed as pathogenic CNV encompassing ISCA curated pathogenic CNV. Case 1: Karyotype result was Trisomy-X. And 3.7Mb deletion of 17p11.2 (Smith-Magenis syndrome critical region). Case 2: Karyotype result was der(13;14). And 7.2 Mb deletion of 10p26.2q26.3 and 8.0 Mb duplication of 17q25.1q25.3 (due to familial chromosome translocation). Case 3: Karyotype result was normal. And 10.7 Mb duplication of 3q27.3q29 and 10.2 Mb deletion of 6q25.3q27 (due to familial chromosome translocation). The karyotype band level that we analyzed was an average of 550. CONCLUSIONS: SNP microarray is useful for the cases with ultrasound abnormalities with karyotypes accompanied with difficult break point. However we should be prudent in diagnoses and counseling patients, because there still be many of variants of uncertain clinical significance (VOUS) and it is essential to investigate familial variants as well as long-term follow up of infants with abnormal SNP. Of course need check Trio (Fetus, maternal and paternal) analysis.

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P-16

Fetal neuronal migration disorder with megalencephaly at 18 weeks related to paternal UPD mosaicism with PTEN mutation

Ritsuko Pooh[1], Hideaki Chiyo[1], Hiroyasu Ohashi[2], Osamu Shimokawa[3], Issei Imoto[4], Shigeru Saito[5]

[1]*CRIFM Clinical Research Institute of Fetal Medicine, Osaka, Japan*
[2]*Osaka, Japan*
[3]*Ritz Medical Co., Ltd, Osaka, Japan*
[4]*Tokushima University, Tokushima, Japan*
[5]*Toyama University, Toyama, Japan*

OBJECTIVES: PTEN (Phosphatase and tensin homolog) gene is located on the 10q23.31. The PTEN enzyme is part of a chemical pathway that signals cells to stop dividing and triggers cells to self-destruct through a process called apoptosis. PTEN also helps control cell movement (migration), the sticking (adhesion) of cells to surrounding tissues, and the formation of new blood vessels (angiogenesis). We presented a rare case of brain disorder associated with unique genetic change. METHODS: A 37-year-old pregnant woman was referred at 18 weeks due to macrocephaly from 16 weeks. Fetal neuroimaging at 18 weeks revealed macrocephaly of +6.2 SD BPD and HC with abnormal cortical sulcation and irregular ventricular wall. Amniocyte SNPmicroarray resulted in paternal UPD mosaicism of 10q however relations between mosaic UPD of 10q and fetal brain abnormality was not explained. At 20 weeks, remarkable cortical change with polygyri appeared. Pregnancy was terminated at 21 weeks. Postmortem investigation of amniocytes-DNA resulted in mosaic homo-PTEN mutation. RESULTS: It is indicated that rare bilateral macrocephaly associated with neuronal migration disorder of fetal megalencephalic brain seen in this case may be strongly related with PTEN de novo mutation on 10q paternal UPD mosaicism. CONCLUSIONS: Neuronal migration disorder during fetal period has been identified by fetal neuroimaging after 28 weeks of gestation when cortical gyration becomes conspicuous. In the present case, migration disorder-related macrocephaly was depicted by fetal neuro-sono-imaging as early as 18 weeks of gestation. Simaltaneous genetic change of both point mutation and genome structural rearrangement may have contributed to neuronal migration and proliferation. The unique molecular genetic results by use of both SNPmicroarray and sequencing contributed to genetic investigation of fetal neuronal migration disorder.

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Novel heterozygous mutations of INSR gene in a familial case with Donohue syndrome

Litao Qin, Shixiu Liao

Medical Genetic Institute of Henan Province, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou, Henan, China

OBJECTIVES: Donohue syndrome (DS), a rare autosomal recessive disease which represents severe insulin resistance, pre- and postnatal growth retardation, hypertrichosis and dysmorphic features, is caused by the mutation in the insulin receptor (*INSR*) gene. In this study, we report the clinical, molecular and biochemical characterization of a patient with DS. Discuss the method of using targeted next-generation sequencing (NGS) in rare diseases molecular diagnosis. METHODS: A 2-year-old Chinese boy with DS and his kinships as healthy volunteers were recruited. Targeted NGS was performed to detect 27genes which were associated with maturity-onset diabetes of the young (MODY). Sanger sequencing was used to validate the positive correlation of these mutations with DS. Bioinformatics analysis and molecular dynamics simulation were employed to predict the pathogenic mutations. RESULTS: Compound heterozygous mutations in the *INSR* gene in the patient with DS, c.62T>G (p.L21R) and c.2563G>T (p.V855F) were detected. The Sanger sequencing results showed these mutations were inherited from maternally and paternally, respectively. Bioinformatics analysis and molecular dynamics simulation results strong suggested that these mutations were pathogenic mutations in this case. CONCLUSIONS: Targeted NGS identified compound heterozygous mutations in *INSR* gene of a Chinese boy with DS. These mutations which considered as pathogenic compound heterozygous mutations were first reported in *INSR* gene in Asian. The finding expands the mutation spectrum of the *INSR* gene and supports the use of targeted NGS in genetic diagnosis of DS with clinical heterogeneity.

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Comparison of pan-ethnic and ethnic-based carrier screening panels for individuals of Ashkenazi Jewish descent

Lynne Rosenblum, Jennifer Teicher, Zhaoqing Zhou, Natalia Leach, Hui Zhu, Ruth Heim

Integrated Genetics, Westborough, Massachusetts, United States

OBJECTIVES: The intent of carrier screening is to identify individuals at risk for a child with a genetic disease. ACMG guidelines currently recommend that individuals of Ashkenazi Jewish descent be screened for carrier status for nine diseases. A joint statement from ACMG, ACOG, NSGC, SMFM, and the Perinatal Quality Foundation acknowledges benefits of screening for more than nine diseases (expanded carrier screening). Here we analyze detection rates for Ashkenazi Jewish individuals screened by panels with different numbers of diseases, to assess the benefit of disease panels targeted to the Ashkenazi Jewish population. METHODS: Array-based hybridization and allele-specific primer extension with a custom Illumina Infinium ™ array (IGv1.1) were used to detect 434 mutations in 87 genes that cause 87 diseases or a subset of 147 mutations in 18 genes that cause 18 diseases. Mutations were confirmed by targeted DNA sequencing. The 87-gene panel was intended for pan-ethnic carrier screening (pan-ethnic panel), and the 18-gene panel was intended for Ashkenazi Jewish carrier screening (AJ panel). The study sample comprised individuals self-identified as Ashkenazi Jewish, 1150 of whom were tested in the pan-ethnic panel and 1248 of whom were tested in the AJ panel. RESULTS: In the pan-ethnic panel 431/1150 (37.5%) individuals were carriers of at least one disease. In the AJ panel 319/1248 (25.5%) individuals were carriers of at least one disease. If positive individuals in the pan-ethnic panel were tested in the AJ panel, the detection rate would be 280/1150 (24.3%). If positive individuals in the pan-ethnic panel were tested for the nine ACMG recommended diseases, the detection rate would be 207/1150 (18.0%). Twenty-one diseases accounted for the difference in carrier rates between the pan-ethnic and AJ panels. Of these, three were most common: familial Mediterranean fever, CGD1a, and Smith-Lemli-Opitz syndrome. CONCLUSIONS: A pan-ethnic expanded carrier screening panel of 87 genes increased the carrier detection rate in Ashkenazi Jewish individuals by approximately 50%, compared with a panel of 18 genes considered to be relevant to the Ashkenazi Jewish population. The detection rate would have increased by approximately 100% if the pan-ethnic panel were compared to just the ACMG recommended genes in this data set. These data suggest that targeted AJ panels are not as effective as a pan-ethnic panel in carrier detection among individuals of Ashkenazi Jewish descent.

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A maternally-inherited triplication within chromosome Xq26.2 encompassing the DXS1187 STR locus confounds the prenatal QF-PCR rapid aneuploidy detection result

Mary Ann Thomas[1], Amy Inkster[2], Bob Argiropoulos[3]

[1]*Department of Medical Genetics, University of Calgary, Calgary, Alberta, Canada*
[2]*Cytogenetics Laboratory, Calgary, Alberta, Canada*
[3]*Cytogenetics Laboratory, Calgary Laboratory Services and Alberta Children's Hospital Research Institute, Calgary, Alberta, Canada*

OBJECTIVES: The aim of the present study was to interrogate the origin of the biallelic trisomic amplification pattern of the DXS1187 locus in an otherwise normal male fetus which was identified on routine prenatal diagnosis by quantitative fluorescent-polymerase chain reaction (QF-PCR) rapid aneuploidy detection (RAD). METHODS: Prenatal diagnosis was performed on a 35-year-old female at 15 weeks 2 days gestation for a positive first trimester screen. QF-PCR, metaphase FISH and chromosomal microarray were performed on both maternal and fetal DNA. RESULTS: Fetal QF-PCR showed a biallelic trisomic pattern for the DXS1187 STR locus with an otherwise normal male amplification pattern at other loci. Chromosome analysis performed on cultured amniocytes showed a normal male karyotype in all metaphases examined. Chromosome microarray analysis identified a maternally-inherited 385 kb copy number triplication (3 copies) within chromosome Xq26.2 encompassing the DXS1187 locus but no genes. CONCLUSIONS: The maternally inherited X chromosome harbors a tandem triplication of the region encompassing the DXS1187 locus. The biallelic trisomic amplification is due to there being one allele of 149.53 bp and two alleles of 153.87 bp. As the triplicated region is devoid of genes and tandem in nature, it was classified as likely benign in the fetus. Post-natal follow-up reported a healthy male newborn. To our knowledge, this is a unique case demonstrating a 'benign' copy number imbalance involving the region encompassing the DXS1187 STR locus which confounded the prenatal QF-PCR RAD result.

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Prevalence of submicroscopic chromosome aberrations in pregnancies without increased risk for structural chromosome aberrations – A systematic review of the literature

Malgorzata Srebniak[1], Marieke Joosten[1], Maarten F.C.M. Knapen[2], Marike Polak[3], Lidia R. Arends[4], Attie Go[5], Diane Van Opstal[5]

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

OBJECTIVES: To estimate the prevalence of pathogenic submicroscopic chromosome aberrations in fetuses that are not at increased risk for chromosomal abnormalities, a systematic literature search was performed. The aim was to determine whether high resolution testing for submicroscopic aberrations has additional value in a general pregnant population. METHODS: On June 3rd 2016 Embase and PubMed databases were systematically searched for all relevant articles on prevalence of pathogenic submicroscopic CNVs in fetuses tested because of advanced maternal age or parental anxiety. Relevant full text articles were analyzed. Based on the extracted data the prevalence of pathogenic CNVs was estimated. RESULTS: Combined data of the relevant studies (n=19) allowed the analysis of a cohort of 13,386 fetuses. In 0.86% (115/13,386) of fetuses a submicroscopic pathogenic aberration was detected prenatally. The prevalence of early onset syndromic disorders due to a submicroscopic aberration was calculated to be 1:294, based on 0.34% (36/10,614) cases where aberrations were specified. CONCLUSIONS: This systematic review shows that a significant proportion of fetuses in a general, pregnant population carry a submicroscopic pathogenic CNV. Therefore, we conclude that each pregnant woman would benefit from high resolution cytogenetic testing in pregnancy. Based on these figures, women should be informed on their individual risk for all pathogenic chromosome aberrations and not only for common trisomies to allow personalized informed choices on the type of prenatal testing.

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Amniotic fluid cytogenetic analysis of 10043 senile gravidas at middle gestational period

Bei Xia

Sichuan University, West China Second Hospital, Chendu, Sichuan Province, China

OBJECTIVES: To analyze the incidence of fetal chromosome abnormalities in senile gravidas, and to probe into the risk of gestating fetuses with chromosome abnormalities in senile gravidas. METHODS: Data of 10043 cases of senile gravidas receiving amniocentesis and amniotic fluid karyotype analysis during middle gestational period in our hospital from January 2014 to October 2016 were retrospectively analyzed. RESULTS: 9929 out of the 10043 cases of amniotic fluid specimens were successfully cultured, with the success rate of 98.9%. Altogether 416 cases of chromosome abnormalities were detected, with the abnormality detection rate of 4.14% (416/10043); among which, 269 cases had chromosome numerical abnormalities, with the detection rate of 2.68% (269/10043), and 67 cases had chromosome structural abnormalities, with the detection rate of 0.67% (67/10043). 475 cases showed chromosomal polymorphism, the incidence of which accounted for 4.72% (475/10043). CONCLUSIONS: Amniotic fluid karyotype analysis in senile gravidas contributes to detecting fetal chromosome abnormalities and thus avoids the birth of abnormal fetuses.

Table 1. Karyotype of 269 cases of amniotic fluid chromosome numerical abnormality

Type	Karyotype result	Case
Chromosome numerical abnormality	47,XN,+21	167
	47,XN,+18	38
	47,XN,+13	8
	47,XXY	16
	47,XXX	8
	47,XYY	2
	47,XN,+mar	5
	47,XN,+mar denovo	2
	45,X	2
	45,X/47,XXX	3
	45,X/46,XX	5
	45,X/46,XY	4
	47,XN,+9/46,XN	1
	47,XN,+20/46,XN	1
	47,XXY/46,XY	3
	47,XN,+12/46,XN	1
	46,XX/47,XXX	1
	47,XYY/46,XY	2

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In total		269

Table 2. Karyotype of 67 cases of amniotic fluid chromosome structural abnormality

Type	Karyotype result	Case
Chromosome structural abnormality	46,XN,inv(1)(p13q21)	4
	45,X/46,X,i(X)(q10)	3
	46,XN,inv(2)(p13q13)	2
	46,XN,r(20)/46,XN denovo	1
	46,X,del(X)(q22)	1
	45,XN,rob(14;15)	1
	45,XN,rob(13;14)	2
	45,XN,rob(15;22)	1
	45,XN,rob(13;15)	1
	45,XN,rob(14;21)	1
	46,XN,inv(20)(p12q11.2)	1
	46,XN,der(21) denovo	1
	46,XN,der(4) denovo	1
	46,XN,inv(10)(p12q23)	1
	46,XN,inv(10)(q23q24)	1
	46,XN,inv(12)(q13q21)	1
	46,XN,del(18)(p11.2)	1
	46,XN,der(14)	1
	46,XN,del(13)(p11.2)	5
	46,XN,del(21)(p11.2)	4
	46,XN,t(7;16)(q22;q13)	1
	46,XN,t(1;13)(q36;q31)	1
	46,XN,t(1;8)(q31;q21.3)	1
	46,XN,t(6;10)(q23;q25)	1
	46,XN,t(3;15)(p22;q23)	1
	46,XN,t(1;20)(p13;p13)	1
	46,XN,t(9;15)(q10;p11.2)	1
	46,XN,t(11;13)(q13;q13)	1
	46,XN,t(7;10)(q31;q24)	1
	46,XN,t(1;17)(q32;q25)	1
	46,XN,t(16;20)(q13;p11)	1

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	46,XN,t(5;8)(q13;q23)	1
	46,XN,t(7;8)(q22;q24)	1
	46,XN,t(7;11)(q21;q14)	1
	46,XN,t(8;14)(q21;q12)	1
	46,XN,t(10;19)(p11;q13)	1
	46,XN,t(1;5)(q34;q14)	1
	46,XN,t(5;8)(q14;q21.3)	1
	46,XN,t(1;17)(q22;q22)	1
	46,XN,t(3;18)(q21;q12)	1
	46,XN,t(12;18)(q22;q12)	1
	46,XN,t(1;2)(q22;q32)	1
	46,XN,t(11;22)(q24;q13)	1
	46,XN,t(1;5)(p34;q31)	1
	46,XN,t(1;2)(p31;q22)	1
	46,XN,t(4;7)(q22;q22)	1
	46,XN,t(3;11)(q22;q24)	1
	46,XN,t(9;22)(p21;q21)	1
	46,XN,t(11;19)(q13;p13)	1
	46,XN,t(2;20)(q12;p11)	1
	46,XN, inv(16)(q23q24)	1
	46,XN,t(1;6)(q21;p11)	1
	46,XN,t(2;8)(q22;p12)	1
In total		67

Table 3. 475 cases of amniotic fluid chromosome polymorphism

Type	Karyotype result	Case
Chromosome polymorphism	46,XN,inv9(p12q13)	109
	46,XN,1qh+	99
	46,XN,9qh+	86
	46,XN,16qh+	29
	46,XN,15pss	30
	46,XN,21pss	22
	46,XN,14pss	16
	46,XN,22pss	23
	46,XN,15p+	15
	46,XN,13pss	14
	46,XN,15ps+	5
	46,X,inv(Y)(p11q11)	16
	46,X,Yqs	7
	46,XN,22ps+	2
	46,XN,13p+	1

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In total	46,XN,22p+	1 475

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P-22

Cytogenetic analysis of 35,592 Chinese women with advanced maternal age

Lai Yi, Liu Hongqian, Liu Zhiying

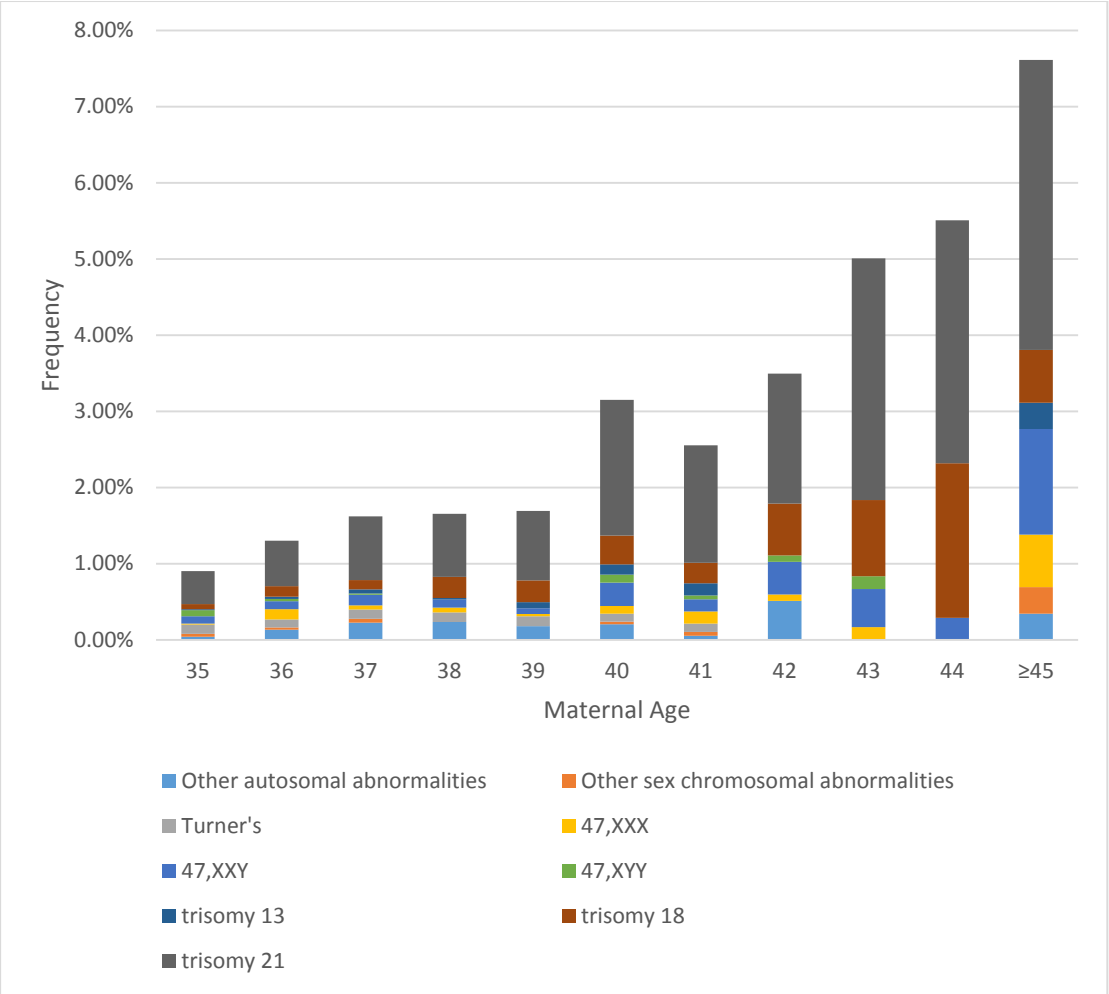
Prenatal Diagnosis Center, Department of Obstetrics and Gynecology, West China Second University Hospital, Sichuan University, Chengdu, China

OBJECTIVES: To examine the prevalence and types of chromosome abnormalities among women aged 35 and over seeking prenatal diagnosis at the West China Second Hospital;Sichuan University. METHODS: We analyzed cytogenetic reports of amniocentesis between October 2008 and September 2016 from women with advanced maternal age (≥35 years at delivery), who had normal sonographic findings for the current pregnancy; without a history of previous congenital abnormalities or other risk factors for chromosome abnormalities; and who sought prenatal diagnosis at the second hospital of West China. We compared the frequency and types of chromosome abnormalities by age. RESULTS: We identified 642 (1.8%) cases of meaningful chromosome abnormalities, including 336, 84, and 18 cases of trisomy(21, 18, 13) respectively, 40 cases of Turner's, 97 cases of 47 chromosomes (XXX, XXY and XYY) ,and 67 cases of other types of chromosome abnormalities. The prevalence of chromosome abnormalities increased from 0.9% in those aged at 35 to 7.6% in those aged at 45 years of older (Figure). There were statistically significant yearly increasing trends for trisomy (21, 18, 13) and 47 (XXX, XXY) while Turner's and 47(XYY) failed to demonstrate statistically significant trend, with p value of 0.28, 0.91. CONCLUSIONS: The number of older pregnant women has been growing in China, particularly after the abandonment of the one-child policy. As shown in our data, the prevalence of chromosomal abnormalities is high and increases with age. Age of the pregnant women was one of the leading indications for amniocentesis; however, amniocentesis is not risk free and cf-DNA analysis is not sensitive to some chromosomal abnormalities. These issues must be carefully considered and discussed with the pregnant women before amniocentesis is ordered.

Figure. Frequency of chromosome abnormalities by age

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Genetic analysis of a fetus with central 22q11.2 deletion encompassing CRKL gene

Shaobin Lin, Yi Zhou

Fetal Medicine Center, Department of Gynaecology and Obstetrics, The First Affilicated Hospital, Sun Yat-sen University, Guangzhou, Guangdong Province, China

OBJECTIVES: To investigate phenotypic characteristics of 22q11.2 deletion syndrome and correlation between *CRKL* on 22q11.2 and cardiac abnormalities.To investigate phenotypic characteristics of 22q11.2 deletion syndrome and correlation between *CRKL* on 22q11.2 and cardiac abnormalities. METHODS: G-banded karyotyping, single nucleotide polymorphism array (SNP) and fluorescence in situ hybridization (FISH) were performed on a fetus with tetralogy of Fallot detected by ultrasound. Genotype-phenotype correlation was reviewed after accurately identification of genomic locations for breakpoints on chromosome 22q11.2. SNP array was also performed on the parental peripheral blood specimens for origin identification of genomic variations detected in the fetus. RESULTS: Karyotype of the fetus showed a normal 46,XY. SNP array performed on fetal blood revealed a 749 kb deletion (chr22:20 716 876-21 465 659) at 22q11.21, encompassing *CRKL* gene instead of *TBX1*, *HIRA*, *COMT* and *MAPK1* genes. And this deletion region was founded to be overlapped with central 22q11.2 deletion syndrome by the identification of genomic locations for breakpoints. SNP array performed on parental peripheral blood specimens demonstrated that 22q11.21 deletion was a de novo variation in the fetus. Besides, FISH analysis on fetal blood confirmed the 22q11.21 deletion revealed by SNP array. CONCLUSIONS: Central 22q11.21 deletion was causative for cardiac abnormalities presented in the fetus, and *CRKL* should be considered as a critical pathogenic gene for cardiac abnormalities in central 22q11.2 deletion region.

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Unexpected finding of a fetus with DMD gene deletion using single nucleotide polymorphism array

Shaobin Lin, Yi Zhou

Fetal Medicine Center, Department of Gynaecology and Obstetrics, The First Affilicated Hospital, Sun Yat-sen University, Guangzhou, Guangdong Province, China

OBJECTIVES: To investigate clinical value of single nucleotide polymorphism array (SNP array) for the identification of de novo mutations in the *DMD* gene in fetuses. METHODS: G-banded karyotyping and SNP array were performed on a fetus with intrauterine growth restriction and without family history of Duchenne/Becker muscular dystrophy (DMD/BMD). Multiplex ligation-dependent probe amplification (MLPA) was subsequently applied on amniocytes and maternal peripheral blood specimen to detect *DMD* gene deletion/duplication mutations. RESULTS: Karyotype on amniocytes showed a normal 46,XY. SNP array on amniocytes detected a 116 kb deletion (chrX: 32 455 741-32 571 504) at Xp21.1 with breakpoints at intron 16 and 30 of *DMD* gene respectively, encompassing an exon 17-29 *DMD* gene deletion. Besides, MLPA analysis of *DMD* gene on amniocytes confirmed the exon 17-29 *DMD* gene deletion identified by SNP array. However, no deletion/duplication mutations were detected by MLPA on maternal peripheral blood. CONCLUSIONS: The de novo exon 17-29 *DMD* gene deletion detected in the fetus may result in BMD or DMD. And SNP array would improve diagnostic efficiency of genomic disorders for fetuses with unidentified pathogenic genes, negative family history and nonspecific phenotypes.

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Enhanced first trimester prenatal screening for trisomy 18 and trisomy 13 using nuchal translucency, maternal serum pregnancy-associated plasma protein A, free β human chorionic gonadotrophin, and placental growth factor

Tianhua Huang[1], Wendy Meschino[1,2], Shamim Rashid[1], Alan Dennis[1], Ellen Mak-Tam[1], Howard Cuckle[3]

[1]*Genetics Program, North York General Hospital, Toronto, Ontario, Canada*
[2]*Department of Pediatrics, University of Toronto, Toronto, Ontario, Canada*
[3]*Obstetrics and Gynecology, Columbia University Medical Center, New York, New York, United States*

OBJECTIVES: Enhanced first trimester screening (eFTS) involves nuchal translucency (NT) and a blood samples taken between 11+0 and 13+6 weeks gestation. The blood sample is measured for serum pregnancy-associated plasma protein A (PAPP-A), free-β human chorionic gonadotrophin (hCG), placental growth factor (PlGF) and αlpha fetoprotein (AFP). eFTS previously shown to be more accurate for Down syndrome than first trimester combined test (FTS). The current study is to assess the performance of eFTS for Trisomy 18 and Trisomy 13, and to compare the results with FTS using model predictions. METHODS: This is a retrospective case-control study using residual serum samples. Eight-three Trisomy 18, 22 Trisomy 13 pregnancies and 588 controls were included in the study. Samples from affected pregnancies were each matched with 5-6 controls and retested for free β hCG, PlGF and AFP. The measurements of NT and PAPP-A were taken from routine screening records. The median multiple of the median (MoM) of screening markers between cases and controls were compared. Multivariate modelling was performed to assess screening performance for Trisomy 18 and 13 with algorithms specific for the two anomalies and for Down syndrome using different marker combinations. RESULTS: The median MoM of PlGF was 0.75, 0.65, and 1.00 for Trisomy 18, Trisomy 13 and controls respectively ($p<0.05$). The median MoM of AFP was 0.96, 1.02 and 1.00 for the three groups respectively ($p>0.05$). At a FPR of 0.5%, 93.7% and 93.6% of Trisomy 18 can be detected using FTS and FTS+PlGF respectively; for either Trisomy 18 or 13, 81.9% and 82.3% respectively. At a 0.2% additional FPR for Trisomy 18, given a 5% FPR for Down syndrome, the DR of FTS+PlGF for Down syndrome, Trisomy 18 and Trisomy 18 or 13 were 90.0%, 91.2% and 84.4%. CONCLUSIONS: PlGF was lower, first trimester AFP was not changed in pregnancies affected by Trisomy 18 and Trisomy 13. Adding PlGF did not improve screening performance for Trisomy 18 alone, but improved the DR for Trisomy 18 or Trisomy 13 combined slightly.

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Enhanced first trimester screening for Down syndrome -preliminary results from a routine screening population

Tianhua Huang[1], Shamim Rashid[1], Alan Dennis[1], Ellen Mak-Tam[1], Wendy Meschino.[1,2]

[1]*Genetics Program, North York General Hospital, Toronto, Ontario, Canada*
[2]*Department of Pediatrics, University of Toronto, Toronto, Ontario, Canada*

OBJECTIVES: To assess the screening performance for Down syndrome using enhanced first trimester screening (eFTS) in a routine prenatal screening population. To compare screening performance of eFTS with that of the FTS. METHODS: Women who have a FTS have nuchal translucency (NT) and a blood sample taken between 11+0 and 13+6 weeks gestation. The blood sample is measured for serum pregnancy-associated plasma protein A (PAPP-A) and free-β human chorionic gonadotrophin (hCG). Women who have a eFTS have blood measured for placental growth factor (PlGF) and α-fetoprotein (AFP) in addition to PAPP-A and free β-hCG. The risk cut-off for eFTS and FTS is 1 in 350 at term. The screening performance of eFTS since its introduction in April 2016 was compared with that of FTS from January 2015 to March 2016. RESULTS: During the study period, 7,353 women had FTS and 10,860 had eFTS. The median maternal age was 32 years; the median gestational age at testing was 88 days for both groups. At a risk cut-off of 1 in 350 at term, the detection rate (DR) was 90.0%, and the false positive rate (FPR) was 9.0% for the eFTS group. The DR was 86.4%, the FPR was 7.4% for the FTS group. The PR of eFTS group was higher than expected. The proportion of women having first trimester screening has increased substantially since the introduction of eFTS. CONCLUSIONS: Our preliminary results show that DR of eFTS was in agreement with those reported in the previous studies. The higher than expected PR might be explained by population bias. Because several types of prenatal screening are offered in Ontario, women with higher risk may choose and complete eFTS as evidenced by higher NT in this population. In addition, eFTS positive women included a larger group who would have received an incomplete test result in the absent of eFTS (e.g. women who had a miscarriage or an amniocentesis before providing the second trimester sample for the integrated screening).

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The implementation of enhanced first trimester screening for Down syndrome in a routine prenatal screening population

Wendy Meschino[1,2], Shamim Rashid[2], Karen Boucher[2], Tianhua Huang[2], Alan Dennis[2], Ellen Mak-Tam[2]

[1]*Department of Pediatrics, University of Toronto, Toronto, Ontario, Canada*
[2]*Genetics Program, North York General Hospital, Toronto, Ontario, Canada*

OBJECTIVES: To summarize our experience in the implementation of enhanced first trimester screening (eFTS) in a routine prenatal screening population. METHODS: Enhanced FTS was introduced at the prenatal screening program of North York General Hospital in April 2016. Women who have an eFTS have nuchal translucency (NT) and a blood samples taken between 11+0 and 13+6 weeks gestation. The blood sample is measured for serum pregnancy-associated plasma protein A (PAPP-A), free-β human chorionic gonadotrophin (hCG), placental growth factor (PlGF) and α-fetoprotein (AFP). The risk cut-off for eFTS is 1 in 350 at term. The study summarized the utilization of eFTS since its introduction and experience in the preparation, implementation and monitoring of eFTS. RESULTS: Education sessions were provided to health care providers to outline the implementation plan and expected performance. Median equations of PlGF and AFP were generated using results from a previous study on fresh samples. Racial specific median equations for PlGF were used from October 2016. This has stabilized the PlGF median and positive rate (PR). As of January 2016, 10,860 women were screened using eFTS. There was a higher than expected PR for the eFTS population. However, the eFTS population appears to have more women with higher NT. There has been a steady increase in eFTS test volume since its introduction. CONCLUSIONS: EFTS was well accepted by pregnant women and health care providers. eFTS has surpassed IPS (Integrated Prenatal Screen) and become the most common screening test at NYGH. This has improved the efficiency of our screening program by reducing the need for sample tracking and shipment between laboratories as required by IPS. Adequate adjustment of PlGF median is essential because of the significant ethnic differences in its measurement. The higher PR observed in the eFTS population could be due to population bias. The median, PR and DR are closely monitored to ensure the quality of the test.

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P-28

Nuchal translucency improves NIPT's predictive value

Marwan Ali, Amelia Kelly, Stephen Chasen

Weill Cornell Medical College, New York, New York, United States

OBJECTIVES: According to ACOG, nuchal translucency (NT) assessment is not necessary for those undergoing noninvasive prenatal testing (NIPT) in the first trimester. There is data to suggest that NT can be useful in identifying abnormalities not screened for by NIPT. In those with high risk NIPT, use of NT could improve predictive values. The objective of this study is to examine the value of adding NT measurement to NIPT in detection of fetal chromosomal disorders. METHODS: This is a retrospective cohort study. We reviewed medical records of all singleton pregnancies who had NIPT, first trimester NT measurement, and invasive testing [chorionic villous sampling (CVS) and/or amniocentesis] between August 2013 and October 2016. Abnormal NT was defined as NT >2 SD above the mean for CRL. Pregnancies were divided into 4 groups: [1] (Low risk NIPT) LR-NIPT/Normal NT; [2] LR-NIPT/Abnormal NT; [3] (High risk NIPT) HR-NIPT/Normal NT; and (4) HR-NIPT/Abnormal NT. The true fetal genetic status was then determined in all groups. Chi-Square test was used to test for statistical significance. RESULTS: 141 pregnancies were included, with abnormal karyotype diagnosed in 15 (11%). 123 had LR-NIPT, and 9 of these had abnormal NT. 18 pregnancies had HR-NIPT, of which 7 had abnormal NT. The karyotype was abnormal in 13/18 cases with HR-NIPT (72%). The PPV of a HR-NIPT was 100% (7/7) in those with abnormal NT compared to 55% (6/11) when NT was normal (p<0.001). There were two abnormal karyotypes in those with LR-NIPT (1.6%), both in those with abnormal NT. The NPV in those with LR-NIPT was 78% in those with abnormal NT, compared to 100% in those with normal NT (p<0.001). CONCLUSIONS: Our data suggest a significant improvement in both the positive and negative predictive value of NIPT when NT thickness measurement is incorporated. This information may be useful when counselling patients regarding the NIPT results, especially those who may perceive false reassurance when a low risk NIPT result is found after an abnormal NT measurement.

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Perinatal outcomes in pregnancies with a low fetal fraction on non invasive prenatal testing

Marwan Ali, Katherine Pryor, Stephen Chasen

Weill Cornell Medical College, New York, New York, United States

OBJECTIVES: 3-13% of the total cell-free maternal DNA is placental in origin. This fraction of cffDNA is commonly known as the fetal fraction (FF). FF increases with gestational age, and decreases with increased maternal weight. FF above a certain threshold is required to provide a NIPT result. Low FF is a risk factor for aneuploidy, and may be a risk factor for poor perinatal outcome. Our objective was to evaluate the relation between low FF, and perinatal outcome using composite perinatal morbidity (CPM). METHODS: This is a retrospective chart review of all women with singleton pregnancies who had NIPT. We compared the median maternal BMI, median gestational age of delivery, median birth weight, and CPM between patients with a fetal fraction <4% on NIPT (low-FF), and those with a normal FF on their NIPT (normal-FF). The CPM was defined as the presence of any of the following complications: spontaneous abortion, fetal demise, hypertensive disorders of pregnancy, placental abruption, IUFD, preterm birth, low birth weight, NICU admission, or neonatal death. Fisher's exact test and Mann Whitney U were used for statistical comparison. RESULTS: Of the 256 patients included, 28 (10.9%) had low FF, while 228 (89.1%) had normal-FF. Median maternal age was 35-years, and median gestational age at delivery was 39-weeks. The rate of CPM in the entire cohort was 26.5%. Median BMI was significantly higher in the low-FF group compared to the normal FF group (27.4 vs. 22.1 kg/m2; *p*<.001). CPM was similar between those with low-FF and normal-FF (25% vs. 28.9%; p=.82), with no differences in the frequencies of any individual outcome. Among the low-FF group, median FF in cases with CPM was significantly lower than those without CPM (2.0% vs. 3.2%; *p*=.04.) CONCLUSIONS: There were no significant differences in obstetric outcomes between the low and normal FF groups. Within the cohort categorized as having low FF, lower FFs were associated with a higher rate of composite morbidity. While we did not observe an overall association between low FF and adverse outcomes, larger studies are necessary to evaluate the association between uncommon outcomes and fetal fraction.

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Aneuploidy testing in pregnancy before and after the availability of non-invasive prenatal screening (NIPS) in predominantly inner city population: Is it different?

Manesha Putra[1], Jing Dai[2], Danielle Finch[3], Leanne Almario[4], Melissa Hicks[5], Jacques Abramowicz[6], Robert Sokol[2]

[1]*Wayne State University/Detroit Medical Center, Detroit, Michigan, United States*
[2]*CS Mott Center for Human Growth and Development, Wayne State University Department Of Obstetrics and Gynecology, Detroit, Michigan, United States*
[3]*Michigan State University College of Osteopathic Medicine, East Lansing, Michigan, United States*
[4]*Detroit Medical Center/Wayne State University Department Of Obstetrics and Gynecology, Detroit, Michigan, United States*
[5]*DMC University Laboratory, Detroit, Michigan, United States*
[6]*University of Chicago, Department of Obstetrics and Gynecology, Chicago, Illinois, United States*

OBJECTIVES: Our objective in this study was to investigate choices in aneuploidy testing among women referred for a genetic counseling, before and after the availability of NIPS in a single tertiary care center setting with predominantly inner city, low socioeconomic status population. We are also looking to examine factors that predict the decision to proceed with prenatal invasive diagnostic testing following genetic counseling in this population. METHODS: We reviewed results of clinical decision making of women referred for genetic counseling at < 24 weeks in a tertiary care center during 2011, prior to the availability of NIDS, and 2013-2015, after NIPS availability. Women seen in January-April each year were included in the sample. We used logistic regression to predict invasive testing from the possible factors advanced maternal age, abnormal ultrasound, previous pregnancy outcomes, and family history while controlling for baseline characteristics of race, age, number of living children, number of fetuses, religion, and city of residence. We employed the likelihood ratio forward stepwise method for covariate selection. RESULTS: Comparing 197 women referred before and 357 after the availability of NIPS (total n=554), there were no significant differences in baseline demography. There was no change in the proportion of women who proceeded with invasive testing before and after the availability of NIPS (19.3 vs 23.2%, p>0.05). The proportion of patients whose final test was maternal serum screening was reduced by almost two thirds (72.1 vs 27.5%). From the logistic regression among 554 women included, only abnormal ultrasound and private insurance were significant predictors of invasive diagnostic testing, respectively (OR 3.62, 95% CI 2.33-5.62) and (OR 1.68, 95% CI 1.08-2.06). CONCLUSIONS: In our low SES environment, NIPS was a popular choice by women after positive maternal serum screening. Despite this choice we did not see a decrease in the uptake of invasive testing, a result differing from several other US studies. Demographic factors could potentially play an important role in this difference and warrant further study. Abnormal ultrasound finding along with being privately insured were found to be correlated with the decision of choosing invasive testing. Despite equal coverage, people with private insurance were more likely to proceed with invasive testing. Insurance status may represent other measures like education or perceived access.

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Features of use of non-invasive prenatal testing in Kazakhstan

Assel Arginbekova

Association of Kazakhstan MD Genetics, Astana, Akmola Region, Kazakhstan

OBJECTIVES: Non-invasive test psychologically better tolerated by pregnant women than an invasive procedure. 3197 cases of invasive procedures, may be noted that 75% of pregnant women have some psychological factors as: fear of the procedure, the tremor, tears, and hypertonicity of the uterus, which begins immediately before the procedure. Almost all pregnant women, who had passed NIPT, are more favorable. Delivering analysis in venous blood has a positive effect on the psyche and mood of women. Even the painful expectation of the results takes place with less stress compared to invasive prenatal procedure, where a woman is waiting. METHODS: For 2 years the introduction of NIPT was examined 158 women. Among them, the average age of the passage was 34,9 ± 4,09, passing gestation period was 15,7 ± 2,65 weeks of pregnancy, a base panel analysis of the past 73% of pregnant women. In the analysis of the expanded panel + 5 microdeletion syndromes was 27%. The percentage of fetal DNA fraction fetal 10,42 ± 1,74%. The result of the risk assessment: low risk was 97.4%. 2.6% was a high-risk group, where the final diagnosis was confirmed by invasive diagnostics. RESULTS: In total, the 6493 in Kazakhstan invasive procedures were carried out in 2 years. Of these, 153 women underwent NIPT, which accounted for only 2%. For 2 years revealed chromosomal aberrations by invasive procedure was 469-7,2%. All were suspended up to 22 weeks of pregnancy. In case of a positive result of a pregnant woman to confirm the diagnosis goes further invasive procedure is mandatory. Getting the result comes with a term of more than 22 weeks (500 grams). As a result, the terms detect abnormal pathologies are lengthened and the question of termination of the fetus with abnormalities excluded. CONCLUSIONS: It is important to rule out (or confirm) the pathology of the fetus in early pregnancy to the couple was free to decide on the termination of pregnancy before fetal viability. Aborting the fetus over the 22 weeks of gestation is not produced. The probability of an error exception is big enough. In addition, the combined prenatal diagnosis in the form of ultrasound and biochemical and genetic screening is not enough to reduce the birth of children with indicators of chromosomal pathology.

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When cell-free DNA testing for fetal aneuploidy does not yield a result: Case reports reflecting the value of assay quality control measures

Annette Batey[1], Jennifer Kaplan[1], Lisa M. Catalano[2], Rhoberta Steeke[1], Karen White[1], Maximilian Schmid[1]

[1]Ariosa Diagnostics, Inc., Roche Sequencing Solutions, Inc., San Jose, California, United States
[2]Emory University Hospital, Atlanta, Georgia, United States

OBJECTIVES: Cell-free DNA (cfDNA) testing has been rapidly adopted due to high sensitivity and low false positive rates for common autosomal aneuploidies. “No call” rates from 0%-11.1% have been published by laboratories offering cfDNA testing commercially (Gil et al, 2014). Rates are influenced by many factors; however, design of an assay’s quality control (QC) impacts the rate observed. A widely-accepted QC requirement is presence of a minimum fetal fraction (FF). Additional QC metrics may also be implemented to identify sample-specific issues that could potentially compromise test accuracy. Laboratory experience supporting the value of “no calls” to ensure reliable results is presented. METHODS: Case reports: Cases that did not pass quality requirements due to biological reasons were identified by a review of communications from care providers. Case 1: A sample submitted from a patient, age 22, at 12 weeks 0 days gestation did not yield a reportable result. Subsequent communication with the genetic counselor revealed that the patient was a liver transplant recipient. Because cfDNA released from a transplanted organ could lead to discordant results, cfDNA testing is not recommended in such patients. As demonstrated here, non-maternal cfDNA present in plasma of transplant recipients may be detected with rigorous assay QC design. RESULTS: Cases 2-8: In this series, ordering providers contacted the laboratory to correct demographic information provided on the test requisition form. Initially, the samples did not meet quality requirements. Reinterpretation with correct IVF status and egg donor ages yielded low probability results. The use of single nucleotide polymorphisms (SNPs) for measurement of FF is a highly accurate method and requires knowledge of the genetic relationship between mother and fetus to interpret informative alleles. Fetal fraction information is used both as a quality metric and in probability calculation. Incorrect interpretation of FF may potentially lead to false positive or false negative results. CONCLUSIONS: The majority of patients receiving no result on the first draw for cfDNA screening will obtain a result upon submission of a second sample; however, these cases demonstrate that reasons for ”no calls” are heterogeneous and dependent on the laboratory quality metrics applied. Although providers routinely confirm patient eligibility and verify the accuracy of clinical information provided to the laboratory, there may be exceptions, for example due to transcription errors, incomplete history information, or patient non-disclosure. QC measures optimize the reliability of test results and may also help mitigate the effect of such sample-specific issues.

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Exploring the etiology of false positive noninvasive prenatal testing (NIPT) for microdeletion syndromes

Amy Breman[1], Andrea Petersen[1], Janice L. Smith[1], Patricia Ward[1], Sandra Peacock[1], Alicia Braxton[2], Weimin Bi[1], Sau Wai Cheung[1]

[1]*Baylor College of Medicine, Houston, Texas, United States*
[2]*Baylor Miraca Genetics Laboratory, Houston, Texas, United States*

OBJECTIVES: We propose data-driven, previously under-appreciated mechanisms for false positive microdeletion syndrome screening on noninvasive prenatal testing (NIPT). METHODS: We reviewed discordant diagnostic testing results from a large cohort of women referred to our lab for confirmatory testing on the basis of positive NIPT results. Confirmatory testing, including fluorescence *in situ* hybridization (FISH), chromosomal microarray analysis (CMA), and/or karyotype was performed by our lab on prenatal (CVS or amniocentesis) or postnatal (blood) samples. RESULTS: Of the 731 cases reviewed, 55 had microdeletion syndrome positive NIPT results, 47 of which had discordant negative diagnostic testing results. Of these, two cases, 3.6% of the total microdeletion cohort, had large regions of absence of heterozygosity (AOH) in a chromosomal region associated with the microdeletion syndrome that screened positive on NIPT: one for 22q11.2/DiGeorge syndrome, and one for 1p36 deletion syndrome. CONCLUSIONS: Select microdeletion syndrome regions have recently been added by some laboratories as additional NIPT screening options. However, the clinical validity and utility of this expanded screening remains to be established and further data-driven exploration is needed. Previously proposed mechanisms for false positive results include placental mosaicism, vanishing twin, maternal copy number variation, neoplastic conditions and disease rarity. We report two cases of false positive NIPT microdeletion results whereby SNP-based chromosomal microarray analysis revealed regions of absence of heterozygosity (AOH) over the respective chromosome region. This mechanism bears consideration in cases of discordant confirmatory testing.

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The clinical validity of noninvasive prenatal testing (NIPT) for chromosomal microdeletion syndromes

Andrea Petersen[1], Janice L. Smith[1], Patricia Ward[1], Sandra Peacock[1], Alicia Braxton[2], Sau Wai Cheung[1], Weimin Bi[1], Amy Breman[1]

[1]*Baylor College of Medicine, Houston, Texas, United States*
[2]*Baylor Miraca Genetics Laboratory, Houston, Texas, United States*

OBJECTIVES: Select microdeletion syndrome regions have recently been added by some laboratories as additional noninvasive prenatal testing (NIPT) options. However, given the rarity of these syndromes in the general population, the accuracy of this expanded screening is largely unknown. Existing studies are limited to either small cohorts of patients, or based on data extrapolated from spike-in samples. Our goal was to establish clinical validity of NIPT for microdeletion syndromes using a large clinical diagnostic laboratory data set. METHODS: Our study includes diagnostic testing results from 731 women initially referred to our laboratory following abnormal or inconclusive NIPT results. Confirmatory testing consisted of fluorescence *in situ* hybridization (FISH), chromosomal microarray analysis (CMA), and/or karyotype analysis performed on prenatal (CVS or amniocentesis) or postnatal (blood) samples. Positive predictive values (PPVs) and false positive rates were computed from tabulated data. RESULTS: Our data revealed poor positive predictive values (0-23%) and high false positive rates (77-100%) for the major microdeletion syndromes: cri du chat/5p- syndrome, Prader-Willi/Angelman syndromes, 22q11.2/DiGeorge syndrome and 1p36 deletion syndrome. Included in these data are two cases that screened positive for a microdeletion by NIPT, one for 22q11.2 and one for 1p36, but confirmatory microarray studies revealed large blocks of absence of heterozygosity (AOH) over the respective chromosomal region. CONCLUSIONS: Our data represent the largest microdeletion-positive NIPT cohort reported to date, and shows a poor overall concordance between NIPT and confirmatory testing, with low PPVs and high false positive rates. Ultimately, clinical utility for NIPT for microdeletion syndromes necessitates additional studies, including those of negative predictive values and false negative rates.

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Experience performing cell-based noninvasive prenatal testing using NGS-based copy number assessment of fetal cells

Amy Breman[1], Liesbeth Vossaert[2], Roseen Salman[2], Sadeem Qdaisat[1], Qun Wang[2], Xinming Zhuo[2], Adriel Kim[2], Jennifer Chow[3], Lance U'Ren[3], Elizabeth Normand[1], Chad Shaw[2], David Henke[2], Elizabeth Chang[3], Ron Seubert[3], Eric Kaldjian[3], Jackie Stilwell[3], Yaping Yang[2], Ignatia Van den Veyver[1], Arthur Beaudet[1]

[1]*Baylor College of Medicine, Houston, Texas, United States*
[2]*Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, United States*
[3]*RareCyte Inc., Seattle, Washington, United States*

OBJECTIVES: Detection of genomic copy number abnormalities from circulating single fetal cells using next generation sequencing (NGS) offers a promising noninvasive alternative for prenatal diagnosis. Towards this goal, we have established a method for performing fetal cell-based, noninvasive prenatal testing (CB-NIPT) during the first trimester. As part of our initial validation study, we show that we can successfully repeatedly recover individual fetal trophoblastic cells during the first trimester and detect clinically important copy number variants by NGS. METHODS: Fetal trophoblasts were isolated from maternal blood collected during first or second trimester, after density-based collection, immunofluorescence staining, custom high-resolution scanning and analysis, and integrated single-cell picking. Whole genome amplification was performed on individual cells and single nucleotide polymorphism-based genotyping studies were carried out for confirmation of fetal origin. NGS on an Illumina platform with approximately 5 million reads per cell was used to generate genome-wide copy number data. RESULTS: From July 2016 to February 2017, 111 samples from 108 pregnancies were studied. Fetal cells were recovered from the vast majority of cases with an average of 0.18 cells/ml. A total of 451 putative fetal cells were identified by microscopic scanning; 383 were picked for further analysis. Genotyping and/or sequencing confirmed fetal origin in 64% of cells examined. The CB-NIPT results agreed with available clinical diagnostic data in all cases, including multiple aneuploidies, one Wolf-Hirschhorn syndrome 18.9 Mb deletion and one 2.7 Mb deletion on chromosome 15. Additionally, likely confined placental mosaicism was detected in two cases. CONCLUSIONS: CB-NIPT by NGS has the potential to detect most clinically significant cytogenetic abnormalities and even, in the future, de novo deleterious point mutations. Further optimization of our method to obtain a more consistent recovery of sufficient fetal trophoblastic cells and a higher throughput is underway to enable the use of CB-NIPT as a routine clinical test.

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The association between anticoagulation therapy, maternal characteristics, and a failed cfDNA test result

Whitney Burns[1], Nathan Koelper[2], Mary Sammel[3], Michael Mennuti[4], Andrea Barberio[5], Mary DeAgostino-Kelly[4], Lorraine Dugoff[6]

[1]*Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, United States*
[2]*University of Pennsylvania School of Medicine Center for Research on Reproductive and Women's Health, Philadelphia, Pennsylvania, United States*
[3]*Hospital of the University of Pennsylvania Department of Biostatistics and Epidemiology, Philadelphia, Pennsylvania, United States*
[4]*Hospital of the University of Pennsylvania Department of Obstetrics and Gynecology, Philadelphia, Pennsylvania, United States*
[5]*Weill Cornell Medical College Department of Obstetrics and Gynecology, New York, New York, United States*
[6]*University of Pennsylvania, Philadelphia, Pennsylvania, United States*

OBJECTIVES: Fetal cell free DNA (cfDNA) is increasingly being used to screen for fetal aneuploidy. Despite advancing technology, a small proportion of women do not receive a test result. Test failure can result from insufficient fetal cfDNA, which has previously been characterized as a fetal fraction (FF) less than 4%, or other technical reasons. Gestational age, maternal obesity, and the use of anticoagulants have been shown to influence FF. The objective of this study was to identify maternal characteristics associated with a failed cfDNA test result due to a low FF. METHODS: Retrospective cohort study of women with singleton pregnancies who had cfDNA screening through the same laboratory at 10-25 weeks gestation between October 2011-January 2016. The primary outcome was a failed cfDNA result due to a low FF. Women who had a spontaneous loss < 20 weeks or a fetal demise at ≥20 weeks, underwent an elective termination of pregnancy, or had unavailable pregnancy outcome data were excluded. Student t test was used to compare continuous variables; Pearson chi-square test was utilized for categorical variables. Multivariable logistic regression was performed to assess the relationship of maternal characteristics with the primary outcome. RESULTS: 2875 women met inclusion criteria. 34 (1.2%) women had failed cfDNA tests; 0.6% (18) of these were secondary to low FF. The women with low FF were more likely to be obese (BMI of 34.6 v. 26.4, $p < 0.0001$), have chronic hypertension (cHTN) (22% v. 7%, $p = 0.0016$), or taking enoxaparin (28% v. 1%, $p < 0.0001$). Effect of obesity and anti-coagulation on receipt of a failed cfDNA test result remained significant after adjusting for confounders. Adjusted odds ratio for failed cfDNA test due to low FF while on anti-coagulation was 37.5 (CI 11.19 – 125.87; $p < 0.0001$). CONCLUSIONS: Anticoagulation therapy and obesity were associated with an increased incidence of a failed cfDNA test result due to low FF. The possibility of a failed cfDNA test result should be included in pretest counseling. Further research is needed to determine the mechanisms by which obesity and anticoagulation therapy impact the FF, evaluate whether this finding varies with diffierent labs, and identify approaches to improve cfDNA performance in these women.

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Characteristics associated with no cfDNA result due to low fetal fraction

Comorbidity	Adjusted Odds Ratio* (95% CI)	*p*-value
BMI (kg/m^2)	1.114 (1.051-1.180)	0.0003
cHTN	1.34 (0.332-5.405)	0.681
Anticoagulation Use	37.531 (11.191-125.865)	<0.0001

* Adjusted Odds Ratio adjusting for BMI, cHTN, Anticoagulation use, and Gestational Age at NIPT

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International trends in patient samples submitted for non-invasive prenatal testing (NIPT)

Kelly Chen, Caroline Loy, Karen White, Maximilian Schmid

Ariosa Diagnostics, Inc. and Roche Sequencing Solutions, Inc., San Jose, California, United States

OBJECTIVES: Initially, NIPT using cell-free DNA (cfDNA) was only recommended by professional society guidelines for women at increased risk of fetal aneuploidy. Landmark studies such as the Noninvasive Examination of Trisomy (NEXT) study in 2015 demonstrated superior performance of cfDNA screening as compared to that of traditional first trimester screening in the routine prenatal population. Subsequently, guidelines were updated to recommend offering NIPT to all pregnant women. In this study, we examine international trends in patients submitting samples for cfDNA screening to determine whether they reflect a shift towards use of NIPT in lower risk populations. METHODS: A retrospective review of demographic information was performed for specimens submitted to the Ariosa Diagnostics Inc. clinical laboratory for the Harmony Prenatal Test® between January 1, 2015 and December 30, 2016. Information from specimens submitted from within the United States as well as outside of the United States was included in the review. The proportion of specimens for patients under 35 years of age was compared with the proportion of specimens for patients 35 years and older. In addition, ICD9 and ICD10 codes were reviewed for claims made to patient insurance in the same timeframe. RESULTS: There was a significant increase in the proportion of specimens submitted from patients under 35 years of age, from 51.9% in 2015 to 60.0% in 2016 (Chi-square test, $p < 0.01$). In addition, billing data demonstrated similar trends with an increasing proportion of specimens from average risk patients in the United States. CONCLUSIONS: The proportion of samples submitted to our laboratory by patients under 35 years has significantly increased since January 2015. This suggests a change in clinical practice and an increase in uptake of NIPT in the average risk population. This trend is likely due to several factors including professional society guidelines, increasing provider and public awareness of the utility of NIPT, and increasing payor and third-party coverage. Similar maternal age trends were seen both in the United States and outside of the United States, supporting the broader uptake of NIPT internationally.

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Genome-wide cfDNA testing: Clinical laboratory experience screening for sex chromosome abnormalities

Jill Rafalko[1], Samantha Caldwell[2], Theresa Boomer[2], Jason Chibuk[2]

[1]*Integrated Genetics, West Orange, New Jersey, United States*
[4]*Sequenom/Integrated Genetics, San Diego, California, United States*

OBJECTIVES: Genome-wide cell-free DNA (cfDNA) testing was introduced for clinical use in August 2015. During the first year following its release, over 17,000 samples were submitted for analysis. We describe the clinical laboratory experience of this screening test for the detection of sex chromosome abnormalities. METHODS: Maternal blood samples submitted to Sequenom Laboratories® for MaterniT® GENOME testing were subjected to DNA extraction, library preparation, and whole-genome massively parallel sequencing as previously described by Jensen *et al*. Sequencing data were analyzed using a novel algorithm to detect aneuploidies and other subchromosomal events as described by Lefkowitz *et al*. For all positive results, outcome data (e.g. cytogenetic/molecular results and/or birth outcomes) were elicited by phone or email from the ordering provider. RESULTS: Analysis of over 17,000 samples yielded at least 155 results that were positive for sex chromosome abnormalities (SCA). The most common SCA detected was monosomy X. Four samples were positive for subchromosomal events involving the X chromosome. The relative positive predictive value was approximately 66.7% for the >36 cases with diagnostic follow-up information. Of the remaining cases without diagnostic confirmation, approximately 72.3% had clinical findings that supported the positive cfDNA result, as determined by ultrasound and/or serum biochemical screening. CONCLUSIONS: Genome-wide cfDNA testing allows for the identification of sex chromosome aneuploidies, as well as subchromosomal events involving the sex chromosomes. Though the uptake of diagnostic testing in these cases is limited, knowledge of a potential chromosome abnormality in these pregnancies may help families arrange for appropriate diagnostic testing at birth. Early diagnosis of sex chromosome abnormalities may improve long-term outcomes for these children.

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First clinical application of a new paired-end MPSS approach for cfDNA screening of common chromosome aneuploidies

Vincenzo Cirigliano[1], Elena Ordoñez[1], Laura Rued Rueda[1], Isabel Castilla[1], Manuel Grau[1], Cristina Puertollano[2], M.Paz Cañadas[1]

[1]*Labco Diagnostics, Barcelona, Spain*
[2]*Labco Diagnostics, Esplugues de Llobregat, Spain*

OBJECTIVES: Paired-end MPSS allows accurate digital cfDNA counting while also determining fragments length. As fetal DNA is shorter than maternal, size differences can be used to determine fetal fraction (FF). Also, in aneuploid cases, it is reasonable to expect that counting differences would appear more evident if confirmed on shorter fragments only. The NeoBona is the first test developed to exploit paired-end MPSS through a novel bioinformatics approach combining conventional counting statistics and assessment of cfDNA size distributions to provide this double check of chromosome counting data. We report the first clinical application on a large cohort of average risk pregnancies. METHODS: A total of 19151 consecutive samples were collected from pregnant women above 10w of gestation (575 twins), regardless their risk category. Samples were screened for autosomal trisomies using the NeoBona test, which allows simultaneous assessment of fetal fraction, cfDNA fragments size distribution and chromosome counting statistics, through a novel Tscore value reflecting the likelihood for chromosome aneuploidy. Chromosome specific pre-established cut-offs were applied at Tscores to classify normal and aneuploid cases. XY aneuploidy screening was carried out as requested in 57% of cases from singleton pregnancies. Samples were tested and results reported within 5 days from collection. RESULTS: Results were obtained for 19015 patients (99.2%), 1.7% after redraw. 288 trisomies 21, 63 trisomies 18 and 27 trisomies 13 were reported, 23 cases with FF between 1 and 3%. Invasive procedures were performed in 99% of cases with 5 FP for T21, 2 T18 and 3 T13 (FPR 0.026%, 0.01% and 0.016%). One T21 was missed (DR 99.6%). XY aneuploidies were reported in 39/11021 samples. Presence of an unrecognised twin was suspected in 3 cases and no XY results reported, 2 maternal aneuploidies were also identified. Follow-up was available for 11 cases with 4 FP results (FPR 0.13%). CONCLUSIONS: Paired-end MPSS coupled with the new analysis algorithm of the NeoBona test, proved highly efficient, allowing cfDNA analysis to be successful on a high proportion of clinical cases. The new multifactorial Tscore allowed detecting aneuploidies with confidence even at fetal fractions as low as 1% while reducing false positive rates. Eliminating the need of a fixed lower limit at fetal fraction for reportable cases extends the benefits of cfDNA screening to a larger population of pregnancies.

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Accurate fetal fraction from NIPS using whole genome sequencing

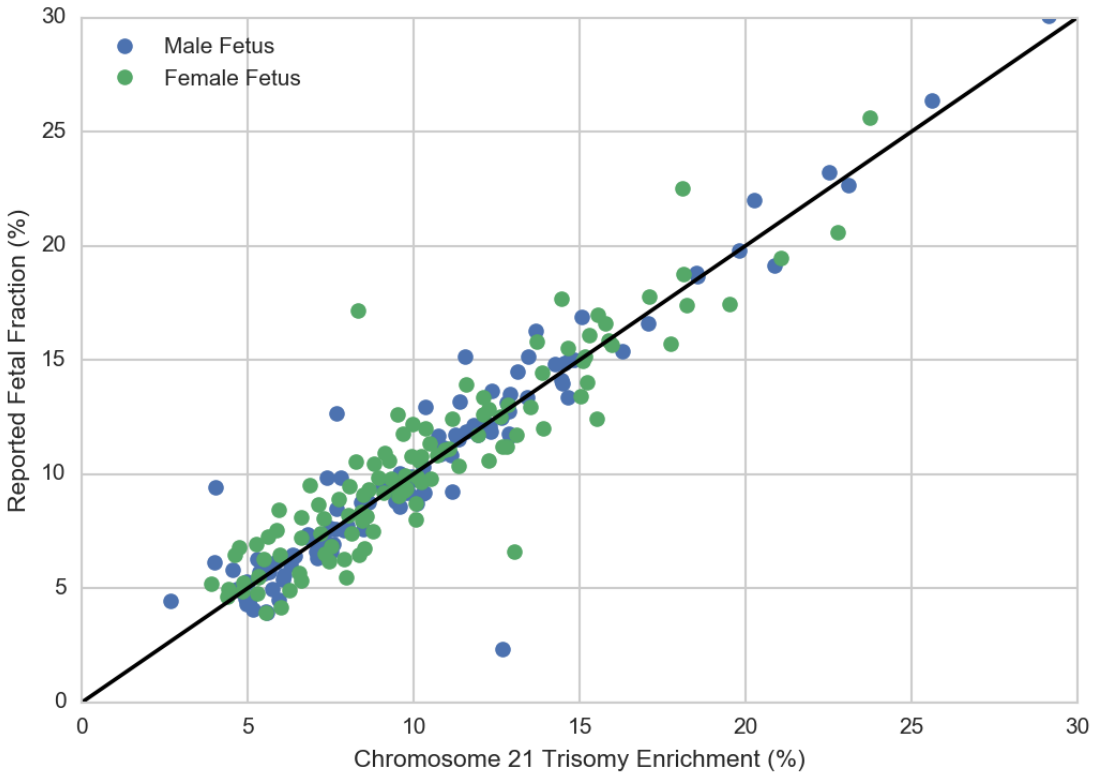
Kevin Haas, Kevin D'Auria, Jeff Tratner, Chuba Oyolu, Carrie Haverty, Dale Muzzey

Counsyl, South San Francisco, California, United States

OBJECTIVES: The ability of noninvasive prenatal screening (NIPS) to identify fetal aneuploidy in a maternal blood sample is proportional to the fetal fraction (FF), which is the percentage of cfDNA derived from the placenta. For NIPSs that utilize whole-genome sequencing (WGS), FF ascertainment in male-fetus pregnancies is straightforward using reads from chrX and chrY; for female-fetus pregnancies statistical inference is required to estimate FF. Since the accuracy, precision, and relative prevalence of different FFs is important for proper interpretation of NIPS results, we endeavored to further improve FF inference from WGS data. METHODS: WGS NIPS data from 16,434 male-fetus pregnancies and 15,064 female-fetus pregnancies was retrospectively analyzed to develop and characterize a FF inference algorithm. For training of the model, we use male samples with FF directly measured on chromosomes X and Y. WGS reads in bins tiled across the genome can detect relative enrichment of fetal-derived cfDNA in specific parts of the genome between mother and fetus. This enrichment is mapped to known FF values using ridge regression followed by both polynomial smoothing and an error reduction scaling process. Model fitting performance was evaluated from 10-fold cross-validated data. RESULTS: The correlation between the FF inferred using our algorithm and the FF observed in male-fetus pregnancies on chrX and chrY is 0.957, in excess of previously published reports. Further, the median absolute error in inferred FF was 0.68%, far smaller than the range of FF. The observed percentiles of FF for the 5th, 25th, 50th, 75th, and 95th percentile are 3.3%, 6.1%, 8.6%, 11.6%, and 17.0%, respectively. Analysis of trisomy 21 samples shows a correlation of 0.954 for male samples and 0.905 for female samples between the inferred FF and the FF calculated from chr21 enrichment (Figure 1). CONCLUSIONS: Although FF is not strictly required for WGS-based NIPS to detect fetal aneuploidies with high clinical sensitivity and specificity, it is recommended by clinical guidelines and provides a valuable quality-control metric that can further improve overall test performance. Here we have demonstrated that a large training dataset combined with careful statistical analysis yields accurate and precise FF prediction. Importantly, the ranges of observed FF, and understanding percentile vs. percentage, from a large cohort of test samples will aid in the interpretation of fetal fraction results.

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Positive NIPT for trisomy 18 and Angelman syndrome in setting of maternal malignancy

Hayley Green-Morfesi, Marie Discenza, Rosemary Reiss, Erica Holland, Louise Wilkins-Haug

Brigham and Women's Hospital, Boston, Massachusetts, United States

OBJECTIVES: To present a novel case of abnormal non-invasive prenatal testing (NIPT) involving autosomal aneuploidy and a microdeletion in the setting of a maternal malignancy. METHODS: NIPT including microdeletion testing on a SNP platform performed on a 25 yo G4P2012 indicated increased risks for trisomy 18 and Angelman syndrome. She declined amniocentesis in 2nd trimester. Second and third trimester ultrasound surveys of fetal anatomy were normal. At 26 weeks she transferred to our tertiary center. She accepted amniocentesis after genetic counseling. Fluorescent in situ hybridization (FISH) and SNP microarray (Affymetrix Cytoscan) were performed on cultured amniocytes. Physical exam revealed a breast mass which was not biopsied until 36 weeks gestation due to initial patient reluctance. The infant was delivered by Cesarean section at term for malpresentation. RESULTS: Amniocentesis showed normal FISH signals for chromosomes 13, 18, 21, X and Y. Microarray on cultured amniocytes was also normal, including examination of the 15q11.2-q13 region. Methylation specific multiplex ligation-dependent probe amplification (MLPA) was also normal. Newborn was non-dysmorphic, with birthweight at the 15th percentile. Placental microarray studies including the Angelman/Prader Willi critical region are pending. Breast biopsy showed invasive lobular carcinoma and associated ductal carcinoma in situ. CONCLUSIONS: Abnormal NIPT results suggesting multiple aneuploidies not attributable to the fetal genome and resulting from maternal malignancy are now well described. However, no prior publications have reported NIPT simultaneously positive for a single aneuploidy and a microdeletion in the setting of maternal malignancy. Although postpartum maternal serum studies and cancer tissue studies are unavailable, the findings raise the possibility that not only multiple chromosome aneuploidy but also aberrancy at the segmental level may be reflective of underlying maternal fragment genomic imbalances related to a malignancy.

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Case study: False negative non-invasive prenatal screening result due to isochromosome 21q

Katie Ellis[1], Nerida Mansfield[2], Michael Bonifacio[2], Paulette Barahona[2], Steven McArthur[2]

[1]*Genea, Deakin, Australian Capital Territory, Australia*
[2]*Genea, Sydney, New South Wales, Australia*

OBJECTIVES: This study presents a rare case of a false negative result on Non-invasive Prenatal Screening (True Seq NIPT) where the resulting baby has a diagnosis of Trisomy 21 attributable to an isochromosome 21q. METHODS: Non-invasive Prenatal Screening (NIPS/NIPT) was performed at 12+6 weeks gestation on a 31 year old Primagravida giving a 'No Aneuploidy Detected' (NAD) result. At birth, the baby was noted to have a subtle Trisomy 21 phenotype including single palmar crease, low birth weight and minor facial features. Repeat testing from the archived sample was performed and both the original adn repeat raw data was also sent to Illumina (Redwood City, CA/USA) for repeat analysis. Sample mix-up was examined and excluded. Combined First Trimester Screening also yielded a very low risk and all ultrasounds performed throughout the pregnancy were unremakable. RESULTS: Repeat testing on the stored sample confirmed the previous result of 46, XX as did analysis at Illumina. The Combined First Trimester Screening returned a very low risk result of 1:8377 for Trisomy 21 (background risk 1:607). Ultrasounds performed at 13+2, 18+6 weeks respectively were unremarkable. The Obstetrician noted a slight drop off in growth in the third trimester and ordered ultrasounds at 32+2 weeks and 34+4 weeks. Both were unremarkable with measurements corresponding to gestational age. Testing at birth revealed 46, XX,i(21)(q10).nucish (DYRK1A/KCNJ6/DSCR4/DSCR8)x3. CONCLUSIONS: This case aims to increase awareness and understanding of rare diagnoses that may arise from NIPS. Given the Trisomy is for the entire q arm of chromosome 21, one expects it should have been detected via the True Seq method. Interestingly, all screening of this pregnancy was unremarkable. At six months old baby has features of Trisomy 21, but is currently on par with standard developmental milestones. At the time of writing, it is likely that there may be two identical cases worldwide. Further studies are required to understand why an isochromosome 21 is not detected by Non-invasive Prenatal Screening.

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Preponderance of females affected with trisomy 18 detected via Non-invasive Prenatal Screening (NIPS)

Katie Ellis[1], Paulette Barahona[2], Michael Bonifacio[2]

[1]*Genea, Deakin, Australian Capital Territory, Australia*
[2]*Genea, Sydney, New South Wales, Australia*

OBJECTIVES: It has long been recognised that there is an increased prevalence of females affected with Trisomy 18 at birth compared to males in the order of approximately 75-80%. Previous studies have reported that this effect is not apparently until after 16 weeks gestation. We present here data showing a preponderance of female fetuses identified at 10 weeks gestation via Non-invasaive Prenatal Screening (NIPS) METHODS: Most 'Aneuploidy Detected' cases of Trisomy 18 identified via GeneSyte (TrueSeq NIPT) were noted to be female over a two year period. Follow-up outcome data was obtained for each case. RESULTS: 13 or 16 'Aneuploidy Detected' cases of Trisomy 18 were found to be female on Non-invasive Prenatal Screening (NIPS). Each case was confirmed as 47, XX+18 via invasive testing. Our discordant sex ratio is similar to that seen at birth (81%). Our population is biased with all women of Advanced Maternal Age, mean 39.9 years. CONCLUSIONS: Our observation is in alignment with the preponderance of liveborn females with Trisomy 18 but refutes some earlier studies suggesting the sex ratio discrepancy is not found until after 16 weeks gestation given more males succumb during the pregnancy. Previous reports of female fetuses with Trisomy 18 due to Meiosis I errors are supported by our results given our population age. Our results also support previous hypotheses that trisomy sex ratios are skewed at conception or during early embryonic development rather than through differential uterine selection. Further comparitive reports from other companies could provide further insights.

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Detection of common aneuploidies by maternal cell-free fetal DNA (cffDNA) as a second-tier screening test after a positive maternal serum screening result

Monica Flessel, Robert Currier, Sara Goldman, Richard Olney

California Department of Public Health, Richmond, California, United States

OBJECTIVES: To evaluate the detection rate of cffDNA when offered as a second-tier screening test following an initial positive result for maternal serum screening for fetal aneuploidy. METHODS: Second-tier cffDNA became an authorized follow-up service within the California Prenatal Screening Program in November, 2013. For this analysis, we included patients who had a positive biochemical screen in the period from November, 2013 to October, 2015 and were seen at a State-approved Prenatal Diagnostic Center. For each of the positive screen cases, clinicians were surveyed regarding the outcome of the pregnancy at the expected date of delivery. In addition to data collected through this survey, aneuploidies detected in a fetus or infant up to age one were reportable to the California Registry of Chromosome Defects. RESULTS: Of the 20,582 patients offered cffDNA screening during the two-year period, 12,799 elected to have this procedure (63.0 %). The detection rate of cffDNA as a second tier screening test in this high-risk population was over 90% for each of the targeted aneuploidies. The detection rate for Down syndrome was 97.6% and the detection rate for trisomy 18 was 91.4%. Detection rates for trisomy 13 and Turner syndrome had wide confidence intervals due to small numbers. CONCLUSIONS: Although cffDNA screening had satisfactory performance as a second-tier test, these data highlight the need to provide patients with genetic counseling to understand that cffDNA is not a diagnostic test and to consider amniocentesis to confirm the cffDNA finding.

	Trisomy 21	Trisomy 18	Trisomy 13	Turner Syndrome (45, XO)
Aneuploidy reported in screened population	1197	310	90	166
Seen at PDC	880	230	52	107
Had cffDNA	375	58	9	11
cffDNA positive	366	53	9	10
cffDNA negative	9	5	0	1(*)
Detection Rate	97.6%	91.4%	100%	90.9%
95% Confidence Interval	(96.1%-99.1%)	(84.2%-98.6%)	(74.1%-100%)	(76.1%-99.5%)

(*) 1 specimen was not analyzed for sex chromosome aneuploidies.

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Detection of genome-wide abnormality by cell-free DNA testing in 15633 consecutive pregnancies

Jia Zhao[1], Hong Yao[2], Yanling Dong[2], Huixin Xu[3], Yuying Yuan[3], Fang Chen[3], Wei Wang[3], Ya Gao[3]

[1]BGI Education Center, University of Chinese Academy of Sciences, Shenzhen, China
[2]Department of Gynecology & Obstetrics, Southwest Hospital, The Third Military Medical University, Chongqing, China
[3]BGI Research, Shenzhen, Guangdong, China

OBJECTIVES: Cell free DNA (cfDNA) testing has been extensively studied for its efficacy of detecting fetal trisomy 21 (T21), trisomy 18 (T18) and trisomy 13 (T13). Whether cfDNA should be used to detect other chromosome abnormalities is in controversy due to the lack of clinical validation. Herein in this study, we aimed to evaluate the performance of cfDNA testing in detecting genome-wide abnormality, including common aneuploidies, sex chromosomal aneuploidy (SCA), chromosome copy number variants (CNV), and other incidental findings (OIF). We also aimed to assess the utility of genome-wide cfDNA testing by analyzing the incremental yield of reporting extra abnormalities. METHODS: We prospectively tested for genome-wide abnormalities by cfDNA testing in 15633 pregnancies with mixed risk factors. A binary hypothesis t-test was used to detect chromosomal aneuploidy and the FCAPS algorithm was used to detect chromosome CNV. CfDNA testing results were accordingly reported and counseled depending on the presence of other chromosome abnormalities. High-risk results of cfDNA testing were confirmed by G-banding, FISH and/or SNP-array, while low-risk results were followed with pregnant outcomes. In the end, we obtained the diagnostic confirmation in 80.1% of the high-risk results, and follow-up in 90.3% of the low-risk results. RESULTS: CfDNA testing identified 191 cases (1.2%) of chromosomal abnormality including 101 common trisomies (T21/T18/T13) and 90 other abnormalities. The sensitivity, specificity and positive predictive value (PPV) for common trisomies were 100.00% (95%CI 93.43%-100%), 99.87% (95%CI 99.79%-99.92%) and 79.31% (95%CI 69.02%-86.96%), respectively. By extending the reporting range to genome-wide abnormalities, cfDNA testing increased the detection yield from 0.5% to 0.76% (p=0.0086), and had the overall sensitivity of 97.14% (95%CI 91.27%-99.26%), specificity of 99.63% (95%CI 99.51%-99.72%), and PPV of 66.67 (95%CI 58.53%-73.93%). Comparing with conventional biochemical screening, genome-wide cfDNA testing had 60 times higher true positive rate. CONCLUSIONS: Our data demonstrated that cfDNA testing can detect genome-wide chromosomal abnormalities with high sensitivity and specificity. By reporting extra chromosomal abnormalities, the ultimate diagnostic yield significantly increased, whereas the cumulative FPR remained low. Furthermore, genome-wide cfDNA testing outperformed conventional biochemical screening by detecting more chromosomal abnormalities with higher true positive rate. Therefore, detection of genome-wide chromosomal abnormality by cfDNA testing may offer clinical benefits of incremental yield at earlier time of pregnancy.

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Comparison between different screening strategies for the detection of all fetal karyotype abnormalities

Francesca Romana Grati[1], Beatrice Grimi[1], Komal Bajaj[2], Livia Marcato[1], Barbara Malvestiti[1], Federico Maggi[1], Giuseppe Simoni[1], Susan Gross[2], Jose Carlos Pinto B. Ferreira[3]

[1]*TOMA Advanced Biomedical Assays, S.p.A., Busto Arsizio, Italy*
[2]*Albert Einstein College of Medicine, Bronx, New York, United States*
[3]*1st Department of Obstetrics and Gynecology, Medical University of Warsaw, Warsaw, Poland*

OBJECTIVES: Cell-free DNA (cfDNA) and traditional serum±ultrasound screening (TSS) are screening tests for T21,T18,T13. For these targets, cfDNA tests have a higher detection rate (DR) and a much lower false positive rate (FPR). The higher FPR is often considered a limitation of TSS when compared with the highly specific, albeit more expensive, cfDNA test. However, authors have identified a distinct advantage with TSS due to nuchal translucency's (NT) ability to pick up additional anomalies in addition to the higher reflex invasive testing rate. As a result, rare, unexpected 'off-target' chromosome abnormalities may be discovered using TSS. The aim of this study is to compare the detection rates of all fetal karyotype abnormalities (target and off-target) at birth by cfDNA and TSS. METHODS: Using a model derived from our laboratory data, we predicted the DR of all karyotype anomalies for seven screening strategies, including different TSSs, cfDNA tests (±SCAs) and contingent approaches with first-tier combined first trimester screening and second-tier cfDNA in different risk groups. Three representative maternal ages (MA) - 25, 35 and 45 years- were considered. Predicted frequencies of any karyotype anomaly in pregnancies with no risk factors other than MA were used to determine a-priori risk for each karyotype anomaly. The DR and FPR for target anomalies were abstracted from several published studies. DRs for off-targets are 5% and 0.09% corresponding to the FPR for T21 of TSSs and cfDNA tests, respectively. The DR for all target+off-target abnormalities using cfDNA was adjusted downward as 1% of 'no result' cases by cfDNA are actually undetected karyotype abnormalities. RESULTS: CfDNA testing for T21,18,13+SCA (cfDNA-TXY) has the highest DR for all karyotype abnormalities at all MAs. In young women, cfDNA for T21,18,13 only (cfDNA-T) has a lower DR than any TSS as the higher 5% TSS FPR results in the identification of a greater proportion of off-target abnormalities, which dominate the overall chromosomal anomaly risk in young women; cfDNA-T equals contingent or combined first trimester screening at a MA of 38y, when trisomies dominate the risk. CONCLUSIONS: Based on our model, the DR for all karyotype abnormalities of any TSS, frontline or contingent strategy, appears to be lower compared to that of cfDNA-TXY but is superior when compared with cfDNA-T (trisomies only). However, concerns remain regarding screening for SCAs of the cfDNA-TXY model. These results are useful for the development of screening implementation economical models for public health policy decision-makers.

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Predicted performances of cell-free DNA testing for sex chromosome aneuploidies

Francesca Romana Grati[1], Valentina Zanatta[1], Francesca Malvestiti[1], Barbara Malvestiti[1], Livia Marcato[1], Beatrice Grimi[1], Federico Maggi[1], Susan Gross[2], Jose Carlos Pinto B. Ferreira[3], Komal Bajaj[2]

[1]*TOMA Advanced Biomedical Assays, S.p.A., Busto Arsizio, Italy*
[2]*Albert Einstein College of Medicine, Bronx, New York, United States*
[3]*1st Department of Obstetrics and Gynecology, Medical University of Warsaw, Warsaw, Poland*

OBJECTIVES: Cell free DNA (cfDNA) testing can screen for homogeneous sex chromosome aneuploidies (SCAs). The unique genetics of sex chromosomes, which may generate mosaic cell lines, have significant implications for cfDNA test performance and management. The aims of this study are to predict the false positive (FPR) and false negative rates (FNR) by cfDNA testing consequent to feto-placental mosaicism for any SCAs and to predict the positive (PPV) and negative predictive values (NPV) of both high and low risk results. METHODS: Retrospective analysis of a chorionic villus sample (CVS) database including serial karyotype results of cytotrophoblast, mesenchyme and amniocytes of samples showing a placental mosaicism. Cases with a SCA cell line identified in at least one placental layer were included. Predictions of cfDNA testing performance were based on cytotrophoblast karyotype results. The percentage of each cell line in the cytotrophoblast was transformed into 'dosage equivalent' mimicking the quantitative counting approach of cfDNA. RESULTS: The cfDNA SCA FPR consequent to type 1/3 confined placental mosaicism (CPM) is predicted to be 0.05%: other phenomena (maternal mosaicism or vanishing twin) can likely justify the published increased FPR for SCAs. The FNR is very low for all non-mosaic SCAs (0-5.7%); it is high for mosaic 45,X with normal ultrasound (70%). The predicted PPV based on amnio results is very high for most SCAs (94.4% for 47,XYY, 99.4% for 45,X cases with abnormal ultrasound); it is much lower for 45,X without ultrasound anomalies. A 45,X high risk result with normal ultrasound can reflect placental 45,X mosaicism in 50% of cases, thus requiring a confirmatory amniocentesis. It may also reflect a mosaic condition or a discordant abnormality on amniocytes in 18% of cases. The NPV for all SCAs is 99.9%. CONCLUSIONS: A positive cfDNA 45,X result should be interpreted and managed in the context of the ultrasound evaluation: if a cystic hygroma or an increased NT is detected, a confirmatory CVS may be considered because the positive cfDNA result most likely reflects a non-mosaic 45,X karyotype in the placenta; if the ultrasound is normal, an amniocentesis will better characterize the cytogenetic composition of the fetus. For the remaining SCAs, a confirmatory CVS could also be a reliable option for patients who require an early diagnosis even in the absence of ultrasound findings, although amniocentesis still best reflects the fetal chromosomal complement. The high NPV means that cfDNA is a good tool at ruling out SCA.

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Positive and negative predictive values of cell-free DNA for noninvasive prenatal testing

John Williams III, Megha Gupta, Steve Rad, John Ozimek, Dalar Ratousi, Sayeh Farivar, Margareta Pisarska, Rhona Schreck

Cedars Sinai Medical Center, Los Angeles, California, United States

OBJECTIVES: Over the past 4 years, noninvasive prenatal testing (NIPT) using cell-free fetal DNA in maternal has been incorporated into routine obstetric practice. Published reports have demonstrated both sensitivity and specificity for detection of trisomy 21 and trisomy 18 of >99%. Recent studies have suggested that the positive predictive value (PPV) of NIPT is much lower than reported by the laboratories performing NIPT. The objective of this study is to determine the PPV and negative predictive values (NPV) for NIPT compared with diagnostic testing in patients referred to our center. METHODS: We conducted a retrospective review of medical records of pregnant women referred to the Cedars-Sinai Prenatal Diagnostic Center for prenatal diagnosis and screening and for screen positive NIPT results between January 1, 2012 and December 31, 2015. Data collected include maternal age, gravidity, parity, gestational age at screening, results of NIPT screening for trisomies 13, 18, 21 and sex chromosome abnormalities, and karyotype results for women undergoing invasive testing with chorionic villus sampling (CVS) or amniocentesis. RESULTS: Of 7,896 women referred for genetic counseling, prenatal diagnosis or screening, 3296 (41.7%) chose to have diagnostic testing with CVS (n=2970) or amniocentesis (n=326). Of 4600 women chose screening, 1543 (33.5%) had NIPT. There were 149 (9.6%) women with positive NIPT results. There were 7(4.7%) women who declined diagnostic testing including 1 woman who terminated without confirmation of the NIPT results. There were 3 (0.19%) false negative results (trisomy 13, 69,XXX, trisomy 22). The true positive and false positive rates for the 142 women who had diagnostic testing are shown in Table 1. CONCLUSIONS: While NIPT has a high NPV, positive results should be interpreted with caution. The likelihood that a positive NIPT result is a true positive is lower than perceived by clinicians based on the sensitivities and specificities reported by the NIPT laboratories. This appears to be particularly true for trisomy 13, sex chromosome aneuploidy and chromosomal microdeletions. There is a continued need to educate clinicians about the difference between specificity and positive predictive value.

Table 1

NIPT Result	No. Of Cases	True Positive	False Positive	PPV
Trisomy 21	76	67/76 (88.2%)	9/76 (11.8%)	88%
Trisomy 18	17	15/17 (88.2%)	2/17 (11.8%)	88%
Trisomy 13	13	5/13 (38.5%)	8/13 (61.5%)	38%

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45,X	11	3/11 (27.3%)	8/11 (72.7%)	27%
47,XXX/XXY	14	8/14 (57.1%)	6/14 (42.9%)	57%
Microdeletion	11	2/11 (18.2%)	9/11 (81.8%)	18%
Total	142	100/142 (70.4%)	42/142 (29.6%)	70%

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Maternal derivative X chromosome with loss of Xq and gain of 4q identified following prenatal cfDNA screening: Implications for clinical management

Carrie Guy[1], Megan Maxwell[1], Renius Owen[1], Guity Ghaffari[1], Mohammed El Naggar[1], Ben Anderson[1], Ke Zhang[1], Douglas Rabin[2], Felicitas Lacbawan[1]

[1]*Quest Diagnostics, San Juan Capistrano, California, United States*
[2]*Quest Diagnostics, Madison, New Jersey, United States*

OBJECTIVES: Female carriers of derivative X of X-autosome translocations may exhibit phenotypic features of a variant of Turner syndrome due to random X inactivation. Phenotypic abnormalities of distal trisomy 4q may include mild intellectual disability, cranial malformations, minor facial dysmorphism, and digital anomalies. We describe a case in which a maternal derivative X chromosome with loss of Xq and gain of 4q was identified following prenatal cell-free (cfDNA) DNA screening. Identification of this chromosomal abnormality in pregnant patients impacts clinical management for both the patient and the fetus. Follow-up recommendations are discussed. METHODS: A specimen from a 40-year-old pregnant woman at 11 week's gestation with an indication of advanced maternal age was submitted to our laboratory for prenatal cfDNA screening. Prenatal history included one prior spontaneous abortion. Prenatal cfDNA screening using massively parallel shotgun sequencing was performed and outcome information was obtained by laboratory genetic counselors. Microarray analysis was performed on maternal blood as follow-up to the prenatal cfDNA screening result. RESULTS: Prenatal cfDNA screening was negative for trisomy 13, 18, and 21 in a female fetus; fetal fraction was 13.13%. The z-score for X chromosome was -8.87 and the z-score for chromosome 4 was 23.03, consistent with a maternal Xq deletion and 4q duplication. Microarray analysis revealed a maternal derivative chromosome Xq4q unbalanced translocation (arr[hg19]4q34.2q35.2(177,446,819-190,957,473)x3,Xq27.1q28(139,172,184-1 55,233,73 1)x1) with a ~13.6-Mb gain of the terminal end of the long arm of chromosome 4 and an ~16-Mb loss of the terminal end of the long arm of the X chromosome. CONCLUSIONS: We identified maternal derivative X chromosome with loss of Xq with gain of 4q following prenatal cfDNA DNA screening. Clinical evaluation of the patient, high resolution chromosomal and subtelomeric fluorescence in situ hybridization (FISH) studies, familial cytogenetic studies, and genetic counseling regarding the option for prenatal diagnosis to determine fetal status were recommended. This case illustrates that identification of maternal derivative translocations by prenatal cfDNA screening and follow-up cytogenetic studies inform clinical management for pregnant patients, their current pregnancy, and at-risk family members.

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Maternal malignancy evaluation following the detection of multiple aneuploidies by cell-free DNA screening

Laura Carlson[1], Emily Hardisty[2], Neeta Vora[2]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States*
[2]*University of North Carolina School of Medicine, Chapel Hill, North Carolina, United States*

OBJECTIVES: Cell-free fetal DNA is a common method for aneuploidy screening. The American College of Medical Genetics has advocated for its use in all pregnant women regardless of risk. Abnormal cell-free fetal DNA results are associated with maternal disease in the setting of a chromosomally normal fetus, including maternal mosaicism and malignancy. In a previous case series, 17% of women with multiple aneuploidies detected on cell-free DNA were diagnosed with malignancy (Bianchi 2015). This report describes the maternal malignancy evaluation protocol pursued after the third case of malignancy was detected at our institution incidentally by cell-free DNA screening. METHODS: Our patient is a 35 year old G2P1001 who presented for genetic counseling at 12w1d. She opted for aneuploidy screening via cell-free fetal DNA. Her cell-free fetal DNA via the Counsyl platform was notable for monosomy 13 and trisomy 21. It was noted on the report that genome wide data revealed multiple aneuploidies, such has been previously reported in the setting of maternal neoplasm. She underwent amniocentesis following a normal 15 week ultrasound. FISH and karyotype were both normal (46,XX). As such, the patient was referred to Maternal Fetal Medicine for a malignancy evaluation. RESULTS: Maternal Fetal Medicine completed a physical examination. This was notable for hoarse voice, left arm larger than right, and mild hepatomegaly. The remainder of her physical exam was normal for a gravid woman. The patient underwent evaluation with CBC, CMP, and a chest x-ray. The chest x-ray was notable for a large mediastinal mass concerning for malignancy (figure). Chest CT then showed a large left sided anterior mediastinal mass with pleural metastases. CT-guided biopsy was consistent with atypical thymic carcinoid tumor. She terminated her pregnancy, has completed a course of whole chest radiation, and enrolled in a clinical trial. CONCLUSIONS: Atypical cell-free DNA results, particularly those with multiple aneuploidies detected, which are discordant with fetal genotype are concerning for maternal malignancy and warrant evaluation. Malignancies reported include hematologic, neuroendocrine, colorectal, and anal cancers. Suggested evaluation includes a thorough physical exam, CBC, complete metabolic panel including liver function testing, pap, heme-occult, and a chest x-ray. Should these be unrevealing, consideration for MRI chest/abdomen/pelvis and colonoscopy may be warranted. A protocol for malignancy evaluation in these patients should be developed between Maternal Fetal Medicine, laboratories analyzing cell-free DNA, and Oncology specialists to optimize a testing strategy.

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Identification of smaller maternal 22q11.2 deletions with SNP-based noninvasive prenatal test

Katelyn Hashimoto[1], Melissa Maisenbacher[1], Melissa Stosic[2], Sheetal Parmar[1], Michael Young[1], Eddie Pham[1], Mary Grace Del Rosario[1], Styrmir Sigurjonsson[1], Stephanie Kareht[1], Kimberly Martin[1]

[1]*Natera Inc, San Carlos, California, United States*
[2]*Columbia University, New York, New York, United States*

OBJECTIVES: Non-invasive prenatal testing (NIPT) for common aneuploidies using cell-free DNA in maternal plasma has been widely adopted. NIPT coverage has expanded to include subchromosomal anomalies such as the 22q11.2 deletion. Currently, SNP-based NIPT allows for screening of the common 3 Mb deletion that comprises 87% of cases of the 22q11.2 deletion syndrome, but not for smaller atypical deletions. The second most common deletion, is 1.5 Mb and accounts for 7% of cases. SNP-based NIPT is known to detect maternal deletion 3 Mb in size, but detection capabilities for smaller maternal deletions were unknown. METHODS: Maternal blood samples submitted to a reference lab for NIPT screening for common aneuploidies and select microdeletion conditions were amplified and sequenced using pooled primers targeting 13,926 distinct genetic loci, including 1,351 SNPs spanning a 2.91 Mb section of the 22q11.2 region. Three cases were identified with screening results suspicious of smaller maternal deletions in this region. These samples were genotyped using Illumina CytoSNP-12b microarrays with bioinformatics to confirm and size the maternal findings. RESULTS: Please see table 1 CONCLUSIONS: These three cases demonstrate the ability of SNP-based NIPT to detect smaller, ~1.5-1.6 Mb maternal 22q11.2 deletions. Although future studies are necessary, this small study changed the NIPT reporting protocol for these types of cases from no result to a high risk result for the 22q11.2 deletion syndrome. This is clinically impactful for recurrence risk information, genetic counseling, and maternal medical management.

Table 1

NIPT Result	No. Of Cases	True Positive	False Positive	PPV
Trisomy 21	76	67/76 (88.2%)	9/76 (11.8%)	88%
Trisomy 18	17	15/17 (88.2%)	2/17 (11.8%)	88%
Trisomy 13	13	5/13 (38.5%)	8/13 (61.5%)	38%
45,X	11	3/11 (27.3%)	8/11 (72.7%)	27%
47,XXX/XXY	14	8/14 (57.1%)	6/14 (42.9%)	57%
Microdeletion	11	2/11 (18.2%)	9/11 (81.8%)	18%

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Total	142	100/142 (70.4%)	42/142 (29.6%)	70%

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Detecting sub-chromosomal abnormalities by use of circulating fetal trophoblast cells in maternal blood

Lotte Hatt[1], Ripudaman Singh[1], Katarina Ravn[1], Ida Vogel[2], Else Marie Vestergaard[3], Rikke Christensen[2], Steffen Sommer[4], Helle Mogensen[5], Marianne Østergaard[6], Hanne S Jensen[7], Niels Uldbjerg[8], Palle Schelde[1]

[1]*Arcedi Biotech ApS, Aarhus, Denmark*
[2]*Aarhus University Hospital, Aarhus, Denmark*
[3]*Department of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark*
[4]*Department of Gynecology and Obstetrics, Horsens Hospital, Horsens, Denmark*
[5]*Department of Gynecology and Obstetrics, Kolding Hospital, Kolding, Denmark*
[6]*Department of Gynecology and Obstetrics, Randers Hospital, Randers, Denmark*
[7]*5Department of Gynecology and Obstetrics, Regional Hospital West Jutland, Herning, Denmark*
[8]*Dept Obstet Gynecol, Aarhus University Hospital, Aarhus, Denmark*

OBJECTIVES: Our aim is to develop a technology for circulating fetal cell based non-invasive prenatal testing (cbNIPT). Fetal cells in the maternal circulation have an advantage over cell free fetal DNA (cffDNA) in that the complete fetal genome is not contaminated with maternal DNA and not degraded. Thus, it should be more accessible for chromosomal and subchromosomal analysis. We present a high throughput method for enriching fetal trophoblast cells from maternal blood, subsequent amplification of the fetal genome, and detection of chromosomal variations in the genome. METHODS: We collected blood samples from low risk pregnancies and pregnancies at risk of chromosomal abnormalities between the gestation ages of 10 and 13 weeks. Fetal trophoblast cells were enriched and stained using a cocktail of 8 antibodies. The enriched cell fraction was scanned, and for samples coming from high risk pregnancies, positively detected fetal cells were picked using the CellCelector, a capillary based cell picking instrument from ALS. Subsequent whole genome amplification was performed on fetal cells and the DNA was analyzed by 180K array CGH. RESULTS: From 190 pregnancies an average of 12.8 (range 1-45) fetal trophoblast cells were recovered from 30 ml of maternal blood. From high risk pregnancy samples, fetal cells were picked and whole genome amplification was performed which resulted in DNA in quantity and quality high enough to generate array CGH profiles. We present data comparing our non-invasive cell based prenatal test results with CVS array CGH results, including a case of trisomy 21, trisomy 2 mosaicism and a case of an unbalanced translocation t(4;8) resulting in a 31 Mb deletion and a 30 Mb duplication. CONCLUSIONS: Results from the genome analyses on fetal trophoblast cells enriched from maternal blood were verified by array CGH on CVS samples. Chromosomal and subchromosomal analyses can be performed on fetal cells enriched from maternal blood. This method developed has the future potential of offering a cell-based NIPT with large genomic coverage and low false positive rate.

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Next-generation counseling: A model for non-invasive prenatal screening results disclosure and patient management

Colleen Schmitt[1], Aishwarya Arjunan[2], Dave Peticolas[3], Elizabeth Denne[3], Gabriel Lazarin[3], Carrie Haverty[3]

[1]*Counsyl, Frederick, Maryland, United States*
[2]*Counsyl, Chicago, Illinois, United States*
[3]*Counsyl, South San Francisco, California, United States*

OBJECTIVES: Non-invasive prenatal screening (NIPS) utilization has grown dramatically since its introduction, and is now increasingly being offered to the general population by non-genetics specialists. All major guidelines recommend that patients with both positive and negative results should be counseled regarding limitations of testing.[1,2] There is, however, a disconnect between these recommendations and the patient's clinical experience. Genetic counseling utilization is limited by, among other factors, inadequate supply of genetic counselors.[3] As a genetic testing laboratory that provides a results delivery system, including telecounseling, we report on how this service is being utilized for patients undergoing NIPS. METHODS: Upon results availability, providers opting into the results delivery system are notified. If negative, patients are contacted by automated text or email to access them through a secure patient portal. Patients may watch tailored informational videos, request "on-demand" genetic counseling, schedule a later consult, or decline all of the above. If a consultation is elected, a summary is subsequently sent to the ordering provider. If results are positive, either the ordering physician or our own genetic counselor contacts the patient directly, according to the physician's choice. Positive results are not automatically released to a patient. RESULTS: Over a 29-month period, 27,827 NIPS results were issued through the system. Of these, 1,975 patients elected genetic counseling, 1,907 (96.6%) of which received negative results. 65.2% (n=1,244) of patients with negative results requested an on-demand consult while 72.1% (n=49) of patients with positive results requested an on-demand consult. Average consultation time was seven minutes (range: 1-40 minutes) for negative results and 15 minutes (range: 3-54 minutes) for positive results. The average patient satisfaction rating for consultations was 4.9/5.0. CONCLUSIONS: Combining web education, genetic telecounseling, and automated notification protocols, we implemented a service that efficiently manages NIPS results disclosure. The majority of patients who chose to schedule a consultation had negative results, demonstrating a desire for post-test genetic counseling, even in the presence of normal results and showed high satisfaction with the service they received. We describe an efficient and scalable means of manifesting medical guidelines on post-NIPS patient management to a large number of patients, which is imperative to quality clinical care as uptake grows among the general population.

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Strategies to avoid false positives caused by maternal copy number variants in noninvasive prenatal screening

Greg Hogan, Eerik Kaseniit, Kevin D'Auria, Carrie Haverty, Dale Muzzey

Counsyl Inc., South San Francisco, California, United States

OBJECTIVES: Maternal copy number variants (CNVs) are reported to be a major source of false positives in noninvasive prenatal screening (NIPS). Maternal CNVs of 250kb and larger were predicted to increase false positive rate by 40- to 1000-fold or more (Snyder et al., NEJM 2015). Two recent studies of trisomies 13, 18, and 21 attributed one-third to one-half of NIPS false positives to maternal duplications (Strom et al. NEJM 2017, Chudova et al. NEJM 2016). Here, we characterize the impact of maternal CNVs on our NIPS analysis and outline a set of best practices for robust aneuploidy screening via whole-genome sequencing. METHODS: Retrospective analysis and simulations were used to characterize the relationship between features of maternal CNVs (chromosome, size and type) and risk for a false call. The size of putative maternal CNVs identified in the whole-genome sequencing data of 30,000 NIPS samples was plotted against the CNV-harboring chromosome's z-score, which reflects the probability of an aneuploid call. Since large CNVs spanning >10Mb are empirically rare in the healthy pregnant population, we supplemented the retrospective analysis with simulations of thousands of maternal CNVs of different sizes. RESULTS: Our computational pipeline uses outlier-robust approaches that effectively mitigate the impact of maternal CNVs, such that their contribution to the false positive rate is much lower than that predicted by the analysis approach of Snyder et al. (NEJM 2015). This result is further supported by a preliminary review of patient outcome data. Our simulations establish the boundary beyond which a maternal CNV could compromise an outlier-robust algorithm; however, such large maternal CNVs can be readily detected with appropriate quality-control metrics and computer-assisted manual review of NIPS calls, which we perform on all clinical samples. CONCLUSIONS: High specificity in NIPS can be achieved—even in the presence of maternal CNVs that range widely in size—using a suite of best practices, including algorithmic omission of outlying bins, fine-tuned quality-control metrics, and manual call review. Critically, the mechanisms that ensure robustness to maternal CNVs do not compromise detection of true aneuploidies, thereby preserving both high sensitivity and a low test-failure rate. High specificity, which enables higher positive predictive value (PPV), is critical to preserve clinical utility as NIPS adoption increases in the average-risk population.

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Population-based trends in the prenatal diagnosis of sex chromosome aneuploidies in the pre- and post-NIPT era

Allanah Howard-Bath[1], Alice Poulton[2], Jane Halliday[2], Lisa Hui[3]

[1]*Murdoch Childrens Research Institute, Warrandyte, Victoria, Australia*
[2]*Murdoch Childrens Research Institute, Parkville, Victoria, Australia*
[3]*Murdoch Childrens Research Institute, Heidelberg, Victoria, Australia*

OBJECTIVES: Non-invasive prenatal testing (NIPT) has revolutionized the diagnosis of fetal aneuploidies and provided unprecedented opportunities for sex chromosome aneuploidy (SCA) detection. The routine inclusion of SCA into prenatal screening has been controversial in some sectors. We aimed to assess trends in the prenatal diagnosis of SCA before and after the introduction of patient-funded NIPT in 2013 that included SCA screening in a state population with > 73,000 annual births. METHODS: We analyzed prospectively-collected data on all women undergoing amniocentesis or chorionic villus sampling < 25 weeks gestation from 1986-2015 in Victoria, Australia. Women with a prenatal diagnosis of whole chromosome SCA were included; sex chromosome mosaics or co-existent autosomal aneuploidies were excluded. Indications for testing from three triennia (1986-88, 2002-04 and 2013-15) were analysed to assess trends in ascertainment of SCA. Annual numbers of total prenatal diagnosis procedures and government birth statistics were used as denominators. Statistical significance was tested using the chi-square test for trend. RESULTS: There were 780 prenatal SCA diagnoses and 1,960,453 births from 1986-2015. The annual rate of SCA/10,000 births increased significantly over the 30-year period ($p<0.001$) but remained steady between 2010-15 ($p=0.71$), despite a concurrent decline in invasive testing. The proportion of diagnostic tests resulting in a SCA diagnosis increased significantly from 0.9% in 2010 to 1.4% in 2015 ($p=0.006$). The common indications for diagnostic testing among women with fetal SCA have evolved from advanced maternal age (58% of indications) and ultrasound abnormality (25%) in 1986-88, to ultrasound abnormality (45%) and high risk of SCA on NIPT (25%) in 2013-15. CONCLUSIONS: The introduction of NIPT for sex chromosomes has not been associated with a significant increase in the detection of prenatal SCA as a proportion of all births in our population. Annual prenatal SCA diagnoses remained steady despite a decline in invasive testing over the past six years. The ascertainment of fetal SCA has changed from an "incidental" finding after testing for Down syndrome based on advanced maternal age, to a diagnosis obtained after abnormal ultrasound or suspected SCA on NIPT. Pre-test counselling for a potential diagnosis of SCA is now more important than ever.

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Population-based impact of noninvasive prenatal testing (NIPT) on screening and diagnostic testing for fetal aneuploidy

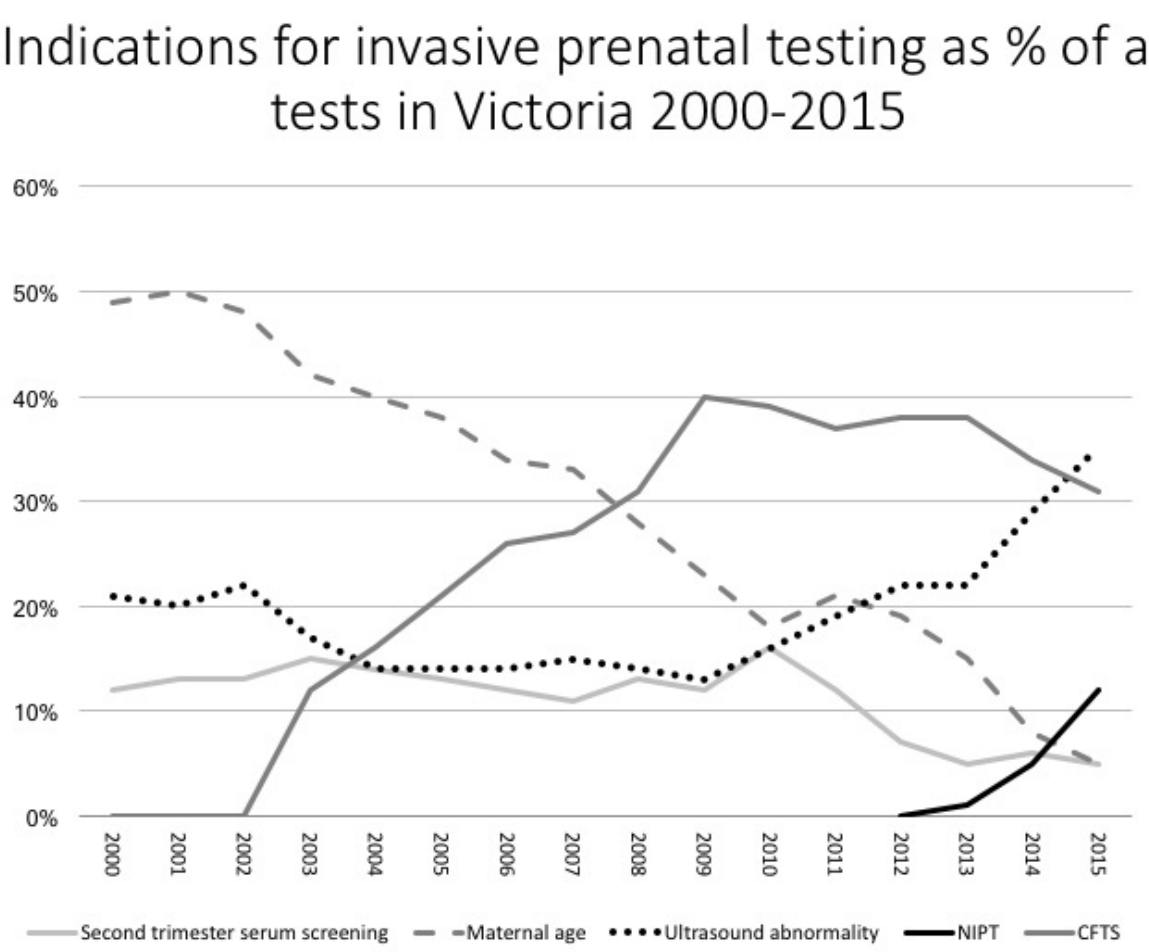
Lisa Hui[1], Briohny Hutchinson[1], Alice Poulton[2], Jane Halliday[2]

[1]*Murdoch Childrens Research Institute, Heidelberg, Victoria, Australia*
[2]*Murdoch Childrens Research Institute, Parkville, Victoria, Australia*

OBJECTIVES: Noninvasive prenatal testing (NIPT) for fetal chromosome abnormalities, also known as cell-free DNA based screening, has been hailed as the vanguard of genomic medicine. Until recently, measuring the downstream effect of NIPT has been largely confined to reporting the decline in invasive testing rates in single center, or multicenter studies. We aimed to perform a detailed population-based evaluation of the impact of NIPT on indications for testing, diagnostic yield and the primary methods of prenatal ascertainment of fetal aneuploidy. METHODS: This study analyzed prospectively collected data from 2000-2015 on prenatal screening, diagnosis and ultrasound from the Australian state of Victoria, with approximately 73,000 births p.a. We analysed these population-based data from 2000-2015: (i) invasive prenatal tests including indications for testing, procedural numbers, and karyotype results (ii) population uptake of CFTS, and (iii) total births. Nuchal translucency ultrasound utilization as ascertained from government statistics were analyzed before and after patient-funded NIPT became available in 2013. We performed 2-tailed chi-squared tests for comparison of two proportions, or chi-squared tests for trend where appropriate, with a p value of < 0.05 being considered significant. RESULTS: The number of diagnostic procedures performed < 25 weeks during the 16-year study period was 62,536. Invasive testing decreased significantly by 39.6% during the NIPT era (2012 to 2015) despite steady births. More than half of all confirmed cases of trisomy 21 were ascertained by NIPT in 2015, despite NIPT comprising only 11.7% of total indications for invasive testing. The population uptake of CFTS declined significantly from 77.5% in 2013 to 68.1% in 2015, but 11-13 week ultrasounds did not. In 2015, ultrasound abnormality replaced CFTS as the most common indication for invasive testing and chromosomal microarray was performed for 85.3% of all prenatal karyotypes. CONCLUSIONS: Prenatal testing is now unequivocally in the genomic era. NIPT is now the screening test that precedes the majority of confirmed diagnoses of trisomy 21 in our population. The increasing use of NIPT and the decline in CFTS uptake were not accompanied by a decline in 11-13 week ultrasounds, suggesting widespread recognition of the value of early fetal morphology assessment. The contributions of NIPT, 11-13 week ultrasound and chromosome microarray has led to unprecedented detection rates of major chromosome abnormalities, now found in 20% of all invasive tests.

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Socioeconomic status and clinical variation in prenatal testing in the NIPT era

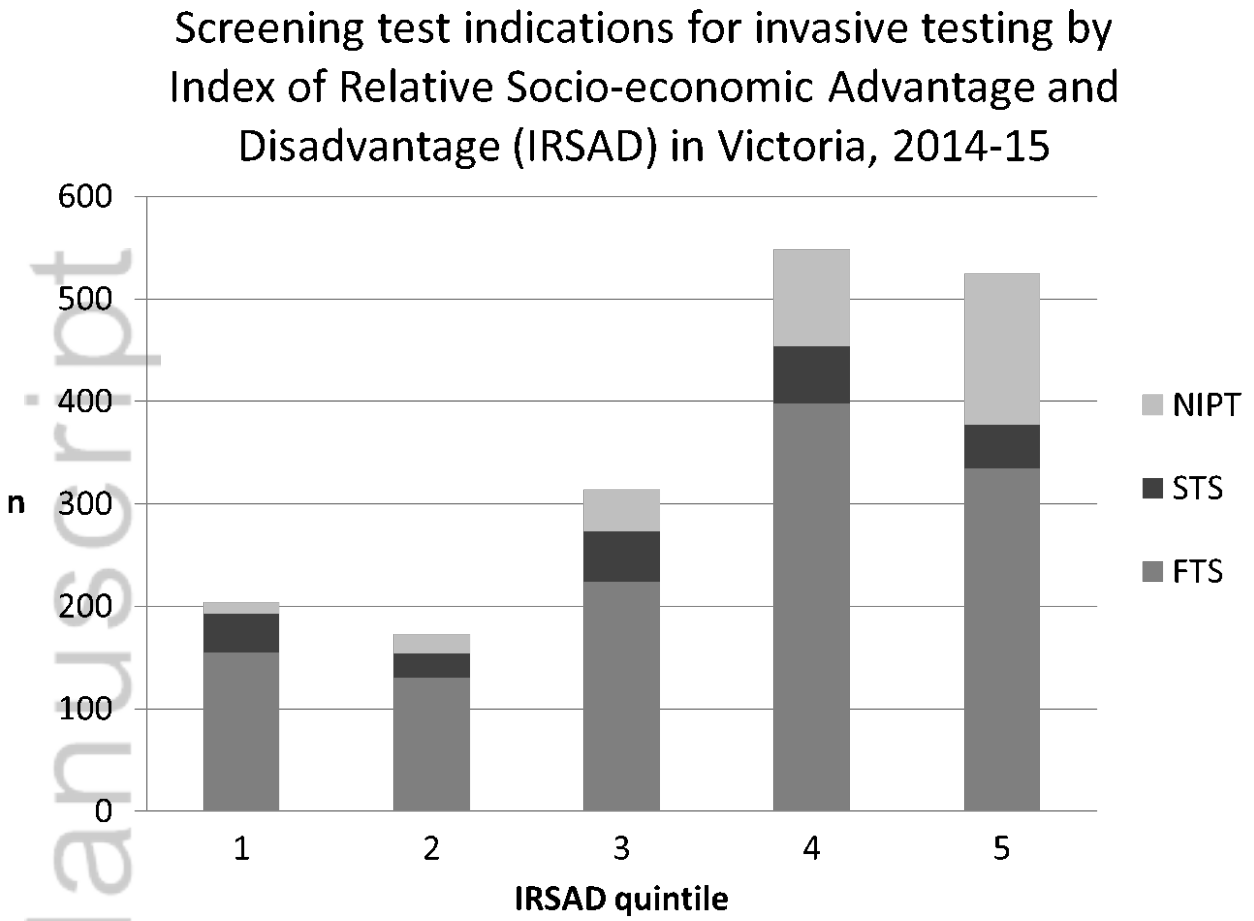
Lisa Hui[1], Jenna Barclay[2], Alice Poulton[2], Briohny Hutchinson[1], Jane Halliday[2]

[1]*Murdoch Childrens Research Institute, Heidelberg, Victoria, Australia*
[2]*Murdoch Childrens Research Institute, Parkville, Victoria, Australia*

OBJECTIVES: Disparity in the utilization of prenatal testing due to socioeconomic status is a well-documented phenomenon. The introduction of patient-funded, non-invasive prenatal testing (NIPT) based on analysis of cell-free DNA in maternal plasma has the potential to exacerbate inequities in prenatal screening, and therefore, in prenatal diagnosis (PNDx). We aimed to analyse variations in screening test indications for PNDx in the NIPT era according to socioeconomic status and geographical location in a state population with more than 73,000 annual births. METHODS: Data on all women undergoing amniocentesis or chorionic villus sampling < 25w from 2014-2015 in Victoria, Australia, were collected. All records of those with an indication of a high risk screening test result (combined first trimester screening (CFTS), second trimester serum screening (STSS) or noninvasive prenatal testing (NIPT)) were assigned an Index of Relative Socio-economic Advantage and Disadvantage (IRSAD) according to residential postcode. The relationship between screening indications for PNDx, diagnostic outcomes and IRSAD quintile were analysed using the chi-squared tests for trend or proportions as appropriate.Geo-mapping was performed with data visualisation software program Tableau v9.3. RESULTS: 1764 PNDx cases were included. CFTS was the most common indication for PNDx, with no significant differences across IRSAD quintiles, or geographical location. Increasing socioeconomic advantage was positively associated with NIPT-indicated PNDx ($p < 0.0001$), while relative socioeconomic disadvantage was associated with STSS-indicated testing ($p = 0.02$). Metropolitan women were more likely than rural women to have NIPT as an indication for PNDx (19.4% v 10.9%, $p = 0.0003$). The diagnostic yield of PNDx increased with socioeconomic advantage, from 11% in the lowest IRSAD quintile to 24% in the highest quintile ($p = 0.007$). CONCLUSIONS: There is significant variation in screening indications for PNDx and diagnostic outcomes according to socioeconomic status and geographical residence in our population. NIPT is an underrepresented indication for diagnostic testing in the lowest IRSAD quintile. This clinical variation resulted in disadvantaged women undergoing more unnecessary invasive prenatal tests due to false positive screening results than advantaged women. This finding has important ethical and public health implications for all prenatal screening programs.

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Technical and clinical implications of maternal chromosome Xp deletion identified by prenatal cell-free DNA screening

Bernard Ilagan[1], Megan Maxwell[1], Barbra Fisher[2], Jeri Milanovich[2], Renius Owen[1], Ben Anderson[1], Ke Zhang[1], Charles Strom[1]

[1]*Quest Diagnostics, San Juan Capistrano, California, United States*
[2]*Northwest Perinatal Center, Portland, Oregon, United States*

OBJECTIVES: With the introduction of microdeletion screening, prenatal cell-free (cfDNA) screening has been validated to detect fetal genomic copy number variations (CNVs) dependent upon factors such as CNV size and sequencing depth. Emerging evidence indicates that prenatal cfDNA screening can also potentially detect maternal CNVs with accuracy similar to chromosomal microarrays, and possibly confirm a related diagnosis of suspicion. Here we present such a case report in which prenatal cfDNA screening identifies maternal variant Turner syndrome involving a deletion of Xp. The technical and clinical implications of this case are discussed. METHODS: A peripheral blood specimen from a 39-year-old pregnant patient at 11 weeks' gestation was sent to our laboratory for prenatal cfDNA screening with the clinical indication of advanced maternal age. Prenatal cfDNA screening was conducted using massively parallel shotgun sequencing (MPSS) to screen for fetal aneuploidy of chromosomes 21, 18, and 13 as well as the sex chromosomes. Subsequent maternal chromosomal microarray analysis (CMA) was conducted on remnant maternal blood using Affymetrix CytoScan® HD SNP-array platform and Chromosome Analysis Suite (ChAS; version 3.0, Affymetrix). RESULTS: The cfDNA sequencing data showed a markedly lower level of cfDNA sequencing products at Xp. At a fetal fraction of 9.73%, the level was lower than expected for a fetal Xp deletion in an unaffected mother. Therefore, the Xp deletion was suspected to be maternal in origin. Follow-up CMA analysis of the patient's DNA confirmed a maternal 39.5-Mb deletion of Xp22.33p11.4, consistent with the prenatal cfDNA screening finding and a diagnosis of variant Turner syndrome (46,XXp-). A recommendation was made to offer the patient confirmatory invasive diagnostic testing to interrogate this deletion in the fetus. CONCLUSIONS: This case report adds to the growing body of evidence supporting accurate detection of maternal CNVs via prenatal cfDNA screening. Identification of maternal variant Turner syndrome due to partial deletions informs clinical management of the patient and the fetus. The patient requires heritable (rather than sporadic) recurrence risk counseling and routine cardiac surveillance due to risk of cardiovascular complications augmented in pregnancy. If the fetus inherits the deletion, early intervention with growth and sex hormone therapies can mitigate short stature and aid development of secondary sexual characteristics.

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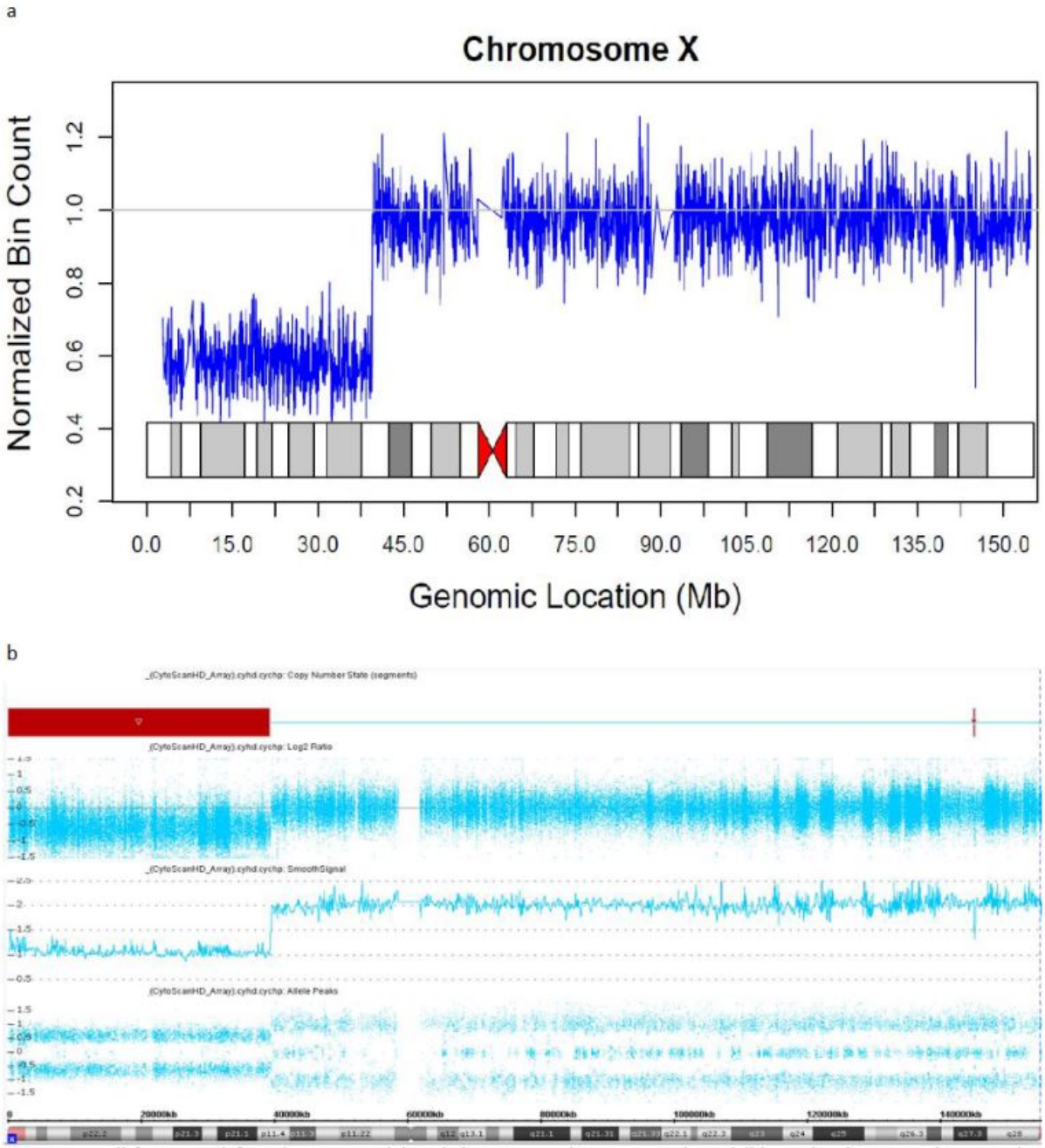


Figure 1. Cell-free DNA (cfDNA) screening (a) and chromosomal microarray (CMA) (b) results showing relative and comparable decreased ratios along the terminal end of chromosome Xp

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Plasmatic extracellular vesicles in first trimester of patients who develop spontaneous abortion

Manuel Varas[1], Paula Correa[2], Stephanie Acuña[2], Sofia Cubillos[2], Pía Venegas[2], Lara Monteiro[1], Sebastian Illanes[1]

[1]*Universidad de los Andes, Santiago, Chile*
[2]*Department of Obstetric and Gynaecology and Laboratory of Reproductive Biology, Faculty of Medicine, Universidad de los Andes, Santiago, Chile*

OBJECTIVES: Spontaneous abortion (SA) is a common complication in early pregnancy. The methods used to identify SA are ultrasound scan, and measurement of human chorionic gonadotropin. Although both methods can identify women at risk of SA, research are focused in find biomarkers to prospectively identify at-risk pregnancies, before the onset of clinical symptoms. Extracellular vesicles (EVs) have been studied as a powerful diagnostic and prognostic tool to placental pathologies since they are released into the circulation. The aim of this study was to investigate the associations between maternal plasma concentration of EVs in the first trimester of pregnancy and subsequent SA. METHODS: A prospective study was conducted to establish EVs concentration in maternal blood of women in the first trimester of pregnancy. Blood samples in the first trimester of pregnancy were collected and plasma was isolated and stored at -80°C. Women were prospectively followed, and clinical variables and pregnancy outcomes were recorded. A retrospectively study was designed involving normal healthy pregnant women (n=34) and women who develop SA (n=10). EVs were isolated with the reagent ExoQuick according to manufacturer instructions, and the EVs were characterized by electron microscopy, western blot, and the concentration and size distribution were analyzed by nanotracking particles analysis. RESULTS: The clinical characteristics were similar in both groups. The EVs isolated from both groups showed the typical morphology described for EVs of placental origin, positive for CD63, Alix, and Flotilin-1, proteins enriched in EVs, and showed a size distribution around 100 nm in diameter. The total amount of EVs didn´t shows any difference between groups. However, when normalized by gestational age at sample, the patients who develop a SA didn´t show the normal increase of EVs in plasma that are observed in patient with normal pregnancies, and this was statistically significant. CONCLUSIONS: Women who develop spontaneous abortion do not show the increase of the EVs concentration in the maternal circulation that is observed in normal pregnancies.

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Improving efficiency of non-invasive prenatal diagnosis for monogenic disorders in a National Health Service laboratory through automation of bioinformatics analysis

Lucy Jenkins[1], Helena Ahlfors[1], Fiona McKay[1], Natalie Chandler[1], Rowenna Roberts[1], Jane Hayward[1], Lyn Chitty[2]

[1]NE Thames Regional Genetics Laboratory, London, United Kingdom
[2]Genetics 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: We offer an ISO 15189:2012 accredited service for non-invasive prenatal diagnosis (NIPD) for monogenic disorders at our National Health Service (NHS) Regional Genetics laboratory. Established in 2013, we now deliver a United Kingdom Genetic Testing Network (UKGTN) approved service for a range of conditions using next generation sequencing (NGS) techniques. Amplicon-based NIPD of de novo / paternal dominant conditions and relative haplotype dosage analysis for definitive diagnosis of recessive conditions are provided for couples at risk of rare monogenic disorders. Here we describe automation of our pipeline to increase reliability and scope, decrease costs and improve turnaround times for NIPD. METHODS: Our original analysis step using Excel macros has been rewritten using bash scripting not only to be able to automate data processing but also include quality controlling, monitoring potential sample contamination and filtering out poor quality data. This pipeline has been applied to NIPD for detection of de novo and paternal mutations, to autosomal recessive conditions or those where presence of maternal mutation requires consideration. Recently the pipeline was expanded to include an R script to perform relative haplotype dosage analysis (RHDO) using pregnancies at risk of cystic fibrosis as an exemplar of testing based on linkage analysis. RESULTS: Automation and incorporation of quality control checks in our bioinformatics analysis pipeline reduced analysis time from three hours to 30 minutes. Validation of the new pipeline was completed by direct comparison with our original pipeline (previously validated against pregnancy outcomes) to show 100% concordance. The RHDO pipeline was validated in 12 pregnancies with known outcomes and was also 100% concordant. These pipelines have also been applied clinically for testing families at risk of rare disorders; bespoke NIPD has been performed for 35 families, with a range of >17 disorders including Osteogenesis imperfecta, Neurofibromatosis type 1 and early infantile epileptic encephalopathy. CONCLUSIONS: Automation and robust quality checking processes for our bioinformatic analysis pipeline has allowed expansion of our NIPD service provision, both in referral numbers and test repertoire. In addition to providing resilience for this growing service, efficiencies in analysis time and reduction in risk of operator error have facilitated the delivery of results within a rapid turnaround time.

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Non Invasive Prenatal Testing – An Indian Study

Priya Kadam[1], Eswarachary Venkatawamy[1], Tulika Tayal[2], Sheela Nampoorthiri[3], Chitra Andrew[4], Madhulika Kabra[5], Rashmi Bagga[6], Mamatha Gowda[7], Meenu Batra[8], Sridevi Hegde[9], Anita Kaul[10], Jayshree Ganapathi Subramanian[11], Shruti Lingaiah[1], Riyaz Akhtar[1], Francis Kidangan[1], Chandran R[1], Kiran C[1], Ravi Kumar G. R.[1], Ramprasad V. L.[1], Ratna Puri[11], Ishwar Chandra Verma[11]

[1]*Medgenome Laboratory Private Limited, Bangalore, India*
[2]*Rainbow Hospital, Hyderabad, India*
[3]*AMRITA Institute, Kochi, India*
[4]*Sri Ramchandra Medical College, Chennai, India*
[5]*AIIMS, New Delhi, India*
[6]*Postgraduate Institute of Medical Education and Research, Chandigarh, India*
[7]*JIPMER, Pondicherry, India*
[8]*CIMAR, Kochi, India*
[9]*Manipal Hospital, Bengaluru, India*
[10]*Apollo Indraprastha Hospital, New Delhi, India*
[11]*Sir Ganga Ram Hosiptal, New Delhi, India*

OBJECTIVES: Noninvasive prenatal testing has revolutionized prenatal screening for chromosomal aneuploidies around the world. In India, the implementation has been sporadic so far. Given the population variation as well as ethnic differences in India compared to rest of the world, we evaluated the performance of this test in India. METHODS: Ten centers in India participated in this project after due institutional ethical approvals. Samples fulfilling the preset inclusion and exclusion criteria were collected after informed consent. The Panorama NIPT was performed in 516 pregnant women, who had previously tested intermediate to high risk on conventional first and second trimester screening (Combined, Triple and Quadruple screening). All samples were processed in the Medgenome laboratory located in Bengaluru, India. Results of the NIPT, both high and low risk, were confirmed either by amniocentesis followed by fluorescence *in situ* hybridization and cytogenetic evaluation or by clinical evaluation of the infant after birth. RESULTS: NIPT results were obtained in 97.7% samples. Of these, 480 (98.2 %) were low risk and 19 were high risk on NIPT. A sensitivity of 100% and specificity of 99.7 % was seen in all tested chromosomal abnormalities combined. Taken together, after confirmation, the positive predictive value for Trisomy 21, 18, 13 and Monsomy X was 84.2 %. The average fetal fraction was 8.2%, which is slightly lower than the average observed in studies elsewhere. NIPT results were available in 97.4% of obese women. The overall no-call rate was 2.3%. There were no false negatives. CONCLUSIONS: This is the first report of NIPT carried out in a developing country, and demonstrated comparable performance in all aspects of testing to the results in the countries that have extensively adopted the testing.

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Beyond the basics: A case series from genome-wide non-invasive prenatal testing (NIPT)

Erica Soster[1], Morgan Verrill[2], Erin O'Toole[3], Theresa Boomer[1], Jason Chibuk[1], Adity Khanna[1]

[1]*Sequenom/Integrated Genetics, San Diego, California, United States*
[2]*Center for Prenatal Diagnosis at St. Vincent Women's Hospital, Indianapolis, Indiana, United States*
[3]*OhioHealth Maternal Fetal Medicine, Columbus, Ohio, United States*

OBJECTIVES: NIPT continues to expand to include additional content. Genome-wide NIPT extended the amount of clinically relevant information available through screening. This case series examines some complex positive results and important clinical and technical points raised. METHODS: Maternal blood samples submitted to Sequenom Laboratories for MaterniT® GENOME testing were subjected to DNA extraction, library preparation, and whole genome massively parallel sequencing as described by Jensen et al.[1] Sequencing data were analyzed using a novel algorithm to detect trisomies and sub chromosomal, genome wide events 7Mb and larger, as described by Lefkowitz et al.[2] RESULTS: 1. MaterniT GENOME identified a 4q25-q35.2 duplication (80Mb) in an anomalous fetus. Amniocentesis confirmed the result and suggested the duplication was part of a Y-chromosome translocation. 2. MaterniT GENOME identified an 8q21.13-q24.3 duplication (63.70Mb) in an anomalous fetus. Sequencing data suggested a possible maternal duplication. Amniocentesis confirmed the fetal result. Peripheral blood microarray confirmed a previously unrecognized maternal duplication. 3. MaterniT GENOME identified a 18q22.1-q23 deletion (13.95Mb) in an anomalous fetus. Postnatal microarray confirmed the deletion and detected an additional smaller deletion below the 7Mb reporting threshold of MaterniT GENOME. Postnatal karyotype detected a normal chromosome 18 and two ring versions of chromosome 18. CONCLUSIONS: Genome-wide NIPT delivers information regarding chromosome conditions beyond traditional aneuploidies. These results may have clinical relevance not only for the fetus, but also for the pregnant patient or her partner. In some cases, this may provide further insight into the etiology of the fetal chromosome abnormality.

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Fetal -Based Risk (FFBR) model in SNP-based NIPT detects triploidy

Shifra Krinshpun, Allison Ryan, Trudy McKanna, Stephanie Kareht, Kimberly Martin

Natera Inc, San Carlos, California, United States

OBJECTIVES: A fetal -based risk (FFBR) score, incorporating maternal weight and gestational age, was developed to determine risk in cases that received 'no results' due to low FF on non-invasive prenatal testing (NIPT). Here we specifically review cases with triploidy. The prevalence of triploidy among conceptuses is approximately 1 –3%. Survival past the first trimester is uncommon. Distinct fetal and placental phenotypes emerge depending on parental origin of the extra set of chromosomes. Digynic triploidy, which has a small, non-molar placenta, is expected to be associated with a very low FF and is therefore likely to receive 'no results' on NIPT. METHODS: Outcome was collected for 1352 pregnancies that received 'no results' due to low fetal fraction on NIPT. The relative FF was compared with three models, ('normal', 'trisomy 13 or 18', 'digynic triploidy') and corrected for gestational age and maternal weight. The FFBR score, a posterior risk estimate, revises the age-based prior risk based on the observed FF. High FFBR score is defined as a >1% estimated risk of trisomy 13, 18 or triploidy. A FF z-score was also calculated; it is defined relative to the normal-hypothesis model by subtracting the model average and dividing by standard deviation. RESULTS: Of the 1352 cases in this cohort, 651 (48%) were found to be high risk by FFBR and 19 (2.9%) were triploid. There was one additional triploid case that was not called high FFBR. Triploid cases had significantly lower FF z-scores compared with other aneuploidies (mean, -3.97 ±0.95 vs. -1.85 ±1.69). Accordingly, FFBR scores were much higher for the triploidies than the other aneuploidies (median, 55% vs. 2.8%). Three of the 20 triploid cases resulted in first-trimester losses, 5 were second-trimester losses, 3 were intrauterine fetal demises at >22 weeks, and 9 were electively terminated (7 in the second or third trimester). CONCLUSIONS: The FFBR model, which estimates risk according to fetal fraction, gestational age, and maternal weight in cases with low FF, enabled ascertainment of most (19/20) triploid pregnancies, which tend to have lower FF. Fifteen of twenty triploid pregnancies in this study survived beyond the first trimester, highlighting the clinical utility associated with identification of triploidy. Therefore, a "no result" from NIPT due to low FF does not mean "no information"—the FFBR score can provide clinically relevant information to providers and their patients.

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Is non-invasive prenatal screening for aneuploidy impacting on the rate of children born with Down syndrome?

Melissa Hill[1], Angela Barrett[2], Mahesh Choolani[3], Celine Lewis[4], Lyn Chitty[5]

[1]*University College London Hospital, NE Thames Regional Genetics Laboratory & UCL Institute for Child Health, London, United Kingdom*
[2]*NUS, Singapore*
[3]*National University of Singapore, Singapore*
[4]*Genetics and Genomic Medicine, Great Ormond Street Hospital NHS Trust and UCL Institute of Child Health, London, United Kingdom*
[5]*Genetics 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: Non-invasive prenatal testing (NIPT) is a highly accurate prenatal screening test for aneuploidy that is being implemented worldwide, raising concerns that the improved prenatal detection and increased uptake might decrease the number of children born with Down syndrome (DS). Here we aim to investigate these concerns by reviewing the available literature on pregnancy outcomes following introduction of NIPT to ascertain reports of impact from across the globe. We also report the results of a prospective audit of outcomes following implementation of NIPT into the DS Screening pathway in maternity units in the UK and Singapore. METHODS: We systematically reviewed the published literature reporting number of live births of children with DS following screening with NIPT from 2011 to December 2016. Publications were included if they described data on numbers of women having NIPT for DS and provided information on pregnancy outcomes. We also reviewed the literature exploring parents' hypothetical choices about NIPT and next steps following a diagnosis of DS. Audits were performed of pregnancy outcomes in maternity units in south-eastern England and in Singapore following implementation of NIPT into the routine DS Screening pathway. RESULTS: We identified 903 articles, after reviewing 62, 12 were included. Pooled results suggest numbers of babies born after prenatal diagnosis of DS was unchanged in the USA, Asia and Netherlands, but increased in the UK. The UK prospective audit of NIPT implementation as a contingent test for women with a standard DS screening risk of >1/150 supports this observation, with 37% women continuing pregnancies after a DS diagnosis. Two of six women continued the pregnancy in Singapore. Psychosocial research suggests a significant number of parents want NIPT for information only. CONCLUSIONS: Several lines of evidence suggest that many parents who take up NIPT will choose to continue the pregnancy when Down syndrome is predicted. Where NIPT uptake is high and termination rates remain unchanged, live-birth rates may fall. In the UK, whilst uptake is high, termination rates following NIPT are lower than pre-NIPT rates so live-birth rates may be unaffected. Where parents are using NIPT for information only we will need to provide appropriate support. To determine the true impact of NIPT prospective audit and reporting is required following implementation alongside population based studies.

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	Total diagnosed	Livebirths (%)	Pre-NIPT termination rate
Asia	37	0	94%
Netherlands	31	2 (6%)	93%
USA	99	32 (32%)	67%
UK	115	24 (21%)	92%

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Incidental NIPT finding +12p was identified a low-grade placental mosaicism after applying the NIPT-method to placental DNA

Merryn Macville[1], Melanie Van Esch-Lennarts[1], Nieke Houben[1], Stijn Ghesquiere[1], Jos Offermans[2], Ingrid Witters[1], Mijntje Pieters[1], Christine de Die-Smulders[1], Yvonne Arens[1], Audrey Coumans[1]

[1]Maastricht University Medical Center, Maastricht, Netherlands
[2]Zuyderland MC, Sittard-Geleen, Netherlands

OBJECTIVES: A +12p incidental finding (27 Mb windowed z-score 5.864) was found with NIPT in a healthy primigravida at 12 wks + 5 dys with increased risk 1:9 for Downsyndrome. This may indicate a (mosaic) isochromosome 12p causing Pallister-Killian syndrome. A positive NIPT may be false due to confined placental mosaicism (CPM). Moreover, iso12p is not described in the literature - to our knowledge - probably due to very low-grade mosaic presence and lack of powerful detection methods. The pregancy was continued and triweekly monitored by ultrasound. A healthy baby girl (AS9/10, 3085 gr) was born without complications at term. METHODS: NIPT aneuploidy screening was performed with shallow whole-genome sequencing and subsequent windowed z-score analysis by WISECONDOR. Invasive testing on amnion fluid was performed with standard molecular cytogenetic methods. When placental tissue was received post partum, chromosomal microarray (CMA) was performed on the DNA of 4 biopsies from every quarter. Since NIPT is able to indicate fetal aneuploidy in a 4% fetal fraction of total cell free DNA in maternal plasma, we hypothesized that the employed method for NIPT would be a suitable way for the detection of low-grade mosaics under 20% in placental tissue. RESULTS: After NIPT, follow-up invasive testing by amniocentesis was performed, but gain of 12p was neither shown with CMA on the DNA of uncultured amnion cells nor with extensive karyotyping and FISH. After birth, the girl had a normal female karyotype 46,XX and no signs of Pallister-Killian syndrome. CMA of placenta did not show the gain of 12p indicating either absence or a low-grade mosaic <20% in those biopsies. In contrast, when subjecting sheared placental DNA to shallow whole-genome sequencing and z-score analysis by WISECONDOR - in the same way as NIPT - we unambiguously showed gain of 12p (17 Mb windowed z-score 3.850). CONCLUSIONS: We describe to our knowledge the first case of CPM +12p, although the isochromosome was not made visible by FISH or karyotyping. The 'NIPT method' is a powerful way to detect low-grade mosaic (<20%) chromosomal aberrations above 15Mb in placental tissue. Furthermore, this method may also apply to any other tissue.

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Cell free fetal DNA testing in Argentina experience after 3 years, updated information

Jose Pablo Marchili

Buenos Aires, Argentina

OBJECTIVES: We have offered cell free fetal DNA (cffDNA) testing in Buenos Aires, Argentina since July 2013. Over 2400 patients were tested. This is an update of the data presented in the last two ISDP's Meetings. METHODS: All samples were shipped to Illumina's CLIA Laboratory in USA to perform the verifi® prenatal test. RESULTS: 2437 cases, 1045 in the last 12 months, 44,13% more over last year (725). Results obtained in 2411 (99,99%). 25 cancelations, only 1 (0.04%) technical. Of 2412 reported cases, 2298 (95,27%) singletons; 114 (4,73%) twins. We tested high and average risk population. Aneuploidy were detected (AD) or suspected (AS) in 84 (3.48%), 75 (3.10%) reported AD and 9 (0.38%) AS. Of the AD cases, 46 T21 (1,91%), 4 T18 (0.17%), 7 T13 (0.29%), 1 T16 (0.04%), 1 T18/MX (0.04%), 7 MX (0.29%), 3 XXX (0.12%) and 1 XXY (0.04%) were reported. 16,84% was low risk . No false negative CONCLUSIONS: This new data confirms the acceptance for this technology from both colleagues and patients. The consistence and performance increased the number of pregnant women which accept it as first line screening for the most prevalent aneuploidies and as a second step ruling out these conditions after an increased risk in a FTCST, positive serum screening or abnormal ultrasound. We expect the number of patients to keep growing as the test becomes more affordable as well as medical education and patient information spreads

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The experience of non-invasive prenatal test in Mexico City; Considering ethical aspects related with genetic counselling

Dora Gilda Mayen, Eva Ramirez, Mariana Hernandez-Gomez, Ricardo Meléndez, Luz Maria Garduño, Antonio Paz-Martinez, Yazmin Lopez-Zavaleta

Genetics Clinic, Hospital Angeles Lomas, Mexico, Huixquilucan, Mexico

OBJECTIVES: Non-invasive prenatal test (NIPT) is employed in the analysis of aneuploidies and fetal sex determination. Massive parallel sequencing (MPSS) detects the origin of each amplified sequence, and analyses over-representation of sequences or any decrease in the fetal chromosomes in maternal plasma. The objective is to present the experience of a prenatal diagnosis center in Mexico, in the use of NIPT and the importance of genetic counselling before and after the test. METHODS: Development of a database of patients to which NIPT was applied from August 2013 to date. RESULTS: We performed genetic counselling in 123 pregnant woman with a gestational age between 10.0 and 18.6 weeks, before the test. We did not performed studies for paternity purposes or microdeletions. Eight of the patients decided to have amniocentesis and another refused the test so there have been 114 patients. We obtained results in 113 pregnant women: 105 low-risk cases and eight with high risk results. Four cases showed high risk for sex chromosomes abnormalities: three X monosomy and one XXY. Two cases showed high risk for trisomy 18 and two high risk for trisomy 21. CONCLUSIONS: There are different and specific ethical considerations in each country, in Mexico the NIPT test is an expensive study for most women so only 10% or less have access to it. We offered genetic counselling in all cases to explain the implications of the NIPT as a screening method and to insist that in the cases of high-risk results, confirmation by an invasive method, before an obstetric decision is indispensable. We are seeing now some of the advantages of the test like the reduction in the number of invasive studies and the risk of fetal loss.

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Genome wide non-invasive prenatal testing – 2,000 samples outcome experience

Jenna Wardrop, Samantha Caldwell, Theresa Boomer, Phillip Cacheris, Ron McCullough

Sequenom Laboratories, San Diego, California, United States

OBJECTIVES: Noninvasive prenatal testing (NIPT) of circulating cell-free DNA (cfDNA) has become part of the standard of care in the screening for fetal aneuploidies in high-risk pregnancies. However, this traditional NIPT analysis has been limited to analysis of chromosomes 13, 18, 21, X and Y. The MaterniT® GENOME test can report on trisomies, monosomies, select microdeletions, as well as genome wide copy number variants larger than 7 Mb. Here we present our experience collecting cytogenetic, molecular and/or birth outcomes on our first 1,997 clinical samples received by the laboratory. METHODS: Maternal blood samples submitted to Sequenom Laboratories® for MaterniT® GENOME testing were subjected to DNA extraction, library preparation, and whole genome massively parallel sequencing as previously described.[1,2] For results reported with a positive screening result, we followed up with the ordering clinician at approximately 22 weeks gestational age to obtain cytogenetic and/or microarray results in addition to any ultrasound abnormalities detected. We also followed up with the ordering clinician at approximately 42 weeks gestational age to obtain birth outcome (clinical exam, cytogenetics, molecular testing, etc.). All outcome information was logged and categorized in a secure database. RESULTS: Of the first 1,997 clinical samples reported with the MaterniT GENOME test, 141 were reported as positive (with follow-up data on over 80%), 1856 as negative. Thirteen discordant positives were identified (0.7% false positive rate) while no discordant negatives were reported. No discordant positives were noted for subchromosomal events; all were noted for whole chromosomal events - Trisomy 3 (1), 7(1), 8(2), 13(1), 15(1), 16(2), 22(2) and monosomy X (3). All but four of the false positive cases were related to mosaic events, had a documented history of vanishing twin or stillbirth, or a maternal abnormality was suspected. CONCLUSIONS: In the first 1997 clinical samples, we observed a high positive predictive value for subchromosomal events as well as the core trisomies (Trisomy 21, 18, 13) with no discordant positives noted for the former and only one for Trisomy 13, in which there was a vanishing twin. Many of the discordant cases involved other autosomes or X chromosome. There are documented biological explanations for discordant cfDNA results, including CPM, co-twin demise, and maternal events. Many of the discordant positive cases in this cohort had clinical findings suggestive of these biological explanations, such as: IUGR, pre-term delivery, and known co-twin demise.

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Implementation of non-invasive prenatal testing (NIPT) for fetal aneuploidy in a UK NHS Regional Genetics Laboratory – the Lucina test

Kirsten McKay Bounford[1], Fiona Togneri[1], Elizabeth Young[2], Samantha Court[1], Rachel Jennings[1], Samuel Clokie[1], Jessica Lander[1], Stephanie Allen[1]

[1]Birmingham Women's and Children's NHS Foundation Trust, Birmingham, United Kingdom
[2]West Midlands Regional Genetics Service, Birmingham, United Kingdom

OBJECTIVES: The UK National Screening Committee has recommended that non-invasive prenatal testing (NIPT) for common aneuploidies is made available to women at high risk following first or second trimester screening. This is to be a modification of the existing NHS Fetal Anomaly Screening Programme. In advance of the anticipated roll-out of NIPT in the NHS, the West Midlands Regional Genetics Laboratory (WMRGL) implemented a privately funded NIPT service in September 2015, using Illumina's Verifi testing method via a technology transfer process. METHODS: The Illumina Verifi technology was selected following a procurement process - the time from technology transfer to implementation was 15 weeks. The technology transfer process involved training from Illumina, validation of the assay using 75 Illumina-provided test plasmas and a competence assessment. Further internal verification was performed using 196 control plasma samples (including 10 trisomy 13, 18 trisomy 18 and 19 trisomy 21 cases). The protocol consisted of plasma separation, cfDNA extraction, library preparation and massively parallel sequencing on a HiSeq 2500. An in-house graphical user interface was developed to display data generated by Illumina's software. RESULTS: Since implementation, 200 patient samples have been tested. There were no technical fails and reportable results were obtained for 199 samples. One case was un-interpretable due to an unexpected chromosomal representation elsewhere in the genome. The NIPT test indicated 8 cases were highly likely to have trisomy 21, 2 cases trisomy 18, and there were no cases of trisomy 13. One sample was reported as having an increased risk of trisomy 21, based on a borderline NIPT result. Screening for monosomy X is also possible using this assay and will be offered to women with indicative ultrasound scan findings. CONCLUSIONS: We have successfully implemented an NIPT assay for common anueploidies using Illumina's Verifi technology – the Lucina test. The assay has proven to be robust, accurate and performs as expected. The experience of provision of a private NIPT service has enabled WMRGL to develop technical expertise, and our referring centres to develop clinical protocols, in advance of the expected roll-out of NIPT in the NHS from April 2018.

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Finger stick self-collection of maternal blood for non-invasive prenatal testing

Haley Milot, Chris Jacob

Gateway Genomics, San Diego, California, United States

OBJECTIVES: Noninvasive prenatal testing (NIPT) is currently limited to maternal venous blood collection by trained phlebotomists, which can restrict accessibility to prenatal screening. We conducted the first evaluation of self-collected capillary blood samples compared with phlebotomist-collected venous blood samples for the determination of fetal sex. METHODS: Pregnant women (9-37 weeks gestation) self-collected capillary blood samples by lancet finger stick. Venipuncture samples were collected from another set of pregnant women to allow for comparison of test results with those obtained from capillary samples. Plasma was separated from whole blood by centrifugation and cell-free DNA was isolated using a commercial DNA extraction kit. Real-time quantitative PCR was performed to detect fetal DNA using a multi-copy sequence on the Y chromosome. An endogenous control gene was used to measure total cell-free DNA (maternal and fetal). Test results for capillary blood samples were compared to venipuncture blood sample results. RESULTS: Cell-free DNA was detected in all capillary and venous blood samples. Y-chromosome specific sequences were detected in capillary and venous blood samples in all pregnancies confirmed to have a male fetus. All gender results were in concordance with known fetal sex, without false-positive or false-negative results. For fetal sex, self-collected capillary blood samples and venous blood samples from pregnant women produced a sensitivity of 100%, specificity of 100% and accuracy of 100%. CONCLUSIONS: Prenatal diagnosis of fetal gender from self-collected capillary blood is simple, accurate, and reliable. The results of this study demonstrate that fetal DNA analysis using self-collected capillary blood is highly feasible and easily adaptable for population screening. This method simplifies blood collection of maternal blood and should increase the accessibility of noninvasive prenatal testing.

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Noninvasive prenatal screening for fetal aneuploidy from cell free DNA in a general obstetrical population: A clinical laboratory experience

Tessa Niemchak, Spencer Fuller, Lindsay Dohany, Christina Settler, Thomas Monroe

Progenity, Ann Arbor, Michigan, United States

OBJECTIVES: In 2015, the Progenity CLIA laboratory validated noninvasive prenatal cell-free DNA screening for chromosomal aneuploidy and began accepting clinical samples. Here we summarize the clinical laboratory performance in >100,000 cases representing a general obstetric population. METHODS: A retrospective analysis was performed for all samples with cell-free DNA screening ordered. The test screens for aneuploidy in chromosomes 13, 18, and 21 for all samples, and for sex chromosome aneuploidy except when excluded by the ordering provider (opt-out). As part of routine laboratory quality assurance, pregnancy outcome data is collected for cases classified as “Aneuploidy Detected” and “Aneuploidy Suspected” through a faxed request to the ordering provider sent 8 weeks after sample submission. Data is also collected for failed analyses and for 5% of negative results following expected delivery date. RESULTS: During the study period >100,000 specimens were received. The mean maternal age was 30 years (range 12-71 years). Overall positivity rate was 1.8%. Follow-up clinical information was received for 58% of positive cases, 37% of failed analyses, and 45% of requested negative cases. Confirmatory chromosome analysis was performed for 47% of patients with results positive for an autosomal trisomy (observed frequency of false positives 0.06%) and for 31% of patients whose results were positive for sex chromosome aneuploidy (observed frequency of false positives 0.1%). Sex chromosome discordance was reported in 0.05% of samples. Observed frequency of false negatives was 0.007%. CONCLUSIONS: Our clinical experience suggests that the noninvasive prenatal cell-free DNA screening by Progenity test performs within the parameters established by validation studies. Reported false positives remain low despite testing in a general obstetric population.

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A direct from plasma approach for non-invasive fetal RhesusD genotyping

Michael Parks

Nonacus Ltd, Birmingham, United Kingdom

OBJECTIVES: Cell free fetal DNA (cffDNA) analysis by quantitative PCR (qPCR) is commonly used in many northern European countries to perform non-invasive prenatal diagnosis of fetal RhesusD (RhD) status in pregnant women at risk of developing Hemolytic Disease of the Fetus and Newborn. Although the cost of this method is relatively low and requires short processing times, additional time and cost could be saved by eliminating the cffDNA extraction process, which is currently necessary. In this study we demonstrate how this is achievable by performing qPCR directly from plasma. METHODS: Plasma from 100 RhD (-) pregnant women was prepared for qPCR using Cell3^{TM}Direct technology (Nonacus Ltd) and tested for fetal RhD status by targeting exons 5, 7 and 10 of the RhD gene. CffDNA was extracted from 15 of these samples and tested using the same approach. Initial assay evaluation was performed using samples artificially obtained by mixing RhD (+) genomic DNA or gBlock gene fragments with RhD (-) genomic DNA. RESULTS: Testing on artificially mixed samples proved that the multi-target qPCR approach could detect down to 3 genomic equivalents of RhD gene copies; and could discriminate between the RhD wild type and RhD-PSI variant. Validation on 100 clinical plasma samples yielded highly accurate and consistent results, with sensitivity and specificity of 100%; and an overall repeat and inconclusive rates of 4% and 2% respectively. The turn-around-time from plasma to report was 3 hours with an estimated hands-on time of 30 minutes. Testing on 15 cffDNA samples also proved that the same assay could be used to perform testing on extracted cffDNA. CONCLUSIONS: Use of a multi-target qPCR approach coupled with direct from plasma testing delivers highly accurate non-invasive diagnosis of fetal RhD status, while reducing time and cost spent on cffDNA extraction procedures.

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Non-invasive fetal platelet genotyping to manage high risk pregnancy of FNAIT: A large cohort study

Yasmine Mammasse, Christophe Chenet, Corinne Martageix, Rachel Petermann

National Institute of Blood Transfusion, Paris, France

OBJECTIVES: Fetal and neonatal alloimmune thrombocytopenias (FNAIT) are caused by maternal antibodies against specific fetal platelet antigens inherited from the father and different from maternal antigens. It occurs at an estimated frequency of 1 in 1000 live births in Caucasians and is the commonest cause of severe isolated thrombocytopenia in the fetus and newborn. The most severe consequence of FNAIT is intracranial hemorrhage, leading to death or neurologic sequelae in approximately 10% of symptomatic FNAIT cases. Currently, fetal platelet genotyping is performed using invasive procedures with a risk of bleeding and miscarriage. METHODS: We developed a new method for non-invasive fetal platelet genotyping using droplet digital PCR (ddPCR). Four platelet antigen systems HPA-1, -3, -5 and -15 were amplified, these systems are implicated in more than 95% of FNAIT and for some of them, serological tests failed to detect alloantibodies. Fetal platelet genotyping was performed on cell free DNA extract from plasma samples collected from 38 women (6-35 WG) carrying fetus at risk of FNAIT. To exclude false-negative results caused by the lack of fetal DNA in maternal plasma, the methylation status of *RASSFA1* gene promotor was used as an internal control. RESULTS: Results showed that 9/38 (24%) pregnant women were compatible with their fetus, 17/38 (45%) were incompatible in one HPA system including 7 cases incompatible in only HPA-1, 7/38 (18%) in two HPA systems and 5/38 (13%) in three combined HPA systems. Predicted fetal HPA genotypes by ddPCR were already confirmed in 24/38 (63%) FNAIT cases either by HPA genotyping performed on amniocentesis or by blood platelet phenotyping after birth. Fetal DNA fraction in maternal plasma was estimated in all samples by using a reliable marker; it ranged from 0.5 to 26% of maternal circulating DNA and increased with gestational age. CONCLUSIONS: This study strongly suggests that non-invasive fetal HPA genotyping using ddPCR appears as a safe and reliable method devoid of risk for the fetus. This technique allows early diagnosis of feto-maternal platelet incompatibility and could be implemented in routine clinical testing because of its high sensibility and specificity. Thus, non-invasive platelet genotyping appears attractive for clinical and therapeutic patient management. Indeed, it may provide a pregnancy risk evaluation, facilitate availability of appropriate phenotyped platelet concentrates at birth, and help preventing complications and unnecessary interventions such as IVIg implementation or serological flow-up during pregnancy in the absence of incompatibility.

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Cost-effectiveness study of prenatal diagnosis after a positive first trimester screening in a developing country

Florencia Petracchi[1], Laura Igarzabal[1], Maria Cecilia Paez[2], Silvina Sisterna[2]

[1]*CEMIC, Buenos Aires, Argentina*
[2]*Sección Genética CEMIC Centro de Educación Medica e Investigaciones Clínicas, Buenos Aires, Argentina*

OBJECTIVES: Non-invasive prenatal testing (NIPT) is currently being offered in Argentina. The samples are sent abroad and payed by patients out of the pocket. The actual cost of NIPT is higher than a monthly universal basic income. First trimester screening and diagnosis are more easily covered by the health care system. The consequences of implementing NIPT in developing countries is required. Objective To perform a cost-effective analysis comparing two different strategies after a positive First Trimester Screening: 1) prenatal invasive diagnosis or 2) NIPT and if positive, follow up with prenatal invasive diagnosis METHODS: A decision-analytic approach modelled a theoretical cohort of 100,000 singleton pregnancies with a positive first trimester screening. The strategies considered were 1) *Standard practice: prenatal invasive diagnosis* and 2) C*ontingent strategy: NIPT and if positive, follow up with prenatal invasive diagnosis.* The number of Down Syndrome (DS) cases detected, procedure-related losses and costs were compared between strategies. The incremental cost per case detected was the primary measure of cost-effectiveness. Two prevalence populations were considered: a cohort with a low prevalence of DS (1:1000 births) and another cohort with a high prevalence (1:100). RESULTS: After a positive first trimester screening the standard practice with prenatal invasive diagnosis resulted in a cost saving strategy compared to contingent NIPT. Per patient diagnosed, this strategy would save from USD 645,476.2 to USD 5,124,242 respectively at high and low prevalence of DS according to maternal age. The contingent strategy with NIPT would have avoided 25 procedure- related losses per 100,000 fetuses. CONCLUSIONS: Prenatal invasive diagnosis after a positive screening for DS was a cost-saving strategy. Contingent NIPT after a positive FTS is still very expensive for a developing country, even in the most favourable scenario with patients 40 years and older. It should be taken into consideration the number of procedure related losses prevented with the NIPT strategy.

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Positive NIPT: An indicator for complex genetic findings

Lynne Rosenblum[1], Natalia Leach[1], Hongli Zhan[2], Stuart Schwartz[2]

[1]*Integrated Genetics, Westborough, Massachusetts, United States*
[2]*Integrated Genetics, Research Triangle Park, North Carolina, United States*

OBJECTIVES: The majority of current non-invasive prenatal testing (NIPT) utilizes next-generation sequence analysis of cell-free fetal DNA in maternal circulation to identify fetal aneuploidy risk for chromosomes 13, 18, 21, X, and Y. More extensive NIPT screening is available for additional aneuploidies and microdeletions, as well as any abnormality in the entire genome (> 7 Mb), but the basic aneuploidy panel can also provide the initial indicator of more complex genetic findings. Here we report three cases in which NIPT positive screening for the basic aneuploidies led to the identification of chromosome abnormalities and/or genetic disorders other simple aneuploidy. METHODS: Our laboratory received amniotic fluid specimens from three patients with positive NIPT results as the primary clinical indication for diagnostic testing. Case 1 was NIPT positive for monosomy X, Case 2 for trisomy 18, and Case 3 for monosomy X. The patients were 27, 28, and 29 years old, respectively. In the first two cases, abnormal ultrasound findings were noted at the time of invasive testing. In each case aneuploidy FISH (chromosomes 13, 18, 21, X, Y) and chromosome analyses were performed. Additionally, cases 1 and 3 had chromosome microarray (CMA) analysis and case 2 had methylation testing. RESULTS: Case 1: FISH was uninformative due to an unclear sex chromosome result and the karyotype was 45,X[11]/46,X,idic(Y)(q11.2)[4]. The CMA result showed a mosaic 22.05 MB gain of Ypter->q11.222; 37.33 MB terminal deletion of Yq11.221->qter. Case 2: FISH was consistent with trisomy 18 in a male. The karyotype was 46,XY,-15,+der(18)t(15;18)(q14;q21.1). Methylation studies were positive for Angelman syndrome. Case 3: FISH showed a normal male result. The karyotype was 46,X,del(Y)(q11.22) and the CMA result was 2.69 MB terminal gain of Xpter->Xp22.33; 5.80 MB interstitial duplication of Xp22.33->p22.31; 43.2 MB terminal deletion of Yq11.221->qter. CONCLUSIONS: These cases illustrate situations where positive NIPT screening for the common aneuploidies ultimately led to the diagnosis of a Y chromosome rearrangement, a derivative chromosome causing partial trisomy for chromosome 18 and partial deletion of 15q resulting in Angelman syndrome, and a derivative Y resulting from translocation involving the X and Y chromosomes. Although such complex genetic findings might not be anticipated following positive NIPT screening for common aneuploidies, we demonstrate that it may lead to the detection of unexpected genetic abnormalities that require diagnostic follow-up and genetic counseling.

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Current status of noninvasive prenatal testing in Japan: Three year experience

Osamu Samura[1], Akihiko Sekizawa[2], Tatsuko Hirose[2], Nobuhiro Suzumori[3], Takahiro Yamada[4], Kiyonori Miura[5], Hideaki Sawai[6], Fumiki Hirahara[7], Haruhiko Sago[8], Japan NIPT consortium[9]

[1]*Department of Obstetrics and Gynecology, The Jikei University School of Medicine, Tokyo, Japan*
[2]*Showa University, Tokyo, Japan*
[3]*Nagoya City University Hospital, Nagoya, Aichi, Japan*
[4]*Hokkaido University Graduate School of Medicine, Sapporo, Japan*
[5]*Nagasaki University, Nagasaki, Japan*
[6]*Hyogo College of Medicine, Hyogo, Japan*
[7]*Yokohama City University School of Medicine, Yokohama, Japan*
[8]*National Center for Child Health and Development, Tokyo, Japan*
[9]*Japan NIPT Consortium, Minato-ku, Tokyo, Japan*

OBJECTIVES: The purpose of this study was to report the 3-year experience of a nationwide demonstration project to introduce noninvasive prenatal testing (NIPT) of maternal plasma for aneuploidy, and review the current status of NIPT in Japan**.** METHODS: Tests were conducted to detect aneuploidy in high-risk pregnant women, and adequate genetic counseling was provided. The clinical data, test results, and pregnancy outcomes were recorded. We discuss the problems of NIPT on the basis of published reports and meta-analyses. RESULTS: From April 2013 to March 2016, 30,615 tests have been conducted at 55 medical sites participating in a multicenter clinical study. Among the 30,615 women tested, 554 were positive (1.81%) and 30,021 were negative (98.1%) for aneuploidies. The implementation status of examinees who received a positive test result was as follows: 279 (96.5%), 106 (82.8%), and 28 (63.6%) of the 289, 128, and 44 women who tested positive for trisomies 21, 18, and 13, respectively, and underwent definitive testing, were determined as true positive. Of the 13,481 negative cases where the woman's progress could be traced, 2 were false negatives. CONCLUSIONS: We described the 3-year nationwide experience with NIPT in Japan. It is important to establish a genetic counseling system to enable women to make informed decisions regarding prenatal testing.

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When should NIPT be carried out after the demise of a twin, and could discordant follow up NIPT results be an indication for undetected vanishing twin?

Kathryn Sanders, Leoni Xanthopoulou, Matthew Wyatt, Anastasios Kotzadamis, Stelia Pissaridou, Aleksandra Golubeva, Sophie Billington, Tony Gordon

Genesis Genetics, London, United Kingdom

OBJECTIVES: Best practice guidelines for Non Invasive Prenatal Testing (NIPT) in cases of a vanishing twin previously involved delaying the blood draw by 4 weeks from the last ultrasound evidence of the vanished twin. However, due to limited data in this area there is debate that this guideline should be extended. We observed two confirmed cases of vanishing twin where initial NIPT result indicated aneuploidy (Trisomy 13 and Trisomy 18), subsequent NIPT result presented euploid results. We discuss patient management and counselling when a vanishing twin event has occurred and implication on producing a false positive result. METHODS: NIPT was performed using the Genesis Serenity test, powered by the Illumina verifi technology, which involves whole genome massive parallel sequencing of cell free fetal DNA in the maternal circulation to detecting aneuploidies involving chromosomes 13, 18, 21, X and Y. For singleton pregnancies patients can also opt in to have gender disclosed. RESULTS: Vanishing twin case 1, the original blood draw from this patient was at 13 weeks 1 day of gestation (4 weeks post co-twin demise), the result indicated suspected trisomy 18, second blood draw was at 15 weeks gestation (6 weeks post co-twin demise), producing a euploid result. Vanishing twin case 2, the original blood draw from this patient was at 13 weeks 6 days of gestation (5 weeks post co-twin demise), the result was consistent with trisomy 13, second blood draw was at 17 weeks 4 days of gestation (9 weeks post co-twin demise), producing a euploid result. Discordant result. CONCLUSIONS: The results seen in the two cases of twin demise show that previous guidelines regarding vanishing twins may not be extensive enough. 4 weeks is not long enough to ensure that the placenta of the demised twin is no longer contributing to the cell free DNA in maternal plasma. It is logical to conclude that the demise of the twin is because of the aneuploidy that is detected in these first blood draws. Further investigation needs to be carried out on vanishing twin cases to detect the rate at which cell free DNA from the demised twin leaves the maternal

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A case of trisomy 21 followed by NIPT false negative by placental chromosomal mosaicism

Hideaki Sawai, Mariko Kamihigashi, Tomoko Ueda, Mina Kashima, Chiho Okada, Hiroaki Shibahara

Hyogo College of Medicine, Nishinomiya-shi, Hyogo, Japan

OBJECTIVES: We report here a case of trisomy 21 followed by NIPT false negative by placental chromosomal mosaicism. METHODS: A 39 year old pregnant woman, primi-gravida, had prenatal diagnosis by NIPT. She had anxiety for chromosome abnormality of her fetus because of advanced maternal agen and IVV pregnancy. She had NIPT, MateriniT 21, Sequenom at 13 weeks and 4 days gestation. The result led to negative for trisomy 13, 18 and 21 after 18 days. The pregnant course was good through second trimester. However in the third trimester, amnionic fluid decreased and presented fetal growth restriction. In 40 weeks gestation, emergency cesarean section was performed because of fetal dysfunction. The newborn was 2648g (-1.3SD), female, Apgar score 8/9. RESULTS: The newborn was suspected as trisomy 21 by her appearance: a small chin, slanted eyes, poor muscle tone, a flat nasal bridge, a single crease of the palm. Later she was found having PDA and ASD. Chromosome testing was performed and trisomy 21 was found. Not only blood sampling but bucchal mucous were tested for chromosome testing, because of negative NIPT result. No mosaic chromosome abnormality trisomy 21 were shown. Placental chromosome was tested by SNP array, then shown to be mosaicism, approximately 30 % was trisomy 21 and 70 % was normal. CONCLUSIONS: We had experienced a case of trisomy 21 followed by NIPT false negative by placental chromosomal mosaicism. Placental mosaicism had been frequently reported, but many of cases are chromosome abnormalities in placenta and normal in fetus. However opposite case like this case may cause mis-diagnosis by NIPT.

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Correlation between maternal BHCG and cell free DNA fetal fraction in choriocarcinoma

Nancy Soliman[1], Jill Nation[1], Philip Wyatt[2], Hilary Racher[3], Jo-Ann Johnson[1]

[1]*University of Calgary, Calgary, Alberta, Canada*
[2]*Consultant in Laboratory and Clinical Genetics, Toronto, Ontario, Canada*
[3]*Scientific and Laboratory Director, Impact Genetics, Bowmanville, Ontario, Canada*

OBJECTIVES: The rapid uptake of noninvasive screening based on cell free DNA (cfDNA) in the maternal circulation has resulted in new information on the relationship between cfDNA and maternal health, including malignancy. We present a case of choriocarcinoma where serial beta human chorionic gonadotropin (BHCG) levels correlated with plasma cfDNA fetal fraction (FF) levels. METHODS: Case report: A 33 year old Gravida 2 Para 1 Spontaneous Abortion 1 woman presented 4 weeks postpartum after a normal term vaginal delivery with vaginal bleeding. The findings of a markedly vascular, hypoechoic mass in the myometrium (3.3 x 3.0 x 3.5 cm) combined with elevated serial quantitative beta human chorionic gonadotropin (BHCG) levels (59,863 IU/L, 77,000 IU/L/ 48 hours) and multiple pulmonary nodules on computerized tomography (CT) scan confirmed FIGO stage 3 choriocarcinoma (WHO score 6). At diagnosis (Time 1), she consented to serial blood samples to be analyzed for cfDNA FF in addition to BHCG. RESULTS: Time 1: BHCG 59,863 IU/L, FF 35%, Time 2 (post-Methotrexate X 4 weeks): BHCG 3094 IU/L, FF 0.05 %, Time 3: (post-Methotrexate X 8 weeks) BHCG 166915 IU/L, FF 1.3 %, Time 4: (2nd line chemotherapy, 4 months) BHCG 50 IU/L, FF 1.6 %, Time 5: (hysterectomy, 3rd line chemotherapy) BHCG negative, FF 0.5%. (“noise”). CONCLUSIONS: This the first report of choriocarcinoma associated with elevated cell free DNA fetal fraction levels. While further study is needed, the close correlation seen between rising and falling BHG and cfDNA FF levels in this case has promising implications for diagnosis and surveillance of this disease.

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Clinical accuracy of a novel NIPT assay using whole-genome paired-end sequencing and a proprietary algorithm to maximize reporting of common fetal aneuploidies

Amy Swanson[1], Kristen Meier[1], Darcy Vavrek[1], Cosmin Deciu[1], Sven Duenwald[1], Meredith Halks-Miller[2]

[1]*Illumina, Inc., San Diego, California, United States*
[2]*Illumina, Inc., Redwood City, California, United States*

OBJECTIVES: To determine the clinical accuracy of a novel, automated noninvasive prenatal test (NIPT) solution that requires reduced sequencing depth and incorporates coverage data and estimated fetal fraction (FF) to detect common fetal aneuploidies. METHODS: An NIPT assay was developed using whole-genome paired-end sequencing (sequencing both ends of cfDNA fragments) to determine fragment size allowing differentiation between maternal and fetal fragments (fetal fragments are typically shorter). An algorithm was developed incorporating fragment size, estimated FF, and coverage data to determine likelihood of aneuploidy. Clinical accuracy of this assay was determined using samples from women ≥18 years of age with singleton pregnancies (gestational age 10–35 weeks) who previously underwent NIPT at the Illumina CLIA Laboratory. Samples were re-blinded prior to testing; results were compared to known clinical outcomes (cytogenetic results or physical exam). RESULTS: Primary analysis of 3,057 samples for trisomy 21, trisomy 18, and trisomy 13 revealed sensitivities of 98.9% (90/91), 90.0% (18/20), and 100% (8/8), respectively, with specificities all ≥99.9%. Results were provided for >99% of samples tested; the quality control failure rate was 0.68% (21/3107). Secondary analysis for sex chromosome classification was performed on 3,082 samples. Concordance for sex chromosome aneuploidies (monosomy X, XXX, XXY and XYY) ranged from 80.0–100%. CONCLUSIONS: A novel NIPT solution, the VeriSeq NIPT Solution, was developed based on whole-genome paired-end sequencing and a proprietary algorithm to detect common fetal aneuploidies. A clinical accuracy study of more than 3,000 maternal plasma samples found this solution had high sensitivity and low false-positive rates for trisomies 21, 18 and 13. The study also reported a low test failure rate. In addition to high performance, laboratories and clinicians using the VeriSeq NIPT Solution will likely realize reduced costs and faster turnaround times due to the reduced amount of sequencing required.

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Positive predictive value (PPV) estimates in SNP-based non-invasive prenatal screening for microdeletions for unselected populations

Elizabeth Valentini[1], Sushma Iyengar[1], Zachary Demko[1], Kimberly Martin[1], Peter Benn[2]

[1]*Natera Inc, San Carlos, California, United States*
[2]*University of Connecticut Health Center, Farmington, Connecticut United States*

OBJECTIVES: The ability to provide effective screening using a single-nucleotide polymorphism (SNP)-based noninvasive prenatal testing (NIPT) for select microdeletions (22q11.2, 1p36, cri-du chat, Prader-Willi, and Angelman deletion syndromes), has recently been demonstrated for a high-risk test referral population. (Gross et al., USOG 2016;47:177. Martin et al., submitted). The objective of this study was to model the expected performance of the SNP-based NIPT methodology for microdeletions in a general patient population, assuming a consistent test sensitivity and specificity. METHODS: Based on 74,938 screening tests performed for 22q11.2 and 39,678 for 1p36, cri-du-chat, Prader-Willi, and Angelman microdeletion syndromes, PPVs were determined based on the formula: PPV = True positives / (True Positives + False Positives). Prevalence for each condition in the population screened was estimated based on the observed number of True Positives, False-Negatives, the expected number of True Positives in cases without follow-up information, and the proportion of affected cases not detectable by the test. From these observed PPVs and prevalence rates, the test likelihood ratios were calculated and these were then applied to general pregnancy population prevalence rates. RESULTS: Table 1 summarizes the observed PPVs and prevalence rates in the referral population together with the modeled PPVs expected for commonly accepted general population prevalence rates. Data is presented for two screening approaches, an original protocol used at the time of screening and an enhanced protocol that involved reflex high-depth resequencing (Martin et al., submitted). For the revised protocol, a general population PPV of 33% was estimated for 22q11.2, and a PPV of 24% was estimated for the other four microdeletions combined. CONCLUSIONS: As expected, PPVs are lower when the screening is applied to general populations with lower prevalence rates. Additionally, because of their rarity, PPVs for individual microdeletions cannot be expected to reach that of trisomy 21. However, the adjusted general population PPVs still compare favorably with those seen in traditional prenatal screening tests. We conclude that this screening is effective for a general population.

	Test referral population			General population		
		Observed PPV			Expected PPV	
Microdeletion syndrome	Observed Prevalence	Original Protocol	Revised Protocol	Population prevalence	Original Protocol	Revised Protocol
22q11.2	1/1,255	15.7%	44.2%	1/2,000	10.5%	33.2%

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1p36	1/4,250	20.0%	50.0%	1/5,000	17.5%	45.9%
Cri-du-chat	1/3,624	8.9%	66.7%	1/20,000	1.7%	26.6%
PWS	-	-		1/10,000	-	-
AS	1/5,820	1.5%	10.0%	1/12,000	0.7%	5.1%
All 5 microdeletions	1/676	9.3%	38.8%	1/1,071	6.1%	28.5%

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Prenatal screening for 22q11.2 deletions using a targeted microarray-based cell-free DNA (cfDNA) test

Maximilian Schmid[1], Eric Wang[1], Patrick Bogard[1], Jacob Zahn[1], Coleen Hacker[1], Susie Wang[1], Jigna Doshi[2], Karen White[1], Jennifer Kaplan[1], Andrew Sparks[1], Jacques Jani[3], Renee Stokowski[1]

[1]Ariosa Diagnostics Inc., Roche Sequencing Solutions Inc., San Jose, California, United States
[2]Roche Sequencing Solutions Inc., San Jose, California, United States
[3]University Hospital Brugmann, Université Libre de Bruxelles, Brussels, Belgium

OBJECTIVES: Cell-free DNA (cfDNA) testing provides high sensitivity for common trisomies and a lower false positive rate than traditional prenatal screening methods. Recently, the scope of testing has expanded to include subchromosomal deletions. Here we aim to determine the performance of a targeted microarray-based cfDNA test (Harmony Prenatal Test®) to screen for pregnancies at increased risk for a 22q11.2 deletion. METHODS: Test performance was determined in two steps including a total of 1,953 plasma samples. Analytical validation was performed in 1736 plasma samples. Clinical verification of performance was performed in an additional 217 prospectively ascertained samples from pregnancies with fetal deletion status determined by diagnostic testing. RESULTS: Analytical sensitivity was 75.4% (95% CI: 67.1-82.2%) based on 122 samples with deletions ranging from 1.96 to 3.25 Mb. In 1614 presumed unaffected samples, specificity was determined to be at least 99.5% (95% CI: 99.0-99.7%). In the clinical cohort, 5 of 7 samples from pregnancies affected with 22q11.2 deletion were determined to have a high probability of deletion. There were no false positive results in the 210 unaffected samples in this cohort. This clinical data is consistent with the performance demonstrated in the analytical validation. CONCLUSIONS: Cell-free DNA testing using targeted microarray quantitation is able to identify pregnancies at increased risk for 22q11.2 deletions of 3.0 Mb and smaller while maintaining a low false positive rate.

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CLIA laboratory experience with over 330,000 samples of non-invasive prenatal testing (NIPT) using a targeted microarray-based cell-free (cfDNA) test for fetal trisomy

Brittany Liedstrand, Karen White, Rhoberta Steeke, Juan Acuna, Junaid Shabbeer, Maximilian Schmid

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

OBJECTIVES: To describe the experience with a targeted microarray-based cfDNA test (Harmony Prenatal Test®) for fetal trisomy in a large CLIA-certified laboratory. METHODS: Targeted cfDNA analysis using DANSR™ and FORTE™ with microarray quantitation was used to evaluate the probability of trisomy 21, 18, and 13 in the Ariosa CLIA laboratory (San Jose, CA, USA). The laboratory performs active follow-up of clinical and diagnostic information for patients with high-probability results. In the present study prospectively collected data on 338,365 reported samples was reviewed. RESULTS: Of 338,365 samples reported, 1.2% received a high-probability result for trisomy 21, 18, or 13. In the entire cohort, mean maternal age was 32.7 years; mean gestational age was 13.8 weeks. 2.5% of samples were from twin pregnancies and 6.4% were from IVF pregnancies. There were 3,958 high-probability results: 3,102 for trisomy 21, 677 for trisomy 18, and 179 for trisomy 13. 1,612 cases were eligible for follow-up. In 655 cases with diagnostic information, 97.0% of trisomy 21, 85.5% of trisomy 18, and 34.1% of trisomy 13 cases were confirmed. 342 cases had other outcome information without karyotype confirmation. CONCLUSIONS: This study complements previously published validation studies that demonstrated high specificity for assessment of fetal aneuploidy using a targeted cfDNA test with microarray quantitation. High positive predictive values for trisomy 21 and trisomy 18 were observed. A lower proportion of confirmed trisomy 13 cases was not unexpected given the lower prevalence of this condition.

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Non invasive prenatal screening: Cautionary tales from the first 1000 cases in a tertiary care centre

Stephanie Winsor[1], Madeline Vandersluis[2], Avi Vandersluis[1], Clara Sacchini[1], Laura Wallace[2], Sarah McDonald[1]

[1]*McMaster University Medical Centre, Hamilton, Ontario, Canada*
[2]*Hamilton Health Sciences, Hamilton, Ontario, Canada*

OBJECTIVES: To share our learning experiences and challenges with the first 1000 NIPS tests performed at our institution. We provide a framework for the interpretation of the NIPS results taking into account the apriori risk and the fetal fraction. We present cautionary tales of "no call" reports, false positive and false negative cases. Protocols for “no call” and low fetal fractions cases are proposed. METHODS: A prospective database was maintained of all consecutive NIPS tests ordered at our institution. The first 1000 cases were collected from March 2013 to January 2016. The following information was analysed: indication for NIPS, “no call” results, false positive and false negative cases. The "no call" results were analyzed for redraw, redraw failure rate and invasive testing rates. Findings are used to formalize counseling and management protocols. RESULTS: The most common indications for NIPS were positive IPS/FTS or MSS. (34.8 %), LMA (≥40)(15.8 %), and isolated fetal anomaly (11.7%).There were 6 false positive cases (0.6%) including 2 for Trisomy 21 (0.2%, 1/500. We also had two false positives for Monosomy X, one for triploidy, and one for Angelman Syndrome. There were 2 false negative for Trisomy 21 (0.2 %, 1/500). We had 31 “no call” reports (3.1 %), subsequent redraw rate was 29 % and redraw failure rate was 44 %. Invasive testing was done in 22 % of these. CONCLUSIONS: NIPS has a high detection rates for trisomy 21, 18 and 13, however, false positives and false negatives still exist. NIPS results need to be interpreted taking into account the a-priori risk and the fetal fraction. “No call” results require urgent genetic counseling due to the high risk for aneuploidy in these cases. Ultrasound can play an important role in the management of the low fetal fraction or no results cases, especially when parents opt against invasive testing.

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Validation of a paired-end sequencing-based NIPT platform for clinical use

Matthew Wyatt, Anastasios Kotzadamis, Leoni Xanthopoulou, Stelia Pissaridou, Aleksandra Golubeva, Evangelia Bakosi, Tony Gordon

Genesis Genetics, London, United Kingdom

OBJECTIVES: We sought to validate the performance of a new PCR-free, paired-end (PE) sequencing-based non-invasive prenatal testing (NIPT) assay for clinical use by comparing the results with a previously validated single-end (SE) sequencing-based assay. METHODS: In the standard practice of NIPT, several aliquots of plasma are taken from each patient blood sample, typically with one sample tested and the others stored at -80c. In order to test the new PE NIPT assay, we performed a retrospective analysis using aliquots of plasma obtained from pregnant women tested previously using the SE assay. Our validation cohort consisted of 282 previously tested samples, containing a mix of euploid and aneuploid samples. PE samples were tested in accordance with supplier recommendations, and the results obtained were compared with SE results for concordance. RESULTS: Our validation cohort (N=282) included 36 detected T21, 15 detected T18, 2 suspected T18, 8 detected T13, 3 Suspected T13 samples (SE results). Of the samples tested, 3 samples failed QC, and with no further aliquots available, were excluded from further analysis. Of the non-excluded samples, the results for all euploid samples and detected trisomy samples were concordant. The five samples reported as suspected trisomies all showed reduction of trisomy scores in the PE assay to below the suspected-trisomy calling threshold, due to increased specificity of the PE assay. Collection of clinical outcome data is ongoing. CONCLUSIONS: The PE results showed strong concordance with SE results, in both aneuploidy calling and in raw data values, with no significant difference between the two assays in either euploid or detected trisomy samples. The assay was designed to improve specificity and further reduce incidence of false positives, and assay performance in the suspected trisomy samples supports such a conclusion. The results obtained here indicate that the new PE assay performs at least as well as the previously validated SE assay, and support introduction of the PE assay into regular usage.

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Examining the stability of blood samples used in NIPT: A pilot study

Anastasios Kotzadamis, Matthew Wyatt, Evangelia Bakosi, Stelia Pissaridou, Sophie Billington, Aleksandra Golubeva, Leoni Xanthopoulou, Tony Gordon

Genesis Genetics, London, United Kingdom

OBJECTIVES: Some NIPT providers require that blood samples be received no later than 5 days post-draw, else they are rejected citing samples stability concerns. This can cause an increase in administrative sample rejection due to long shipping times, particularly in samples undergoing international shipment. Streck tubes are commonly used for collection and transport of NIPT samples, and Streck advertises a two-week sample stability at room temperature. We sought to assess a small batch of samples for stability/suitability for testing beyond the 5-day recommendation as proof-of concept for a larger study examining longer-term sample stability. METHODS: Blood samples were split into three aliquots upon receipt, and assigned to each of three groups: the first was processed immediately (<5 days post-draw), the second to be processed 9-11 days post-draw, and the third to be processed 12-14 days post-draw. Each of these samples was processed using our standard NIPT protocol, and results, including yield, sequencing quality, foetal fraction, and aneuploidy status, were then compared and assessed. All samples were stored at room temperature. RESULTS: All of the samples were able to be processed successfully, with no samples haemolysing. We also saw no significant difference between resulting library concentrations, no significant difference in sequencing quality, and full concordance between results of all time-points tested. CONCLUSIONS: The initial cohort tested here (11 samples at time of abstract submission) is too small to draw any meaningful conclusions regarding policy change, but supports the undertaking of a larger study. Should a larger study of sufficient sample number demonstrate no significant difference in data quality over a 14-day period, acceptance guidelines can then be updated to reflect this. Such a change would relax shipping requirements and reduce the number of administrative sample rejections, thereby reducing unnecessary redraws.

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Implications for management and counseling with noninvasive prenatal testing

Juan Xiao, Suhua Chen

Department of Obstetrics & Gynecology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

OBJECTIVES: This study aimed to summarize the clinical information of noninvasive prenatal testing (NIPT) on fetal aneuploidy at chromosomes 13, 18, 21, X, and Y, and aneuploidy at other chromosomes and genome-wide copy number variants. It is also to demonstrate the management and counseling in further prenatal diagnosis. METHODS: A total of 9050 patients performed NIPT in this study during a time period from May 2015 to October 2016 in Tongji hospital in Middle China. Among the total patient population, 103 patients showed a positive result from NIPT of fetal aneuploidy at chromosomes 13, 18, 21, X, and Y, besides 24 patients with aneuploidy at other chromosomes and genome-wide copy number variants were detected. Clinical outcome information was reviewed according to prenatal diagnosis. RESULTS: From 103 patients with aneuploidy at chromosomes 13,18,21,XY, 58.25%(60/103) were T21, 16.5%(17/103) were T18, 9.7%(10/103) were T13, and 15.55%(16/203) XY. Of these cases, 58.3%(35/60) of T21 were found from invasive prenatal diagnosis, 48.3% (29/60) were to confirm positive results, all of them performed induced labor, 10%(6/60) after amniocentesis showed false positive results, three gave birth to normal neonates, and other 3 are still in pregnancy. The rest 25 of 60, eighteen did abortion without counseling or invasive prenatal diagnosis, five rejected to contact, one insisted to give birth and had a healthy boy and a girl with congenital heart disease. CONCLUSIONS: This study has demonstrated that NIPT can provide specific detection of a wide range of chromosomal abnormalities. However, the loss of follow up and counseling is a critical hidden danger in prenatal diagnosis.

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Noninvasive prenatal diagnosis of achondroplasia and thanatophoric dysplasia using multiplex PCR-based targeted sequencing in plasma cell free DNA

Yifan Xie[1], Yuan Ren[2], Jia Zhao[1], Ruibing Li[2], Lin Yang[3], Wei Wang[3], Ya Gao[3], Yanping Lu[2]

[1]BGI Education Center, University of Chinese Academy of Sciences, Shenzhen, China
[2]Department of Obstetrics and Gynecology, Chinese PLA General Hospital, Beijing, China
[3]BGI, Shenzhen, China

OBJECTIVES: Mutations in the gene *fibroblast growth factor receptor-3* (FGFR3) on chromosome 4p16.3 are known to cause several autosomal dominant human diseases, mainly achondroplasia (ACH) and thanatophoric dysplasia (TD). Conventionally, prenatal diagnosis of these diseases relies on genetic testing of samples obtained by amniocentesis or other invasive procedures. The recent advance of noninvasive prenatal diagnosis (NIPD) using plasma cell-free DNA (cfDNA) provides an opportunity to detect the diseases in a safer and earlier manner. In this study, we aimed to develop and validate a novel NIPD method to detect ACH and TD, using multiplex PCR-based targeted sequencing of plasma cfDNA. METHODS: We designed 10 pairs of PCR primers targeting 19 FGFR3 loci causing ACH (OMIM 100800), TD I (OMIM 187600), and TD II (OMIM 187601). Multiplex PCR was performed in maternal plasma cfDNA by two rounds of nested PCR, followed by next generation sequencing. Maternally homozygous SNPs and sequencing depth of each locus were used to call mutation. The method was validated in artificial plasma samples of c.1138G>A, with the simulating fetal fraction of 0%, 5%, 10%, and 20%. Six clinical cases, including five suspected ACH and one TD, were further tested, and the NIPD results were confirmed by diagnostic results. RESULTS: In artificial samples, mutation c.1138G>A with the simulating fetal fraction of 5%, 10% and 20% were all successfully identified, while the artificial sample with 0% fetal fraction (negative control) had a negative result. Based on simulating data, the minimal sequencing depth was 1000x for target region and 100x for each locus. Among six clinical cases, three cases were identified to contain the ACH causing mutation c.1138G>A, and were classified as ACH affected. One case contained the TD-associating mutation c.1118A>G. Two cases had wild-type genotypes. All results were consistent to diagnostic confirmation. CONCLUSIONS: Dominant mutations of FGFR3 associating ACH and TD could be accurately identified with the fetal fraction as low as 5% using our method. It is possible that the larger number of mutations can be detected with even lower fetal fraction, yet further validation tests are required. With the development of multiplex PCR-based targeted sequencing and proper bioinformatics analysis, it is promising to noninvasively test for different autosomal dominant diseases in plasma cfDNA.

Case No.	Gestational week	Fetal fraction	Mean error rate (%)	Mean depth of target region	Depth of mutation loci	Mutation cut-off	NIPD result	Diagnostic result

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AMP449	14	6.07	0.3158	20129.67	-	-	Negative	Negative
AMP868	20	5.02	0.2918	20121	5787	875.14	c.1138G>A	c.1138G>A
AMP13	13	5.07	0.2732	26933.34	-	-	Negative	Negative
AMP18	18	5.5	0.328	25978.3	1748	872.28	c.1138G>A	c.1138G>A
AMP28	28	6.01	0.2857	24927.74	3437	695.32	c.1138G>A	c.1138G>A
AMP354	13	4.74	0.2486	25774.48	1490	661.57	c.1118A>G	c.1118A>G

P-89

Maternal age specific risk for trisomies based on clinical performance of NIPT and empirically derived NIPT age specific positive predictive value and negative predictive value in Japan

Takahiro Yamada[1], Akihiko Sekizawa[2], Tatsuko Hirose[2], Osamu Samura[3], Nobuhiro Suzumori[4], Kiyonori Miura[5], Hideaki Sawai[6], Fumiki Hirahara[7], Haruhiko Sago[8], Japan NIPT consortium[9]

[1]Hokkaido University Graduate School of Medicine, Sapporo, Japan
[2]Showa University, Tokyo, Japan
[3]Department of Obstetrics and Gynecology, The Jikei University School of Medicine, Tokyo, Japan
[4]Nagoya City University Hospital, Nagoya, Aichi, Japan
[5]Nagasaki University, Nagasaki, Japan
[6]Hyogo College of Medicine, Hyogo, Japan
[7]Yokohama City University School of Medicine, Yokohama, Japan
[8]National Center for Child Health and Development, Tokyo, Japan
[9]Japan NIPT Consortium, Minato-ku, Tokyo, Japan

OBJECTIVES: The purpose of this study is to empirically estimate the maternal age specific risk for trisomies by using data collected from NITP patients and maternal age specific NIPT positive predictive value (PPV) along with negative predictive value (NPV) based on clinical experience of NIPT in Japan. METHODS: This was a multicenter prospective cohort study across Japan. Data were collected from a total of 22659 pregnant women undergoing NIPT (massively parallel sequencing) with advanced maternal age (age: 38.5±2.49, gestational weeks: 13.4±1.50) between April 2013 and September 2015 at 47 medical institutions in Japan. For the calculation of NPV, data (n=17677; 78.0%) from the patients whose birth outcomes were confirmed were exclusively used. RESULTS: The number of NIPT positive cases of trisomy 21, trisomy 18, and trisomy 13 were 190, 91, and 32 while the numbers of cases confirmed by invasive testing were 182 (PPV 95.8%), 70 (PPV 76.9%), and 21 (PPV 65.6%) respectively. PPVs increased with maternal age as theory explains. When estimated empirically, PPVs were generally

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higher than theoretical figures especially among younger age groups. NPVs obtained were 100%. Maternal age specific risk for trisomy 21 was lower than reported in Western societies while risk for trisomy 18 and 13 were almost the same. CONCLUSIONS: Maternal age specific risk for trisomy 21 was lower than conventional wisdom. When estimated empirically, NIPT/PPV might turn out much higher than theoretical figures especially among younger populations. More research is needed to further accumulate empirical data.

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P-90

Implementation of non-invasive prenatal diagnosis (NIPD) for single gene disorders into routine clinical practice in a UK NHS laboratory

Elizabeth Young[1], Benjamin Bowns[1], Amy Gerrish[1], Michael Parks[2], Samantha Court[1], Siobhan Cleary[1], Samuel Clokie[1], Chipo Mashayamombe-Wolfgarten[1], Julie Hewitt[1], Denise Williams[1], Trevor Cole[1], Michael Griffiths[1], Fiona MacDonald[1], Stephanie Allen[1]

[1]*West Midlands Regional Genetics Service, Birmingham, United Kingdom*
[2]*Nonacus Ltd, Birmingham, United Kingdom*

OBJECTIVES: We aimed to develop methods for NIPD of multiple single gene disorders. Through the Health Innovation Challenge Fund (HICF) funded NIPSIGEN project we have developed relative haplotype dosage (RHDO) assays for NIPD of spinal muscular atrophy (SMA), Duchenne and Becker muscular dystrophies (DMD/BMD), cystic fibrosis (CF) and congenital adrenal hyperplasia (CAH). A diagnostic service was launched for SMA and DMD/BMD in September 2016 and has been successfully implemented into clinical practice. For SGDs where NIPD by RHDO is not possible yet, we have also launched a bespoke NIPD service. METHODS: The RHDO test involves targeted enrichment of thousands of SNPs across multiple genomic regions and massively parallel sequencing (Illumina MiSeq) of cfDNA extracted from maternal plasma, followed by RHDO analysis. Maternal, paternal and proband genomic DNA samples extracted from leukocytes are tested alongside the cfDNA for haplotype phasing and to measure fetal fraction. Bespoke assays are developed prior to a couple becoming pregnant and are designed to directly detect the mutation in question. When required, prenatal testing is performed by massively parallel sequencing of cfDNA extracted from maternal plasma. RESULTS: To date, we have received RHDO referrals that include 9 pregnancies at risk of SMA and 6 pregnancies at risk of DMD/BMD. Samples have ranged in gestational age from 8 to 13 weeks and results have been issued with an average turnaround time of 11 calendar days. Overall, we have reported 4 normal, 6 unaffected carrier and 2 affected pregnancies. 3 pregnancies at risk of BMD/DMD did not have RHDO analysis as fetal sex determination showed the presence of a female fetus. We have also validated 21 bespoke assays for 13 different SGDs and performed 8 prenatal diagnostic tests. CONCLUSIONS: We have successfully launched RHDO and bespoke assay services for NIPD of multiple SGDs. Validation of an RHDO assay is ongoing for CF and CAH, with the aim to launch a clinical service in due course. We are able to multiplex 2-3 patients for multiple disorders on a single sequencing run, thus increasing capacity and decreasing testing costs. The development of an automated analysis pipeline has increased our capacity further. Provision of these services allows implementation of NIPD for SGD testing into routine clinical practice in the UK and is also available for patients outside the UK.

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Non-invasive prenatal testing: Women's experience in the private sector in Wales, UK

Shihui Oliver Zhu[1], Heather Strange[1], Robert Bryan Beattie[2]

[1]*Cardiff University, Cardiff, Wales, United Kingdom*
[3]*Cardiff and Vale University Health Board, Cardiff, Wales, United Kingdom*

OBJECTIVES: This study aims to gain a better understanding of women's experiences of non-invasive prenatal testing (NIPT) in Wales, UK, where NIPT for aneuploidy is currently offered through the private sector. Although NIPT testing has been available to purchase within the UK since 2012, few studies have addressed the private patient experience directly. This study seeks to understand how women find out about NIPT, how they negotiate access to NIPT testing and why they choose private NIPT testing over more established, routine prenatal screening methods. Also exploring more broadly how women understand and approach NIPT testing, their concerns and suggestions. METHODS: This study was conducted as part of an MSc in Genetic Counselling. It employs a qualitative methodology: semi-structured interviews were conducted and analysed thematically, to identify emerging and cross-cutting themes. Women who had previously used NIPT through the private sector in Wales were eligible for inclusion, and a total of six participants were recruited. Participants were encouraged to reflect on and recount experiences in their own words, allowing an in-depth, open exploration of their experience with NIPT, and facilitating the development of new knowledge in an area where little previous research has been conducted. RESULTS: Six participants were interviewed: three had previous experience of 'high-risk' pregnancy, the remaining three were 'low-risk'. Many aspects of the NIPT experience were described positively: participants felt the care and information they received was high-quality, they felt supported by NIPT-providers, they perceived the accuracy and trustworthiness of NIPT to be high, and they expressed desire to see NIPT implemented more widely. A number of issues and concerns, however, were raised: 'high-risk' participants experienced anxiety and distress whist waiting for NIPT results, and the current lack of continuity between private and NHS care services impacted negatively on experiences of NIPT. CONCLUSIONS: These study findings correlate with the wider literature: whilst participants' overall response to both the test and the care provided by the private clinic were positive, concerns were raised. The time spent waiting for NIPT results, the lack of consistency between private and public sector care, the lack of support relating to questions around termination, and the possible volume and complexity of the information NIPT may provide were experienced as problematic. This small-scale study of the private patient experience therefore underscores the relevance and practical significance of a number of critical issues, already raised within the wider literature.

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Prenatal indications of mucolipidosis type II (I-cell disease): Report of two cases

Gregory Costain[1], Michal Inbar-Feigenberg[1], Maha Saleh[1], Shimrit Yaniv-Salem[2], Greg Ryan[2], Eric Morgen[2], Elaine Goh[3], Gen Nishimura[4], David Chitayat[2]

[1]*The Hospital for Sick Children, Toronto, Ontario, Canada*
[2]*Mount Sinai Hospital, Toronto, Ontario, Canada*
[3]*Trillium Health Partners, Mississauga, Ontario, Canada*
[4]*Tokyo Metropolitan Children's Medical Center, Tokyo, Japan*

OBJECTIVES: Mucolipidosis type II (ML-II; I-cell disease; MIM #252500) is a rare autosomal recessive lysosomal storage disorder caused by deficiency of GlcNAc-1 phosphotransferase, an enzyme encoded by the gene *GNPTAB.* There are no proven treatments that alter the progressive disease course, with general fatal outcomes within the first decade of life. While distinctive skeletal and placental manifestations can sometimes facilitate early diagnosis, prenatal diagnosis is a challenge in the absence of a known family history. The purpose of this case series is to highlight the prenatal indications of two unrelated molecularly confirmed ML-II cases. METHODS: The prenatal ultrasound findings and the postnatal X-ray findings were reviewed. Postnatal placental histopathology, analysis of hexosaminidase enzyme activity and DNA analysis of the *GNPTAB* gene were conducted. RESULTS: Both cases were born to consanguineous Pakistani couples. Case 1 showed shortened long bones at 32 + 1 weeks after a 21 week ultrasound showed markedly echogenic kidneys and mildly thickened femoral diaphyses. Case 2 showed normal growth at 20 weeks gestation but shortened long bones were found at 30 weeks gestation with progressive growth lag discrepancy. Postnatally, both cases presented with prominent gingiva, shortened long bones, talipes, and placental pathology showing vacuolization of the syncytiotrophoblast. Both showed very high levels of total hexosaminidase and homozygous changes in the *GNPTAB* gene (c.3335+1G>A in Case 1 and c.3503_35504delTC in Case 2). CONCLUSIONS: ML-II should be considered in the differential diagnosis of prenatal late onset shortened long bones suggestive of skeletal dysplasia. Investigation of ML-II should be considered, especially if the families are consanguineous. Identifying the condition even late in the pregnancy can benefit families in view of the significant morbidity and mortality associated with ML-II.

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Clinical practice and experience in prenatal diagnosis for hereditary deaf family in China: 1332 Cases

Mingyu Han

Dept. Otolaryngology, Head & Neck Surgery, Chinese PLA General Hospital, Beijing, China

OBJECTIVES: Deafness is the most prevalent sensory deficit primarily originating from genetic etiologies. Genetic testing for deafness has been applied world-wide which makes prenatal diagnosis for hereditary deafness possible.Here we introduce our 11-year clinical practice and summarize the workflow, strategy, experience and changes of prenatal diagnosis for hereditary deafness in China. METHODS: 1332 deaf families were applied prenatal genetic testing from 2005 to 2016. Genomic and mitochondrial DNA of each subject was extracted from whole blood. The etiology and recurrent risk in 1332 families were confirmed by the genetic testing on deafness. The prenatal diagnosis was carried out during the pregnancy of all mothers from 11 to 30 weeks, and the following genetic counselling were supplied based on the results. The hearing status of babies after birth was followed up. RESULTS: 1309 families had 25% recurrent risk: 1306 couples were GJB2/SLC*26A4* carriers and 3 were *PCDH15/USH2A/OTOF* carriers. 14 families had 50% recurrent risk: ones were *GJB2/SLC26A4* deafness and others were same gene's carriers in 9 couples, and 5 families were DFNA. 2 deaf couples had 100% recurrent risk and wives got pregnant by artificial insemination with sperm from Sperm Bank. 1464 times of prenatal testing were applied in all 1332 families. 376 times showed fetuses had high risk and 1088 times showed the extremely low risk, and following visit showed 968 babies with normal hearing had been given birth. CONCLUSIONS: Prenatal diagnosis for hereditary deaf family can precisely predict offspring's hearing status and has been accepted as a clinical routine testing. The normative workflow and strategy highly guarantee the safe and favorable application in clinical practice.

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Case report: Prenatal presentation of genitopatellar syndrome

Nicole Martin[1], Resham Ejaz[2], Maha Saleh[3], Greg Ryan[4], Karen Chong[5]

[1]*Department of Obstetrics and Gynecology and the Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, Canada*
[2]*Division of Clinical & Metabolic Genetics, Department of Paediatrics, The Hospital for Sick Children, Toronto, Ontario, Canada*
[3]*The Hospital for Sick Children, Toronto, Ontario, Canada*
[4]*Mount Sinai Hospital, Toronto, Ontario, Canada*
[5]*University of Toronto, Toronto, Ontario, Canada*

OBJECTIVES: To describe the prenatal and postnatal findings in a case of Genitopatellar syndrome (GPS) found to have a pathogenic variant in the *KAT6B* gene. METHODS: A detailed review of the medical records of a patient with GPS was carried out. RESULTS: GPS is an autosomal dominant genetic condition characterized by severe intellectual and developmental disability with multiple congenital anomalies including ambiguous genitalia in males, cardiac defects, flexion contractures of the hips and knees, agenesis of corpus callosum with microcephaly and hydronephrosis. The patient was a 29 year-old G2P1L1 woman who presented in clinic with fetal ultrasound findings of agenesis of the corpus callosum with colpocephaly and delayed maturation of the brain, hydronephrosis with suspected bilateral urinary tract obstruction and small bladder. Postnatally, the male infant was identified to have a micropenis with scrotal hypoplasia, dysmorphic facial features, fixed flexion of lower extremities and a small muscular VSD. Hearing assessment revealed moderate to severe hearing loss. Given the prenatal and postnatal findings, variant analysis was performed on the *KAT6B* gene, and analysis revealed a heterozygous pathogenic variant, c.3606_3609delAACA. The pathogenic variant is predicted to result in a premature stop codon due to frameshift, causing features consistent with GPS. One previous report identified 2 fetuses with findings of microcephaly and renal anomalies and a subsequent clinical diagnosis of GPS. CONCLUSIONS: The combination of agenesis of the corpus callosum and hydronephrosis suggests a diagnosis of GPS and highlights the importance of a targeted fetal ultrasound for other features related to this condition, which may assist with the diagnosis of this rare condition prenatally.

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Prenatal diagnosis of 17-hydroxylase/17,20-lyase deficiency (17OHD) in a case of 46, XY sex discordance and low maternal serum estriol

Lauren Mohnach, Deborah Berman

University of Michigan, Ann Arbor, Michigan, United States

OBJECTIVES: A discrepancy between the fetal karyotype and the appearance of genitalia on ultrasound can be a diagnostic challenge. In these cases, it is difficult to shorten the extensive list of differential diagnoses without information on internal anatomy and endocrine profile. Here, we describe a diagnosis of 17-hydroxylase/17,20-lyase deficiency (17OHD), which was suspected based on low maternal serum estriol. 17OHD is a rare form of congenital adrenal hyperplasia(CAH) characterized by glucocorticoid deficiency, hypergonadotrophic hypogonadism, and severe hypokalemic hypertension. This case illustrates how prenatal diagnosis of this rare condition led to reduced parental stress and seamless transition to postnatal care. METHODS: Patient presented at 17 5/7 weeks' gestation with low estriol level (0.06 MoM) on maternal serum screen consistent with increased risk for Smith-Lemli-Opitz syndrome (SLOS). Ultrasound imaging revealed normal anatomy and female-appearing external genitalia. Amniocentesis excluded a diagnosis of SLOS and revealed 46, XY karyotype. Counseling was provided on possible etiologies of discordance between karyotype and genitalia including defects in androgen action and gonadal dysgenesis. Although a less common cause, a diagnosis of 17OHD was considered given low maternal serum estriol. Cultured amniotic cells were sent for 46, XY ambiguous genitalia gene panel. RESULTS: Results returned at 35 2/7 weeks' gestation showing two variants in the *HSD17B3* gene, consistent with a diagnosis of 17OHD. Through collaboration between Maternal-Fetal Medicine and Disorders/Differences of Sex Development (DSD) teams, the patient was counseled about the diagnosis and postnatal management plans were made. After birth, physical examination identified bilateral labial hydroceles and palpable gonads in the inguinal canal but otherwise female appearing genitalia. Endocrine evaluation was consistent with the diagnosis. The family decided on female gender of rearing. She will be followed long-term in the DSD clinic. CONCLUSIONS: This case demonstrates that disorders of steriodogenesis should be considered when there is low maternal serum estriol in the setting of 46, XY genitalia discordance. To our knowledge, this is the first prenatal diagnosis of 17OHD in the absence of family history. Most cases of complete deficiency are not diagnosed until adolescence. With increased use of cell-free DNA screening, the need to perform diagnostic evaluation in this clinical scenario may present more frequently. Having this information before delivery allowed for education, parental adjustment, early knowledge of recurrence risks, and collaboration between care teams minimizing stress in the neonatal period.

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Prenatal diagnosis and genetic counseling of a case of GJB2 dominant hereditary deafness family

Ren Shu Min

The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

OBJECTIVES: Gene diagnosis were performed in a case of *GJB2* dominant hereditary deafness family, give them clinical consultation, reduce the risk of deafness birth. METHODS: Obtain the fetus specimens through interventional biopsy guided by ultrasound, test fetus' realtive deafness gene with Sanger sequencing in combination with short tandem repeat(STR) test to eliminate maternal blood pollution. RESULTS: The patients were detected *GJB2* c.223C>T(p.R75W) heterozygous mutation.The fetus did not detect the variation.The hearing evaluation result were consistent with the result of prenatal diagnosis. CONCLUSIONS: Application of deafness gene test with Sanger sequencing in prenatal diagnosis can accurately diagnose fetus' genotype, effectively reduce the birth of deafness child.

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Increased nuchal translucency and Noonan syndrome spectrum – A Mount Sinai Hospital experience

Pierre Sinajon[1], David Chitayat[2], Karen Chong[3], Abdul Noor[3]

[1]*The Hospital for Sick Children, Toronto, Ontario, Canada*
[2]*Mount Sinai Hospital, Toronto, Ontario, Canada*
[3]*University of Toronto, Toronto, Ontario, Canada*

OBJECTIVES: To examine a cohort of patients seen at the PNDMG program - Mount Sinai Hospital between 2013 – 2015, with fetal ultrasound findings of an increased nuchal translucency (NT ≥ 3.5mm), and to:

1. Correlate between the NT measurements and a diagnosis of Noonan Syndrome Spectrum (NSS)
2. Conduct a systematic literature review to determine the correlation between NT measurement and a diagnosis of NSS
3. To create a clinical protocol to guide physicians in the investigation of an increased fetal NT

METHODS: A cohort of patients presenting between 2013 – 2015 with fetal ultrasound findings of increased NT. All patients were offered amniocentesis/CVS for QF-PCR as a first line test. If negative, patients proceeded to karyotype/microarray analysis and NSS panel testing. Patients were also offered fetal ultrasounds at 16 weeks and 18 – 22 weeks gestation (GA) along with fetal echocardiogram. A systematic review was conducted in accordance to PRISMA criteria. Pubmed, Embase, Ovid MEDLINE and Web of Science were searched from January 2005 – August 2016 for articles involving NSS and increased NT. Seventeen papers were included for analysis. RESULTS: 226 patients with increased fetal NT were seen. In 116/226 patients, chromosomal aneuploidy was detected through QF-PCR. The remaining 110/226 patients had further testing. 8 had karyotype abnormalities, 13 had abnormal microarray findings and 5 had NSS findings through DNA analysis. CONCLUSIONS: Based on the cohort findings and literature review the following guidelines were created regarding the best approach to a fetus with an increased NT:

1. QF-PCR and microarray should be performed for NT ≥ 3.5 mm
2. If QF-PCR/microarray analysis is normal, DNA analysis for NSS should be performed for NT ≥ 4.0 mm
3. Early anatomic ultrasounds at 16 weeks GA
4. Fetal echocardiogram at 18 – 22 weeks GA
5. Detailed fetal ultrasound at 18 – 22 weeks GA

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Prenatal diagnosis of Sotos syndrome charactered by intrauterine growth restriction

Zhang Yi, Qun Fang

Fetal Medicine Center, Department of Obstetrics and Gynaecology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China

OBJECTIVES: To present prenatal diagnosis and molecular characterization of a fetus associated with Sotos syndrome. METHODS: This was the second pregnancy of a 29-year-old woman. At 25 weeks of gestation, mild bilateral ventriculomegaly, left pyelectasis, ectopic kidney and multicystic renal dysplasia on the right kidney were first detected by ultrasound. At 28 weeks of gestation, bilateral ventriculomegaly, vague fornix in the cavity of septum pellucidum, ectopic kidney and multicystic renal dysplasia on the right kidney and fetal growth restriction were prominent by ultrasound. The woman underwent cordocentesis at 26 weeks of gestation. Karyotype analysis and multiplex ligation-dependent probe amplification(MLPA) were performed on cord blood. SNP array was also performed on cord blood and parental peripheral blood. RESULTS: G-banded chromosome analysis revealed a normal karyotype. SNP array on cord and parental blood showed a de novo 1.899Mb microdeletion at 5q35.2q35.3 encompassing NSD1. MLPA analysis on the cord blood confirmed the deletion of NSD1 gene at 5q35.2. CONCLUSIONS: Fetuses with Sotos syndrome may present fetal growth restriction, ventriculomegaly, multicystic renal dysplasia, ectopic kidney and pyelectasis. SNP array and MLPA are useful for diagnosis of 5q35 microdeletion associated with Sotos syndrome.

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A case report for prenatal diagnosis of primary carnitine deficiency

Zhang Yi, Qun Fang

Fetal Medicine Center, Department of Obstetrics and Gynaecology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China

OBJECTIVES: To present prenatal diagnosis of a fetus associated with primary carnitine deficiency. METHODS: This was the third pregnancy of a 34-year-old woman. The woman and her husband was the fifth degree of consanguinity. In her first and second pregnancies, infants both died at six months for unknown hepatosplenomegaly and pyrexia. In this pregnancy, the nuchal translucency was 0.9mm in the 12 weeks and no malformations were detected by ultrasound. The woman underwent amniocentesis at 17+2 weeks of gestation. karyotype analysis, SNP array and whole exome sequencing were performed. karyotype analysis, SNP array and whole exome sequencing were also performed on parental peripheral blood. RESULTS: G-banded karyotype analysis on this fetus revealed a normal karyotype. SNP array on this fetus showed a total 38.794 Mb loss of heterozygosity in 5q23.1q23.3(116,181,558-127,347,815), 11p15.1p14.3 (18,792,735-25,671,869) and 12q14.2q21.31 (64,274,650-85,023,899). While karyotype analysis and SNP array on parental blood were both normal. The exome sequencing results on parents found the same heterozygous mutation in SLC22A5（760C>T）gene. The exome sequencing results on this fetus reported homozygous mutation in SLC22A5（760C>T）gene. CONCLUSIONS: Whole exome sequencing is useful for diagnosis of mutation in SLC22A5 gene associated with primary carnitine deficiency.

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A novel deletion mutation in ASPM gene in primary microcephaly: Case report and literature review

Hui Zhu[1], Linhuan Huang[1], Zhi-ming He[1], Zou Zhiyong[2], Yanmin Luo[1]

[1]*Fetal Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*
[2]*First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*

OBJECTIVES: Primary microcephaly is a neurodevelopmental and etiologically heterogeneous disorder with
environmental and genetic causes. The aim of this study was to report a case of prenatal microcephaly with a novel ASPM gene mutation and to undertake literature review to assess the prevalence of causes and the accuracy of prenatal imaging for the associated abnormalities in cases of primary microcephaly diagnosed before or at birth. METHODS:
The clinical data, laboratory results and perinatal outcomes of a primary microcephaly case subjected to invasive prenatal diagnostic testing was reported. Scientific literatures about primary microcephaly diag nosed before or at birth from 2000 to 2016 were reviewed. RESULTS: Here we report on a non consanguineous Chinese family in which sequence analysis identified novel compound heterozygous mu tations within the ASPM gene (c.10060C>T/p.Arg3354Ter and c.6854_6855 delTC/p.Leu2285GlnfsX32). A total of 135 studies included 629 cases with primary microcephaly were included. Microcephaly etiology was ascertained in 48.01% of all patients. Genetic causes and virus infection were identified in 23.21% a nd 9.54% of the cases respectively. 16.13% of the cases was detected only by prenatal MRI but missed at prenatal ultrasound, while 22.28% of microcephaly diagnosed at birth was missed at prenatal imaging, a nd over half of these cases were monogenetic disorders. CONCLUSIONS:
Our case report expands the mutation spectrum of ASPM. The most common etiology in primary microc ephaly were genetic abnormalities and virus infection. MRI and invasive prenatal diagnosis is essential in the cases of fetal microcephaly.

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Evidence of SOX3 duplication as a cause of neural tube defects

Jennifer Goldkamp[1], Emanuel Vlastos[2], Rachael Bradshaw[3]

[1]*St. Louis University, St. Louis, Missouri, United States*
[2]*St. Louis Fetal Care Institute, St. Louis, Missouri, United States*
[3]*St. Louis University School of Medicine, St. Louis, Missouri, United States*

OBJECTIVES: We present two cases of duplication of Xq27.1 including the SOX3 gene in the presence of an open neural tube defects (ONTD) and an otherwise normal microarray. METHODS: Introduction: Duplication Xq27.1 involving the SOX3 gene is known to cause X-linked panhypopituitarism, and sometimes developmental delays in males (1). However, there is a growing evidence that this gene duplication may also be linked to open neural tube defects (2, 3). RESULTS: 1. 20yo G1 @ 19w diagnosed with an open fetal myelomeningocele (MMC) of L4 - S2. Amniocentesis resulted as 46XX, and microarray showed a 577.19kb gain at X27.1, including duplication of SOX3. The patient underwent prenatal repair at 24w gestation. She delivered a female infant weighing 1905g via cesarean at 35w. This duplication was of maternal origin. 2. 29yo G4P1021 @ 22w diagnosed with an open fetal MMC of L3 - S2/3. Amniocentesis resulted as 46XX, and microarray showed a 590kb gain at X27.1, including duplication of SOX3. The patient underwent prenatal repair at 25w gestation. The pregnancy is ongoing. CONCLUSIONS: Duplication of the SOX3 gene of Xq27.1 has been linked to ONTD in other case reports; however, this duplication is currently not recognized as a cause of ONTD (2,3). This duplication has been shown to have variable penetrance as exhibited in male siblings with different levels of hypopituitarism and developmental delays (1), and likely exhibits variable penetrance for ONTD as the genetic anomaly was of maternal origins in case 1. This adds to the growing literature of identifiable genetic causes of ONTD.

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Effective fetal epigenetic biomarkers for non-invasive fetal trisomy 21 detection

Ji Hyae Lim[1], Bom Yi Lee[1], Shin Young Kim[1], You Jung Han[2], Jin Hoon Jung[2], Moon Young Kim[2], So Yeon Park[1], Hyun Mee Ryu[2]

[1]Laboratory of Medical Genetics, Medical Research Institute, Cheil General Hospital and Women's Healthcare Center, Seoul, South Korea
[2]Department of Obstetrics and Gynecology, Cheil General Hospital and Women's Healthcare Center, Dankook University College of Medicine, Seoul, South Korea

OBJECTIVES: The discovery of differentially methylated DNA regions between maternal blood cell and fetal (placental) DNA is one of the main areas for the development of fetal-specific biomarkers for the non-invasive prenatal test. Recently, we analyzed DNA methylation change in chromosome 21 of maternal blood and fetal placenta using the methylated-CpG binding domain (MBD) protein based high-resolution array of chromosome 21 and identified three novel fetal-specific methylated DNA regions (FSMRs) for detection of non-invasive fetal trisomy 21 (T21). The aim of this study was to evaluate the diagnostic accuracies of three FSMRs for the noninvasive prenatal test of T21. METHODS: A nested, case-control study was conducted with maternal plasma collected from 167 pregnant women carrying 155 normal and 12 T21 fetuses in the first trimester. In this study, we used the MBD based-protein method to extract methylated DNA regions in maternal plasma. The maternal plasma levels of FSMRs were simultaneously measured by multiplex quantitative real-time PCR. Overall accuracies of FSMRs were estimated by the area under the receiver operator characteristic curve (AUC). RESULTS: The maternal plasma levels of three FSMRs were obtained in all cases. The levels of three FSMRs were not different according to maternal age and body mass index at maternal blood sampling (P>0.05 in both). The levels of three FSMRs were significantly increased in women with a T21 fetus compared with controls (P<0.01 for all). In non-invasive fetal T21 detection, the AUC of FSMR1, FSMR2, and FSMR3 showed 0.860 (95% CI: 0.748-0.972), 0.919 (95% CI: 0.857-0.982), and 0.869 (95% CI: 0.747-0.990), respectively. CONCLUSIONS: The findings of this study suggest that all FSMRs may be useful as potential biomarkers for non-invasive fetal T21 detection, regardless of maternal age and body mass index. To conclude, we suggest that the FSMR2 may be a highly effective biomarker for non-invasive fetal T21 detection.

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Evaluation of extraction methods for effective isolation of methylated cell free fetal DNA from maternal plasma

Ji Hyae Lim[1], Bom Yi Lee[1], Hyun Jin Kim[1], Min Hyoung Kim[2], Dong Wook Kwak[2], So Yeon Park[1], Hyun Mee Ryu[2]

[1]Laboratory of Medical Genetics, Medical Research Institute, Cheil General Hospital and Women's Healthcare Center, Seoul, South Korea
[2]Department of Obstetrics and Gynecology, Cheil General Hospital and Women's Healthcare Center, Dankook University College of Medicine, Seoul, South Korea

OBJECTIVES: The cell-free fetal DNA (cff-DNA) in plasma of pregnant women represents an alternative source for non-invasive prenatal test. However, the use of cff-DNA is limited due to their low concentration in maternal plasma. Therefore, the choice of method for their extraction and quantification is important for downstream analyses. Recently, DNA methylation differences between fetal placenta and maternal blood are applied in the development of new biomarkers for quantification of cff-DNA. The aim of this study was to evaluate the efficiency of magnetic bead-based kit in comparison with the spin column-based kit for effective isolation of methylated cff-DNA from maternal plasma. METHODS: In our study, maternal plasma was extracted from normal pregnant women within the gestational age of 10~13 weeks (n=12). Total cell-free DNA was extracted using the spin column-based kit (QIAamp DSP Virus Kit) and the magnetic bead-based kit (NextPrep-Mag cfDNA Isolation Kit) from the plasma of the same pregnant woman, respectively. And then, methylated cff-DNA was isolated from extracted total cell-free DNA using a methyl-CpG binding domain-based protein method. The amount of methylated cff-DNA was quantified by multiplex real-time polymerase chain reaction using four fetal placenta-specific epigenetic markers. RESULTS: The spin column and the magnetic bead-based kit displayed similar efficiencies of both time and cost. However, the results of isolation efficiency of methylated cff-DNA indicated significant differences between two extraction methods. The Ct-values using spin column-based kit was significantly lower than those using the magnetic bead-based kit (P<0.001 for all epigenetic markers). The quantity of methyl cff-DNA was significantly higher using spin column-based kit than the magnetic bead-based kit (P<0.001 for all epigenetic markers). CONCLUSIONS: Our findings demonstrated that spin column-based kit was more effective than the magnetic bead-based kit for methylated cff-DNA isolation. To conclude, the spin column-based kit presents highly efficient extraction kit for isolation of fetal placenta-specific methylated cff-DNA from maternal plasma.

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Placental textural analysis in the growth-restricted fetus using 3D MRI

Nickie Andescavage[1], Sonia Dahdouh[2], Marni Jacobs[2], Samantha Bauer[2], Sara Iqbal[3], Dorothy Bulas[4], Adre J. Du Plessis[5], Catherine Limperopoulos[2]

[1]Children's National Medical Center, Washington, DC, United States
[2]Children's National Health System, Washington, DC, United States
[3]MedStar Washington Hospital Center, Washington, DC, United States
[4]Division of Diagnostic Imaging and Radiology, Children's National Medical Center, Washington, DC, United States
[5]Fetal and Transitional Medicine, Children's National Medical Center, Washington, DC, United States

OBJECTIVES: Placental insufficiency (PI) is a major cause of fetal-neonatal mortality and the leading cause of fetal growth restriction (FGR). Ex vivo placental pathology reveals inflammatory and vascular abnormalities in FGR, however, there is a paucity of in vivo tools to assess microstructural placental development. Textural analysis is a graphic imaging technique capable of detailed quantitative image analysis and has been used in biomedical imaging to distinguish pathologic from surrounding healthy tissue. The objective of this study was to compare quantitative geometric and textural analysis techniques to compare placental development in pregnancies complicated by FGR and uncomplicated, healthy pregnancies. METHODS: We recruited women with FGR and women with normal pregnancies. T2-weighted single shot fast spin echo sequences of the placenta were acquired in the axial or coronal plane using 4mm slices. 3D reconstructions of the placenta were obtained using ITKSNAP software. Shape features were computed automatically based on an in-house 3D reconstruction method. Textural features were calculated based on the previously segmented masks and extracted for analysis. Linear mixed models with robust variance estimate were used to evaluate the association between textural properties of the placenta and fetal growth restriction and adjusted for gestational age at the time of assessment. RESULTS: We studied 82 pregnant women with a mean gestational age (GA) of 31 completed weeks at fetal MRI; 21 underwent serial scans for a total of 130 images (88 controls, 42 FGR). 13 features were significantly different in FGR compared to controls (Table 1), reflecting smaller placentas (volume, thickness, elongation), increased heterogeneity (mean, variance, kurtosis, short run emphasis), lower grey levels (low and high grey level emphases) and decreased textural symmetry (skewness and cluster prominence). CONCLUSIONS: We report for the first time differences in in vivo quantitative measures of placental shape and texture in the growth-restricted fetus compared to healthy fetuses. Such alterations likely reflect microstructural alterations in placental development that result in placental insufficiency and may serve as an in vivo biomarker of placental function.

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Table 1: Differences in textural features between FGR and control fetuses[a]

Feature	Control (n = 49)		FGR (n=33)		
	Mean	95% CI	Mean	95% CI	p-value[b]
Placental volume (cm^3)	668	643, 693	454	433, 477	< 0.01
Thickness	58	56, 60	51.32	50, 53	< 0.01
Elongation	182	179, 185	162	159, 166	< 0.01
Normalized mean	3.09	2.92, 3.28	4.29	4.06, 4.53	< 0.01
Normalized variance	0.54	0.52, 0.56	0.64	0.61, 0.68	0.01
Kurtosis	0.23	0.21, 0.25	0.14	0.13, 0.15	< 0.01
Skewness	0.16	0.14, 0.18	0.06	0.03, 0.10	0.03
Energy	0.25	0.24, 0.26	0.27	0.25, 0.29	0.30
Entropy	2.45	2.35, 2.55	2.32	2.22, 2.42	0.37
Inverse different movement	0.84	0.84, 0.85	0.85	0.84, 0.86	0.79
Inertia	0.32	0.30, 0.34	0.31	0.29, 0.34	0.97
Cluster shade	1.20	0.85, 1.59	0.63	0.44, 0.83	0.17
Cluster prominence	12.48	10.85, 14.35	6.30	5.26, 7.55	<0.01
Short run emphasis	0.75	0.74, 0.77	0.84	0.83, 0.86	< 0.01
Long run emphasis	2.95	2.74, 3.17	2.48	2.24, 2.74	0.17
Grey level non-uniformity	6205	5672, 6789	5413	5139, 5701	0.19
Run length non-uniformity	10213	9104, 11458	10311	9321, 11406	0.95
Low grey level emphasis	0.15	0.14, 0.16	0.20	0.18, 0.23	0.04
High grey level emphasis	10.10	9.36, 10.90	7.65	6.91, 8.48	0.03
Short run low grey emphasis	0.11	0.10, 0.12	0.17	0.15, 0.19	< 0.01
Short run high grey emphasis	7.93	7.24, 8.69	6.72	6.02, 7.50	0.25
Long run low grey emphasis	0.48	0.41, 0.57	0.58	0.46, 0.72	0.53
Long run high grey emphasis	25.56	24.40, 26.77	15.53	14.54, 16.58	< 0.01

[a] Means controlling for gestational age estimated using restricted maximum likelihood estimation
[b] p-value based on repeated measures ANCOVA using log-transformed variables

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Ultrasound detection of encephalocele during prenatal care: Perinatal outcomes and prognosis

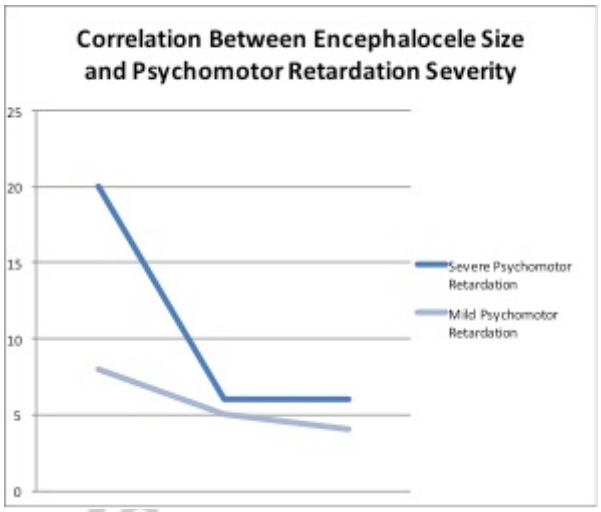
Eduardo Nava[1], Tayde Arroyo-Lemarroy[2], Rosario Staines-Alarcon[3], Edelmiro Garza-Padilla[2], Felipe Zuñiga-Villarreal[2], Maria A Tijerina-Torres[3]

[1]*Hospital Regional Materno Infantil de Alta Especialidad, Secretaria de Salud de Nuevo León, Monterrey, Mexico*
[2]*Tecnologico de Monterrey, Monterrey , Nuevo León, Mexico*
[3]*Hospital Regional Materno Infantil de Alta Especialidad, Guadalupe, Nuevo León, Mexico*

OBJECTIVES: Nowdays, encephalocele prevalence has been estimated at 0.8 to 5 per 10,000 live births. The objective of the research is to describe the perinatal outcomes at delivery and short term prognosis of eight neonates with encephalocele detected during a routine ultrasound in the prenatal care consults, after medical and surgical management in a tertiary-level hospital. METHODS: An observational and retrospective study included eight patients with an encephalocele diagnosis by ultrasound during prenatal care consults. These patients were registered on the fetal medicine consult database from 2014 to 2015 on a tertiary-level hospital and analyzed in detail. The maternal and perinatal variables were evaluated from the electronic medical records, including: maternal age, previous gestations, defect size and location, gestational age at diagnosis and delivery, weight and APGAR score, and associated malformations. After pediatric and surgical interventions in most cases, the severity of psychomotor retardation and mortality in each case were gathered. RESULTS: 63% of patients were between 20-35 years old, 50% primiparous. Most were initially diagnosed during second trimester ultrasound (88%, n=7). Seven cases had an occipital and one a frontal bone defect. All pregnancies were delivered at term, 88% by cesarean section (n=7). One of the neonates died after birth with a trisomy 13 diagnose. The rest of the neonates had APGAR scores >6 and were discharged after surgical management (encephalocele plasy of defects <10cm, two cases with a ventriculoperitoneal shunt). The size of the encephalocele was associated with higher psychomotor developmental affection (*graph 1*). CONCLUSIONS: These findings suggest that the encephalocele diagnosis is not necessarily associated with neonatal mortality and that these products can be treated at birth and live with mild psychomotor retardation at short term. The obstetric ultrasound during prenatal care is a key tool for an early neurological evaluation and possibly referral to a tertiary facility at birth for surgical management.

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The misdiagnosis of achondroplasia in a neonate with skeletal anomalies, narrow chest, and respiratory distress

Victoria Berger, Anne Slavotinek

University of California, San Francisco, San Francisco, California, United States

OBJECTIVES: An accurate diagnosis for skeletal dysplasias can be clinically challenging prenatally due to variability in skeletal dysplasia presentations and the relative rarity of its occurrence. Fetuses and neonates with macrocephaly and rhizomelia can be misdiagnosed with achondroplasia, the most common skeletal dysplasia, by imaging modalities and clinical exam. We report a clinical diagnosis of achondroplasia in an infant who was later determined to have a novel mutation in the *COL2A1* gene. METHODS: Prenatal ultrasound on a 32 year old gravida 3 para 1 mother at 21+4 weeks gestation demonstrated macrocephaly (head circumference > 99th percentile) severe micromelia, (length <1st percentile) and thoracic circumference in the 80th percentile. Infant was delivered at term by cesarean delivery and intubated for respiratory distress. A radiographical diagnosis of achondroplasia was made based on short humeri and femurs and flared iliac wings on radiographs and computed tomography scanning. *FGFR3* sequencing for the p.Gly380Arg mutation in achondroplasia was negative. Given narrow chest and severe respiratory compromise, alternative diagnoses were considered and trio whole exome sequencing was performed. RESULTS: Whole exome sequencing revealed a pathogenic, de novo heterozygous c.1835G>T variant, predicting p.Gly612Val, in the collagen type II, alpha 1 (COL2A1) gene. The c.1835G>T variant has not been previously reported and results in substitution of valine in a highly conserved glycine position. In silico analysis demonstrated the change was likely damaging to the protein function. Mutations in COL2A1 can cause a wide range of phenotypes. In the prenatal period, COL2A1 mutations can present with platyspondyly, cupping of anterior rib ends, and short ribs, while in the neonatal period, significant respiratory morbidity, short long bones, and vitreal abnormalities may be seen. CONCLUSIONS: Skeletal dysplasias are a heterogeneous group of disorders with overlapping phenotypes that can be difficult to diagnose with imaging and clinical exam alone. Misdiagnosis may cause inaccurate or incomplete counseling and may have negative impact on management and prognosis of individual, as well as recurrence risk counseling. Our case demonstrates that, although achondroplasia is a very common cause of macrocephaly and rhizomelia in the perinatal period, a thorough consideration of other entities is warranted in patients with skeletal anomalies, especially in circumstances where atypical clinical findings, such as a narrow chest or respiratory distress, are present.

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A case of fetal Fraser syndrome caused by novel mutations in FREM2 gene

Zhi-ming He, Qun Fang, Yan-min Luo

Fetal Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China

OBJECTIVES: Fraser syndrome (FS) (OMIM 219000) is a rare autosomal recessive disorder, characterized by cryptophthalmos, syndactyly, malformation of the larynx and abnormal genitalia. Here we report a case of fetal congenital high airway obstruction syndrome was identified as Fraser syndrome and have novel genetic mutations. METHODS: A 30-year old woman was referred to the genetic counseling at 26 weeks for fetal malformations. The ultrasound scan showed that the fetus was characterized by mass ascites, bilateral enlarged hyperechogenic lungs with distended trachea, which indicated the existence of congenital high airway obstruction syndrome (CHAOS). Similar situations happened twice before. After abortion, the fetal samples was sent to comprehensive genetic tests. RESULTS: Low-set ears, micromandible, cryptophthalmos of the left eye, syndactyly, laryngeal stenosis, renal dysplasia, imperforate anus and ambiguous genitals were showed in fetal autopsy, which indicated Fraser syndrome. The chromosome karyotype showed a normal female karyotype, and microarray analysis did not show any meaningful duplication or deletion. The next generation sequencing results identified heterozygous mutations of *FREM2,* c.2689C >T (p.Gln897Ter) in Exon 1 and c.7542_7543 insG (p.Pro2514ProfsX12) in exon 15. Both are inherited from the parents. CONCLUSIONS: Recurrent fetal CHAOS sydrome should be taken into consideration as candidates of genetic disease like Fraser syndrome. The technology of next generation sequencing can help us to identify a couple of OMIM diseases in one time.

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P-108

Discordant expression of NRF2 in different types of selective intrauterine growth restriction in monochorionic diamniotic twins

Wu Jing[1], Qun Fang[2]

[1]*The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China*
[2]*Fetal Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*

OBJECTIVES: Selective intrauterine growth restriction (sIUGR) causes higher risks of prenatal mortality and morbidity. Almost all monochorionic diamniotic (MCDA) twins are monozygotic, sharing an identical genetic background and a similar intrauterine environment, but discordant oxidative stress and NRF2 expression were found in placental shares of sIUGR in our previous study. Different types of sIUGR show different abnormalities in the umbilical artery (UA) Dopplers. To investigate relationship between NRF2 expression and the different UA Dopplers of sIUGR, we detected NRF2 levels in placentas of different types of sIUGR. METHODS: Eighteen pairs of sIUGR placentas were collected in our research, including 6 sIUGR type Ⅰ, 6 type Ⅱ and 6 type Ⅲ. sIUGR was defined as the estimated fetal weight (EFW) or birth weight of one twin being below the 10th percentile, and the EFW or birth weight of the co-twin was in the normal range for gestational age. Real-Time PCR and Western Blot assays were used to detect placental NRF2 expression at mRNA and protein levels. Data were represented as the mean ± standard deviation. Student and Paired t-test were used to analyze the results. RESULTS: RT-PCR results showed that NRF2 mRNA was elevated in placental shares of the smaller fetus than larger fetus in sIUGR type Ⅰ ($P < 0.001$). Moreover, NRF2 protein expression was also higher in placental shares of the smaller fetus compared to larger fetus ($P < 0.01$). However, no significant differences of NRF2 were found in placental shares of twins in sIUGR type Ⅱ or Ⅲ at mRNA and protein levels. CONCLUSIONS: NRF2 was elevated in placental shares of the smaller fetus in sIUGR type Ⅰ, without abnormalities in the UA Dopplers. NRF2 showed no significant differences in placental shares of twins in sIUGR type Ⅱ or Ⅲ, with persistently or intermittent absent end-diastolic flow velocities in the UA of the smaller fetus. The discordant expression of NRF2 may related to the different types of sIUGR with different UA Dopplers.

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P-109

Identification of mesenchymal stem cells derived from human placental tissue

Kiyonori Miura, Naoki Fuchi, Tao-Sheng Li, Hideaki Masuzaki

Nagasaki University, Nagasaki, Japan

OBJECTIVES: We tried to identify the mesenchymal stem cells (MSCs) derived from various placental tissues. METHODS: This study protocol was approved by the IRB for Ethical, Legal and Social Issues. Normal placentas were obtained at the third trimester following written informed consent. We expanded MSCs primarily from chorionic villi (CV-MSC), chorionic plate (CP-MSC) and decidua basalis (DB-MSC) by using explant method, and analyzed their characterization by flow cytometry and immunocytochemistry. RESULTS: We isolated and expanded adequate numbers of cells with characteristic features of MSCs from various placental tissues. These cells positively expressed with MSC markers (CD44, CD73, CD90 and CD105), but negatively with hematopoietic markers (CD34 and CD45), in addition, these could be differentiated toward adipogenic, osteogenic and chondrogenic lineage, confirmed the characterization of MSCs. CONCLUSIONS: We successfully expanded CP-MSCs, CV-MSCs, and DB-MSCs from placental tissue, resulting in cells with high proliferative potency and obvious characters of MSC. Placenta-derived MSCs may represent a potentially useful tool for mechanistic understanding of pregnancy-related disorders.

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The expression of C19MC microRNAs in mesenchymal stem cells primarily expanded from various placental tissues

Naoki Fuchi, Masashi Noguchi, Kiyonori Miura, Hideaki Masuzaki, Tao-Sheng Li

Nagasaki University, Nagasaki, Japan

OBJECTIVES: Circulating placenta-specific C19MC miRNAs has been demonstrated to be the potential biomarkers of pregnant disorders. We investigated the expression of C19MC miRNAs in mesenchymal stem cells (MSCs) from various placental tissues. METHODS: This study protocol was approved by the IRB for Ethical, Legal and Social Issues. Normal placentas were obtained at the third trimester following written informed consent. We expanded MSCs primarily from amnion (AM-MSC), chorionic villi (CV-MSC), chorionic plate (CP-MSC) and decidua basalis (DB-MSC). The expressions of miR-517a and miR-518b, two representative members of C19MC miRNAs were measured by quantitative real-time RT-PCR, and normalized with the endogenous U6. RESULTS: These MSCs positively expressed with MSC markers (CD44, CD73, CD90 and CD105). The relative expression levels of miR-517a and -518b, were 0.24 and 0.036 in AM-MSCs, 32.09 and 7.04 in CP-MSCs, 36.47 and 6.49 in CV-MSCs, and 0.38 and 0.062 in DB-MSCs, respectively. The C19MC miRNAs miR-517a and -518b were clearly expressed in CP-MSCs and CV-MSCs, and barely expressed in AM-MSCs and DB-MSCs ($P<0.001$ vs. CP-MSCs and CV-MSCs) CONCLUSIONS: Placenta-specific C19MC miRNAs were clearly detected in MSCs from different placental tissues. Primarily expanded MSCs may serve as useful tools for uncovering the molecular mechanism on pregnant disorders.

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P-111

Hsa-miR-138-5p and its target EZH2 was involved in fetal hippocampus with Down syndrome

Weili Shi[1], Shixiu Liao[2]

[1]*Henan Provincial People's Hospital, Zhengzhou, China*
[2]*Medical Genetic Institute of Henan Province, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou, Henan, China*

OBJECTIVES: Down syndrome (DS), caused by triplication of human chromosome 21, is the most common aneuploidies. The main characteristic of DS patients is intellectual disability. MicroRNAs (miRNAs) play important regulatory roles in various biological processes, such as embryonic development, cell differentiation, proliferation and apoptosis. And several miRNAs have been involved in DS. In this research, the pathological role of miRNA in DS fetus was investigated. METHODS: In this research, total RNA extracted from fetal hippocampal tissues was used to analyze miRNA and mRNA expression via Affymetrix miRNA 4.0 and PrimeView Human Gene Expression Array, respectively. Then miRNA and gene expression profiles were integrated by correlation analysis to identify dysregulated miRNAs with their predicted target mRNAs. Microarray data were further validated by real-time PCR. Regulation of zeste homolog 2 (EZH2) expression by hsa-miR-138 was determined by luciferase reporter assay. RESULTS: We integrate miRNA expression and mRNA expression in hippocampus of trisomy 21 fetus to elucidate the mechanism that underlying DS abnormalities. We characterized the repertoire of specific miRNAs involved in hippocampus in trisomy 21 patients, highlighting hsa-miR-138 and hsa-miR-409, in particular the importance of hsa-miR-138, especially the -5p strand. Furthermore, the expression level of predicted target genes of hsa-miR-138-5p in trisomy 21 fetus, including zeste homolog 2 (EZH2) were further confirmed. In addition, luciferase assay indicated that EZH2 was a direct target of hsa-miR-138 in HEK293T cells. CONCLUSIONS: The function of hsa-miR-138-5p and its target EZH2 was involved in hippocampus in DS fetus. As how hsa-miR-138-5p and its target EZH2 play roles in DS fetus need further investigation.

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A case of placental mesenchymal dysplasia

Naoki Takeshita, Izumi Sasaki, Chiho Tagui, Masahiro Nagaoka, Tomohiro Adachi, Kei Yokokawa, Megumi Manrai, Myumi Urita, Akihiro Ishida, Ayami Yokoyama, Toshihiko Kinoshita

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

OBJECTIVES: In the situation that placental vesicular changes with a coexistent fetus are noted, diagnostic differentiation should be made among a partial hydatidiform mole with a viable fetus, a twin pregnancy with a normal fetus and a complete hydatidiform mole, and placental mesenchymal dysplasia (PMD). This paper deals with a case we have encountered in which placental vesicular changes on diagnostic imaging and chromosomal test results raised the strong suspicion of PMD and the patient was delivered of a live child under cautious perinatal management. METHODS: It is a case report in our Hospital. RESULTS: [Case] A 36-year-old nullipara was found elsewhere to have cystic changes in the placenta on ultrasonographic examination from an early gestational stage onwards, and was referred to our institute at week 14 for further workup. At 16 weeks 1 day, a 7 cm subchorionic hematoma was noted and the patient was admitted here for management to undergo an imminent abortion. A magnetic resonance imaging (MRI)[A1] scan of the uterus showed a single placenta so that either a partial hydatidiform mole or a twin pregnancy with a normal fetus and a complete hydatidiform mole was suspected. As a chromosomal karyotype test. CONCLUSIONS: PMD presents with features resembling a hydatiform mole on imaging and macroscopic examination but, histopathologically, it is differentiated from trophoblastic disease in that it is devoid of chorionic villus cell proliferation. In the case where vesicular cyst formation is noted at an early stage of gestation, there is the possibility that a diagnosis of partial hydatidiform mole may be made to allow a needless induced abortion. Inasmuch as PMD has a relatively favorable prognosis for both the mother and the offspring, it is of importance to exercise management with adequate perinatal genetic counseling, bearing in mind the other disorders

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First trimester diagnosis of a cesarean scar pregnancy with placenta accreta: Case report

Camilo Torres, Diana Alfonso, Mantilla Andrea

Clínica del Country, Bogotá, Colombia

OBJECTIVES: To describe the first trimester diagnosis and assessment of a rare case involving a caesarean scar ectopic pregnancy associated with placenta accreta. METHODS: A G2C1, 35 yo pregnant woman was admitted at emergency room complaining of 15 days of lower abdominal pain and slight vaginal bleeding. Transvaginal ultrasound evaluation revealed an 8 week´s pregnancy with a chorionic hematoma as large as 80% of the size of the gestational sac, subjective thinning of the myometrium overlying the placenta and increased vascular spaces with turbulent blood flow on Doppler evaluation at the site of implantation. MRI and conventional abdominal ultrasound showed the chorionic hematoma towards the cervical canal and a lower implantation of the placenta with compromise of the caesarean scar without extra uterine invasion. RESULTS: As an adjunctive treatment, the patient receives a single dose of Methotrexate and with previous informed consent overwent to a total hysterectomy without complications. Pathological evaluation confirmed the diagnosis of caesarean scar ectopic pregnancy and its association with placenta accreta due to localization, absence of decidua basalis and presence of chorionic villi at myometrium. CONCLUSIONS: Despite the rare co-existence of these entities, usually considered as differential diagnosis, the association of these life-threatening pathologies must be keep in mind to lead an accurate diagnosis and a proper management to minimize morbidity and mortality.

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A malignant fetal facial tumor: Prenatal diagnosis and pathological correlation

Camilo Torres[1], Diana Alfonso[1], Clara Alvarado[1], Carlos Bonilla[1,2], Eduardo Acosta[1]

[1]*Clínica del Country, Bogotá, Colombia*
[2]*Hospital San José, Bogotá, Colombia*

OBJECTIVES: To describe the diagnosis, follow up and pathological correlation of a rare and giant fetal facial tumor. METHODS: A G2P1, 32 yo woman with a previous 15 weeks normal ultrasound, went to her second ultrasound at 19 weeks. It revealed a 3cms homogenous echogenicity and multi-lobulated image, without any established Doppler pattern, located at the middle of the face, covering the nose and the upper lip suggesting a glioma. Further examinations showed a rapid growing mass and at 32 weeks a similar image was seen into the cranium ahead the frontal lobe. Any other finding was ruled out. Due to important size and location of the tumor an EXIT procedure was planned with parent's authorization after properly counseling. RESULTS: At 33 weeks an emergency C-section was performed due to premature rupture of membranes and a non-reassuring fetal status. A promptly orotraqueal intubation reached the neonatal air way but, the giant multi lobulated bleeding opened mass with brain appearance reminded an anterior encephalocele and all reanimations efforts were ceased. Pathological evaluation confirmed the ultrasound findings. Although at first sight the brain appearance guided to an encephalocele, there was not brain compromise and histologic evaluation was compatible with a high mitosis rate and poor differentiated sarcomatoid carcinoma. CONCLUSIONS: Information obtained at serial evaluations with conventional 2D and 3D ultrasound and its post process tools improves imaging details of the fetal morphological evaluation and can be helpful in parental and multidisciplinary decision making at neonatal period, especially in novel cases.

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Fetal adrenal hemorrhage: Prenatal diagnosis and neonatal correlation

Camilo Torres, Diana Alfonso

Clínica del Country, Bogotá, Colombia

OBJECTIVES: To describe the prenatal diagnosis and follow up of a benign adrenal pathology which diagnosis is rare in-utero. METHODS: A 31 yo primigravida, with a history of multiple sclerosis but not receiving any medication, who went to an ultrasound evaluation at 37 weeks that revealed an anechoic and rounded image occupying the left Nyberg´s triangle and in close relation to the fetal adrenal gland. Further ultrasound evaluations showed a slight change in echogenicity but not in size, location or vascularization. Any hemodynamic changes, other masses and maternal compromise were ruled out. RESULTS: At 38 weeks, a spontaneous vaginal birth was performed and a 3020 gr female newborn was obtained. At neonatal physical examination no abdominal masses were found but due to ultrasound findings history the baby was taken to abdominal ultrasound and MRI evaluations. Ultrasound neonatal images were similar to prenatal ones and MRI showed increased signal intensity on T1 and T2 weighted sequences images avoiding the use of contrast. No adrenal insufficiency features were found. Further ultrasound evaluations evidenced that hemorrhage had been solved completely by the second month of live. CONCLUSIONS: Information obtained at serial ultrasound evaluations can lead to make differential diagnosis more accurate and can be helpful in multidisciplinary decision making at neonatal period, avoiding extra or unnecessary studies.

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Fetal measures of pulmonary and genito-urinary development and pregnancy outcomes in suspected posterior urethral valves

Gillian Abrams[1], Anna Blask[1], Pesacreta Lindsay[1], Amy Rice[1], Hans Pohl[1], Dorothy Bulas[2], Nickie Andescavage[3]

[1]Children's National Health System, Washington, DC, United States
[2]Division of Diagnostic Imaging and Radiology, Children's National Medical Center, Washington, DC, United States
[3]Children's National Medical Center, Washington, DC, United States

OBJECTIVES: Posterior urethral valves (PUV) are one of the most common causes of lower urinary tract obstruction in neonates. Morbidity is largely secondary to the presence and severity of pulmonary hypoplasia, which remains difficult to predict in the prenatal period. For survivors, ongoing renal dysfunction remains a significant morbidity. Given the wide range of outcome, the ability to provide accurate prognostic information to families prenatally remains a significant challenge. The objective of this study was to identify specific radiographic characteristics of fetal development in pregnancies complicated by PUV that were associated with neonatal mortality and morbidity. METHODS: We performed a retrospective chart review of neonates prenatally evaluated for posterior urethral valves in the Divisions of Fetal Medicine and Diagnostic Imaging between 2012-2016. Using fetal ultrasound images, a single radiologist (AB) blinded to outcome measured the amniotic fluid index (AFI), thoracic circumference (TC) and 9 markers of genitourinary development (See Table 2). We performed descriptive statistics using one-way between-groups ANOVA and independent t-tests to study fetal radiographic measures with pregnancy outcome using SPSS. RESULTS: We studied 27 fetuses that underwent ultrasonography between 18-39 weeks gestation (mean 28 ± 6 weeks). PUV was confirmed in all surviving pregnancies. While all pregnancies were complicated by oligohydramnios (AFI <5), surviving infants had greater AFI compared to terminated pregnancies and non-survivors (p=0.01). Similarly, surviving infants had a great thoracic circumference compared to non-survivors (p<0.01, Table 1). For non-terminated pregnancies, surviving infants were less likely to demonstrate enlarged kidneys (p=0.02), abnormal echogenicity (p=0.01), cysts (p=0.01) or evidence of keyhole bladder (p<0.01). CONCLUSIONS: Radiographic measures of fetal lung and genitourinary development in pregnancies complicated by PUV are associated with neonatal mortality and morbidity. These data may provide important resources in prenatal counseling to better predict neonatal outcomes, although further studies are needed.

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Table 1: Demographic and Radiographic Data for Pregnancies Complicated by Posterior Urethral Valves

	Termination (n=6)	Neonatal Survival (n=17)	Neonatal Demise (n=4)	p-value
GA at exam (weeks)	21 ± 6	31 ± 5	28 ± 6	< 0.01
GA at birth (weeks)	n/a	37 ± 2	32 ± 0.6	0.01
BW (grams)	n/a	3308 ± 620	2025 ± 450	0.02
Respiratory distress	n/a	2 (12%)	4 (100%)	< 0.01
AFI	2.5± 0.6	2.8 ± 0.5	1.75 ± 1.0	0.01
TC (mm)	136 ± 67	217 ± 45	135 ± 79	< 0.01
TC % for GA*	44.6 ± 29	28 ± 28	15 ± 23	0.26
Renal evaluation				
Enlarged kidneys	n/a	12 (71%)	4 (100%)	0.02
Thin parenchyma	n/a	12 (71%)	3 (75%)	0.87
Abnormal echogenicity	n/a	11 (65%)	4 (100%)	0.01
Poor CMD	n/a	14 (82%)	4 (100%)	0.08
Cysts	n/a	2 (12%)	4 (100%)	0.01
Bladder evaluation				
Thickened	n/a	11 (65%)	3 (75%)	0.71
Distended	n/a	15 (88%)	3 (75%)	0.52
Keyhole appearance	n/a	7 (41%)	4 (100%)	<0.01
Hydronephrosis				
SFU Grade (mean ± SD)		1.6 ± 0.88	2.8 ± 0.53	0.234
Grade 4		0 (0%)	1 (25%)	
Grade 3		4 (23%)	0 (0%)	
Grade 2		2 (12%)	1 (25%)	
Grade 1		11 (65%)	2 (50%)	

GA=gestational age; AFI=amniotic fluid index, TC=thoracic circumference; BW=birth weight; CMD=corticomedullary differentiation; Values represent n(%), unless noted otherwise; * See reference #5

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Non-immune hydrops fetalis: Do placentomegaly and polyhydramnios matter?

Victoria Berger[1], Teresa Sparks[1], Angie Jelin[2], Jeanty Cerine[3], Gosnell Kristen[1], Mackenzie Tippi[1], Gonzalez Juan[2]

[1]*University of California, San Francisco, San Francisco, California, United States*
[2]*Johns Hopkins Hospital, Baltimore, Maryland, United States*
[3]*St. Luke Hospital, San Francisco, California, United States*

OBJECTIVES: Placentomegaly and polyhydramnios are commonly observed in association with hydrops fetalis; however, whether or not their sonographic identification is clinically relevant for prognosis is controversial. Our objective was to evaluate the outcomes of fetal or neonatal death and preterm birth in cases of non-immune hydrops fetalis (NIHF) alone, compared to those with concurrent polyhydramnios and/or placentomegaly (P/PM). METHODS: Retrospective cohort of singletons with NIHF evaluated at an academic center between 1994-2013. NIHF was defined as ≥2 abnormal fetal fluid collections: ascites, pericardial effusion, pleural effusion, or skin edema. Cases attributable to isoimmunization were excluded. Primary outcomes were intrauterine fetal demise (IUFD) and neonatal death within 30 days. Secondary outcomes were preterm birth (PTB, <37 weeks) and spontaneous PTB. Primary and secondary outcomes were examined for NIHF alone compared to NIHF with concurrent placentomegaly (≥4cm in second trimester, ≥6cm in third trimester) and/or polyhydramnios (amniotic fluid index ≥24cm). Chi square test compared proportions and logistic regression generated odds ratios. RESULTS: 153 NIHF cases were identified, 21% (32/153) had NIHF alone and 79% (121/153) had NIHF with P/PM. Comparing NIHF alone to NIHF with P/PM, there was no significant difference in neonatal death (38.1% vs. 43.0%, p=0.809), but IUFD was seen more frequently in NIHF alone (34.4% vs. 17.4%, p=0.049). Cases of NIHF with P/PM were significantly more likely to deliver preterm <37 weeks (80.0% vs. 57.1%, p=0.045), <34 weeks (60.0% vs. 28.6%, p=0.015) and to undergo a spontaneous PTB (64.4% vs. 33.3%, p=0.042). Odds ratios generated with multivariate logistic regression supported these findings after adjusting for the suspected NIHF etiology. CONCLUSIONS: Compared to NIHF alone, pregnancies with NIHF with P/PM had a lower risk of IUFD, which may be largely attributable to their earlier gestational age at delivery. Cases of NIHF with P/PM were at increased risk of PTB <37 and <34 weeks, as well as of spontaneous rather than iatrogenic PTB. This information is valuable for providers in counseling patients with NIFH, and supports the need for close antenatal surveillance for both preterm birth and IUFD.

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Outcomes of NIHF alone compared to NIHF with P/PM

	NIHF alone N= 32	NIHF with P/PM N= 121	P value
GA at diagnosis, weeks	25.0 (18.1-37.6)	26.2 (16.7-38.1)	0.080
GA at delivery, weeks	36.0 (27.1-39.9)	32.3 (24.0-41.0)	**0.001**
Birth weight, grams	2900 (1260-3600)	2190 (600-5220)	**0.049**
NICU stay, days	39.5 (14-72)	31 (5-180)	0.849
Known or suspected etiology			0.084
Chest	6 (18.8%)	48 (39.7%)	
Aneuploidy	1 (3.1%)	8 (6.6%)	
Lymphatic/anemia	3 (9.4%)	14 (11.6%)	
Cardiac	9 (28.1%)	14 (12.4%)	
Primary hydrothorax	3 (9.4%)	16 (13.2)	
CDH	4 (12.5%)	8 (6.6%)	
Other	6 (18.8%)	12 (9.9%)	
Intrauterine demise	11/32 (34.4%)	21/121 (17.4%)	**0.049**
Neonatal death	8/21 (38.1%)	43/100 (43.0%)	0.809
PTB < 37 wks	12/21 (57.1%)	80/100 (80.0%)	**0.045**
PTB < 34 wks	6/21 (28.6%)	60/100 (60.0%)	**0.015**
PTB < 28 wks	1/21(4.8%)	15/100 (15.0%)	0.300
Spontaneous PTB[a]	5/15 (33.3%)	47/73 (64.4%)	**0.042**

GA, gestational age. NIHF, non-immune hydrops fetalis. P/PM, placentomegaly or polyhydramnios. PTB, preterm birth.
[a] Sufficient information to confirm spontaneous versus iatrogenic preterm was available for 15 NIHF alone cases and 73 NIHF with P/PM cases.
Continuous variables are presented as medians with ranges

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Correlation between ultrasound diagnosis and autopsy findings of fetal bilateral schizencephaly

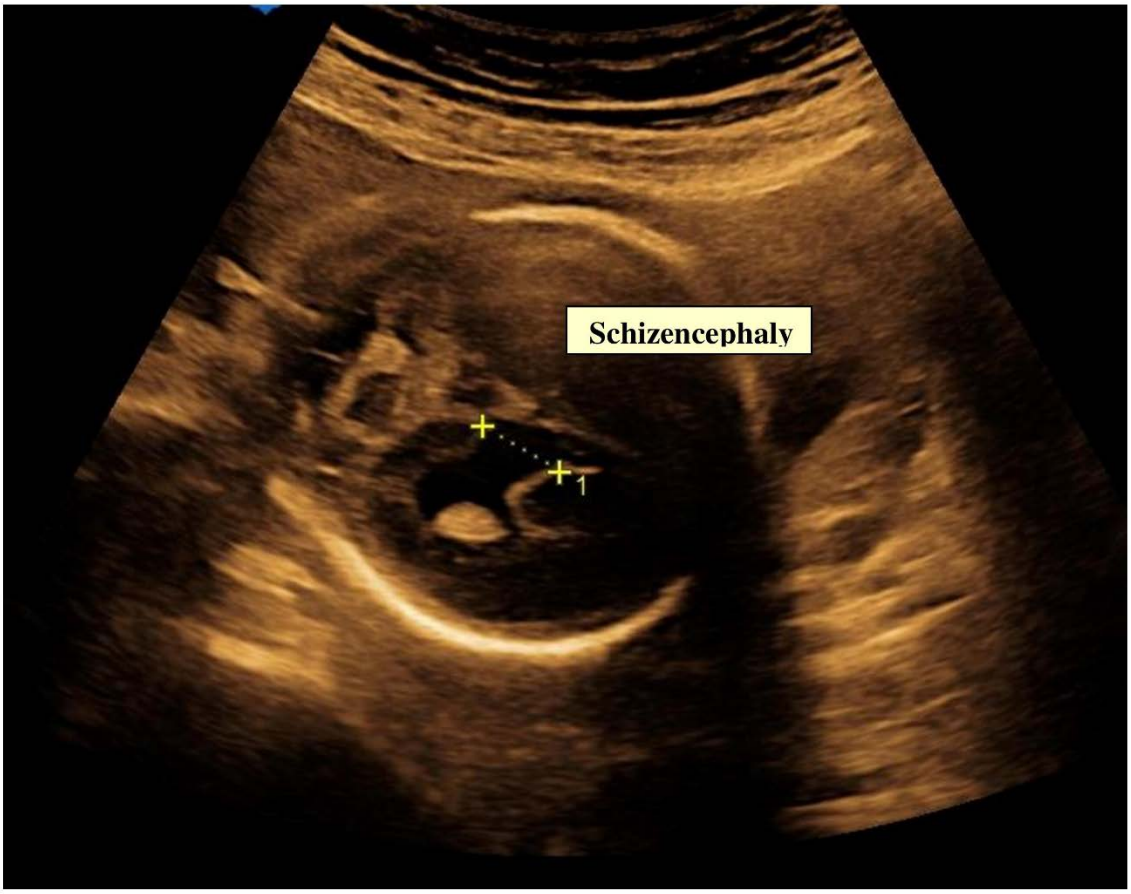
Sihem Darouich[1], Nadia Boujelbène[2], Nadia Aloui[3], Souhir Bouzguenda[4], Dhikra Kacem[2], Radhia Ben Ghorbel[2], Hédi Reziga[5], Aida Masmoudi[4]

[1]*Fetopathology Unit, Bizerte, Tunisia*
[2]*Pathology Department, Tunis, Tunisia*
[3]*Radiology Department, Tunis, Tunisia*
[4]*Embryo-Foetopathology Department, Tunis, Tunisia*
[5]*Department 'B' of Gynecology and Obstetrics, Tunis, Tunisia*

OBJECTIVES: Schizencephaly is a rare congenital malformation of the brain characterized by a gray matter-lined defect that extend from the pial membrane to the ependymal surface of the ventricle, as a result of neuronal migration and organization disorders. The purpose of this study is to correlate fetal ultrasound diagnosis of schizencephaly and associated anomalies with autopsy findings. METHODS: We report a case of bilateral schizencephaly in a 26-week-old male fetus, which was revealed by two-dimensional ultrasound and confirmed by autopsy. RESULTS: A healthy 28-year-old pregnant woman was referred for a detailed ultrasound examination at 26 weeks. The fetal cranial axial sonograms detected a large open-lip cleft defect of the right frontoparietal lobe and agenesis of the corpus callosum and septum pellucidum. The parents requested termination of pregnancy.
The neuropathological examination confirmed the sonographic abnormalities. In addition, it revealed the presence of a closed-lip schizencephalic cleft of the left frontoparietal lobe. Microscopically, the clefts were lined by heterotopic grey matter. The fetal karyotype was normal, and the infection screen was negative. CONCLUSIONS: Prenatal diagnosis of schizencephaly is rare, with very few cases reported in literature. Most cases are still diagnosed after mid-pregnancy, with important implications for prenatal diagnosis. Our case shows the best correlation between antenal imaging and autopsy findings of schizencephaly.

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Correlation between prenatal ultrasound and autopsy findings in two fetuses with femoral facial syndrome

Sihem Darouich[1], Jihen Amraoui[2], Nadia Boujelbène[3], Naima Amraoui[4], Dhikra Kacem[3], Radhia Ben Ghorbel[3], Karima Mrad[3], Aida Masmoudi[5]

[1]*Fetopathology Unit, Bizerte, Tunisia*
[2]*Department of Obstetrics and Gynecology, Centre Hospitalier, Sens, France*
[3]*Pathology Department, Tunis, Tunisia*
[4]*Clinique Raouebi, Bizerte, Tunisia*
[5]*Embryo-Foetopathology Department, Tunis, Tunisia*

OBJECTIVES: Femoral facial syndrome (FFS) is a rare condition, which has been most frequently described in females. Only few cases of prenatal diagnosis have been published. FFS is characterized by facial dysmorphism and femoral hypoplasia, which can be associated with other various malformations. We aim to highlight the role of ultrasound in the detection of this disorder. METHODS: We describe the prenatal ultrasound and autopsy findings of FFS in two female fetuses born to diabetic mothers at 23 weeks' gestation. RESULTS: Prenatal ultrasound showed micrognathia, low-set dysplastic ears and very short femora. A complete fetopathological examination was achieved. Characteristic dysmorphic cranio-facial features were observed, including hypertelorism, posterior cleft with glossoptosis, severe micrognathia, small anteverted nose, thin lips, long filtrum and auricular anomalies. Short neck, short and bowed lower limbs and malposition of the extremities were also noticed. Radiographs demonstrated short bones, bilateral severe hypoplasia of the femora and vertebral sacral anomalies. Autopsy showed increased subcutaneous fat, in both cases, associated with meconium ileus, renal hypoplasia and cardiovascular anomalies in one case, and with uterine malformation and intraventricular hemorrhage in the other case. CONCLUSIONS: FFS has a poor prognosis, justifying medical termination of pregnancy. It is important to ascertain early prenatal diagnosis in order to offer counseling for parents. Ultrasound can easily detect this condition and enable differential diagnoses that especially include osteochondrodysplasias.

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Prenatal diagnosis of right ventricular hypoplasia and correlation with autopsy findings

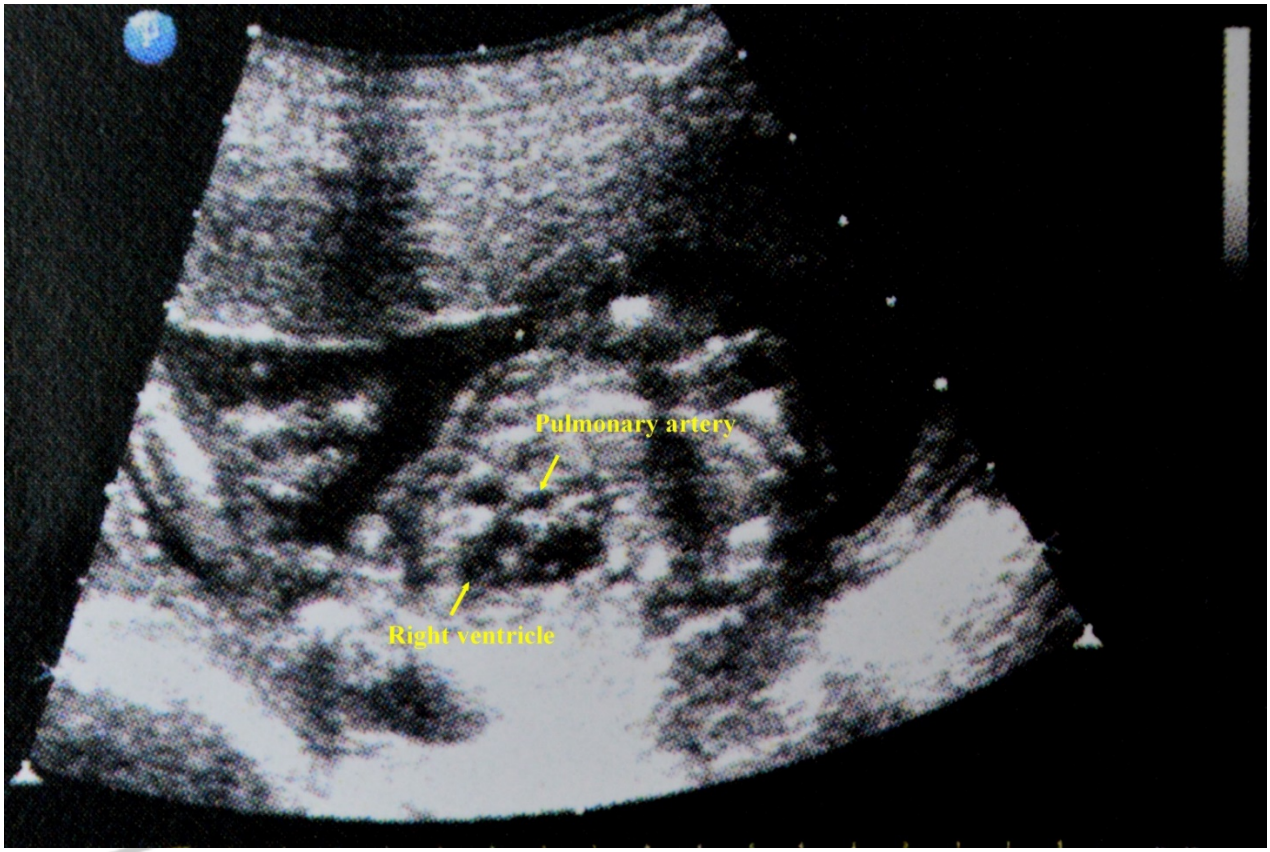
Sihem Darouich[1], Nadia Boujelbène[2], Heykel Kchouk[3], Houda Azaiez[2], Hana Guizani[2], Karima Mrad[2]

[1]*Fetopathology Unit, Bizerte, Tunisia*
[2]*Pathology Department, Tunis, Tunisia*
[3]*Private Practice, Tunis, Tunisia*

OBJECTIVES: The right ventricular hypoplasia is a rare congenital malformation. It is usually associated with a patent foramen ovale or secondum atrial septal defect. It was described in cyanotic young children who died in infancy because of severe heart failure usually complicated by serious cardiac arrhythmias. Here, we describe this rare disease in a female fetus which was aborted at 29 weeks' gestation. METHODS: We report the prenatal echocardiography findings in a fetus with hypoplastic right ventricle and correlate them with those of the fetopathological examination. RESULTS: A healthy 27-year-old pregnant woman was referred for a detailed ultrasound examination at 25 weeks. The latter showed hypoplastic right ventricle, underdeveloped pulmonary artery and dysplastic tricuspid valve. The pregnancy was interrupted at 29 weeks'gestation. Karyotype was normal. The autopsy demonstrated symmetrical intrauterine growth restriction, facial dysmorphism, pulmonary hypoplasia, moderate pericardial effusion and complex congenital heart disease. The hypoplasia of both right ventricle and pulmonary artery was confirmed. But, all the valves were normal in morphology and insertion. In addition, right atrial enlargement, ostium secundum defect and arterial duct agenesis were observed. Histology examination revealed diffuse visceral congestion. CONCLUSIONS: The fetopathological examination is essential to diagnose congenital heart defects that remain difficult to detect by antenatal ultrasound. In this report, the autopsy allowed to confirm the right ventricle hypoplasia and to reveal

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associated cardiac anomalies. Besides, it ruled out the differential diagnoses including tricuspid atresia, pulmonary atresia, Ebstein's malformation and Bernheim's syndrome.



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Isolated fetal ascites: Prenatal diagnosis and autopsy findings

Sihem Darouich[1], Nadia Boujelbène[2], Jihen Amraoui[3], Naima Amraoui[4], Houda Azaiez[2], Hana Guizani[2], Karima Mrad[2]

[1]*Fetopathology Unit, Bizerte, Tunisia*
[2]*Pathology Department, Tunis, Tunisia*
[3]*Department of Obstetrics and Gynecology, Centre Hospitalier, Sens, France*
[4]*Clinique Raouebi, Bizerte, Tunisia*

OBJECTIVES: Fetal ascites is commonly associated with fetal hydrops. But, it can also occur as an isolated anomaly without accumulation of fluid in body cavities or subcutaneous tissues. Despite the fact that

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the isolated fetal ascites is not necessarily considered as a serious condition, the prognosis may be poor depending on its etiology. METHODS: We described a case of isolated fetal ascites with late gestational onset and poor outcome. The autopsy findings were detailed. RESULTS: A 36-year-old healthy woman was referred for extensive antenatal evaluation as ultrasound detected isolated fetal ascites in mid third trimester. Echocardiography of the fetus was normal. The mother has type A positive blood. Serologic tests were negative. Unfortunately, the fetal outcome was fatal. Postmortem examination was achieved. The male fetus was eutrophic of 34 weeks, with no organ malformations. The placenta was small with velamentous cord insertion and presence of a large hematoma in fetal membranes. Histology showed diffuse visceral congestion and thymic cortical lymphocyte depletion, suggesting acute fetal anoxia. Placental examination revealed chronic and diffuse villous hypoxic-ischemic changes. CONCLUSIONS: This case report shows that isolated fetal ascites may occur as a result of anemia due to the rupture of vasa praevia. Thus, color Doppler imaging is needed to identify velamentous cord insertion and vasa praevia.



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Case report: Prenatal diagnosis of a fetus with Proteus syndrome

Lauren Fogarty[1], Lauren Currie[2], David Skidmore[3], Angelica Oviedo[4], Jillian Coolen[5]

[1]*Dalhousie University, Halifax, Nova Scotia, Canada*
[2]*Maritime Medical Genetics Service, IWK Health Centre, Halifax, Nova Scotia, Canada*
[3]*Maritime Medical Genetics Service, IWK Health Centre; Department of Paediatrics, Dalhousie University, Halifax, Nova Scotia, Canada*
[4]*IWK Health Centre; Department of Anatomic Pathology, Dalhousie University, Halifax, Nova Scotia, Canada*
[5]*IWK Health Centre; Department of Obstetrics and Gynaecology, Dalhousie University, Halifax, Nova Scotia, Canada*

OBJECTIVES: Proteus syndrome is a rare condition characterized by mosaic overgrowth of tissue types derived from all three germ layers. It has a highly variable presentation and is generally diagnosed by clinical criteria after birth, as manifestations typically begin to progress within the first 6-18 months. Here, we present a case of Proteus syndrome detected prenatally by ultrasound. We describe the ultrasound and autopsy findings of a fetus with AKT1 related proteus syndrome. METHODS: We report a case of Proteus syndrome detected prenatally using ultrasound at 22 weeks and 5 days' gestation. Following termination of the pregnancy, autopsy and genetic analysis were completed. Rapid aneuploidy detection was normal in the male fetus. Somatic overgrowth gene panel sequencing was used to identify a pathogenic mutation in ATK1. No further clearly causative mutations in other genes associated with segmental overgrowth were noted. RESULTS: A 35-year-old G3P2 woman was first seen at 22 weeks plus 5 days' gestation. A genetic sonogram was performed revealing multiple congenital anomalies including macrocephaly, parallel lateral ventricles, choroid plexus cysts, nuchal thickening, frontal bossing, hypertelorism, bell-shaped chest, acromelia, and bilateral clubfeet. The patient elected to terminate the pregnancy and an autopsy was performed further identifying cutaneous syndactyly of both feet as well as an enlarged brain with cortical periventricular nodular heterotopia and segmental capillary malformations. Genetic testing of a micro-dissected sample of abnormal neural tissue revealed a p.E17K mutation in ATK1 associated with Proteus syndrome at near heterozygous levels. CONCLUSIONS: Proteus syndrome has rarely been described prenatally. In this case, the severity of the fetal abnormalities obscured the expected mosaic presentation on ultrasound and gross autopsy examination, with segmental changes only being cleanly identified on microscopic examination. The confirmation of a mosaic condition will significantly alter genetic counselling for this family. This case outlines how severe presentations of overgrowth conditions can appear non-mosaic on gross examination and provides additional ultrasound findings associated with fetal presentation of Proteus syndrome.

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Spontaneous compared to iatrogenic delivery in cases of prenatally diagnosed gastroschisis?

Anna Girsen[1], Alexis Davis[2], Susan Hintz[3], Paula Trepman[4], Elizabeth Fluharty[4], Katie Sherwin[4], Arti Desai[5], Trina Mansour[5], Giang Truong[5], Allen T. Merritt[5], Bryan Oshiro[6], Yair Blumenfeld[7]

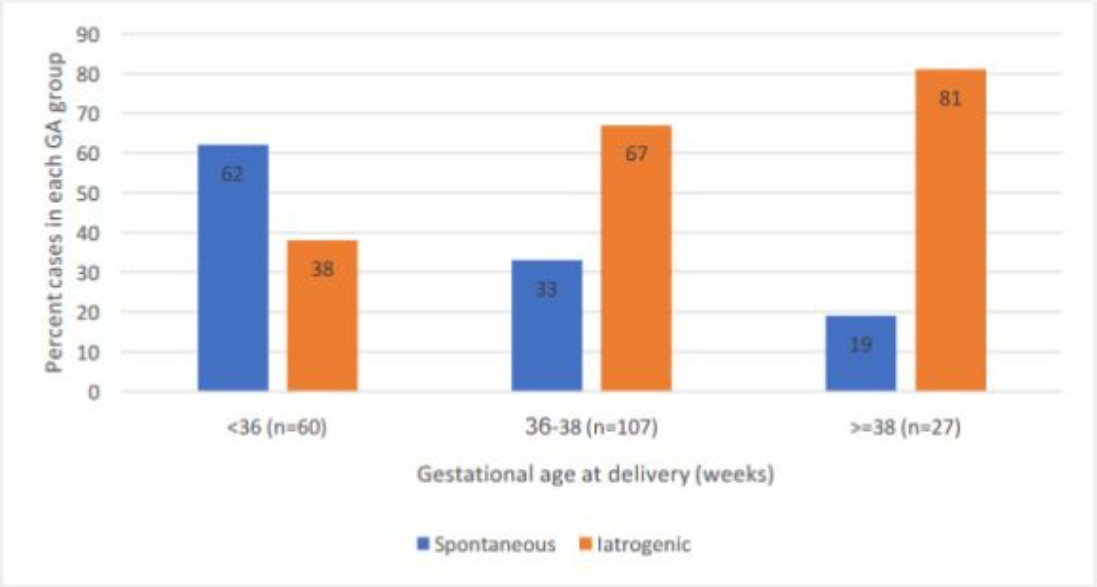
[1]*Stanford University, Stanford, California, United States*
[2]*Pediatrix Medical Group , San Jose, California, United States*
[3]*Department of Pediatrics, Division of Neonatal and Developmental Medicine, Stanford University School of Medicine, Stanford, United States*
[4]*Stanford University, Stanford, California, United States*
[5]*Loma Linda University, Loma Linda, California, United States*
[6]*Health Catalyst, Salt Lake City, United States*
[7]*Department of Obstetrics & Gynecology, Stanford University School of Medicine, Stanford, California, United States*

OBJECTIVES: Controversy exists over optimal delivery timing for fetal gastroschisis cases, with many undergoing iatrogenic late preterm and early term delivery. We aimed to investigate whether spontaneous vs. iatrogenic delivery results in different neonatal outcomes in cases of prenatally diagnosed gastroschisis. METHODS: A retrospective cohort of prenatally-diagnosed gastroschisis referred to two academic centers 1/09-10/16. We compared neonatal outcomes of spontaneous vs. iatrogenic delivery among the following gestational age (GA) groups: <36 0/7; 36 0/7 – 37 6/7; ≥38 0/7 weeks. The primary outcome was defined as any of: neonatal sepsis, short bowel syndrome, prolonged mechanical ventilation (≥75th percentile) or death. Secondary outcome was prolonged (≥75th percentile) length of stay (LOS). RESULTS: 200 neonates with gastroschisis were included: 60 delivered at <36 weeks, 107 at 36 0/7 – 37 6/7 weeks and 27 at ≥38 weeks. Iatrogenic delivery occurred in 38% (n=23/60) of gastroschisis cases delivered before 36 weeks, in 67% (n=72/107) of those delivered between 36 0/6-37 6/7 weeks, and in 81% (n=22/27) among those delivered after or at 38 weeks (p<0.0001) (Figure). There was no significant difference in primary neonatal outcome between spontaneous vs. iatrogenic delivery in any of the GA groups. In addition, the rate of prolonged LOS was similar between spontaneous vs. iatrogenic delivery among the GA groups. CONCLUSIONS: Neonates with gastroschisis delivered after 36 weeks of gestation had a higher rate of iatrogenic delivery compared to those delivered before 36 weeks. No significant difference was seen in the rate of composite adverse neonatal outcome between spontaneous vs. iatrogenic delivery at any gestational age.

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Figure. Percent neonates with spontaneous (blue) vs. iatrogenic (orange) delivery in each gestational age group



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A case of one couple's children with PSS

Mariko Kamihigashi[1], Hideaki Sawai[1], Riya Sakane[2], Yuji Ukita[2], Kayoko Harada[2], Hiroyuki Tanaka[1], Hiroaki Shibahara[1]

[1]*Hyogo College of Medicine, Hyogo, Japan*
[2]*Obstetrics and Gynecology, Nishinomiya, Japan*

OBJECTIVES: Pena-Shokeir syndrome (PSS) type Ⅰ is rare lethal disorder characterized by multiple arthrogryposis, facial anomaly camptodactylia and pulmonary hypoplasia. Half of cases of this disorder are thought to be due to autosomal recessive inheritance and the remaining half are sporadic cases. Today, genetic analysis is quite advanced but the cause gene(s) of this disorder has not been identified. We here report a case of one couple's children with PSS. METHODS: See below RESULTS: During the first pregnancy, the fetus was observed to have no stomach bubble and also showed polyhydramnios, leading us to suspect esophageal atresia. Caesarean section was performed at 34 weeks of pregnancy, but the baby died 8 hours after birth due to sever pulmonary hypoplasia. This and the baby's appearance resulted in a diagnosis of PSS. The second pregnancy followed the same course and PSS was diagnosed prenatally by ultrasound, MRI and chromosomal analysis. Although emotional distress of parents was greater, the parents were saved for healthy third baby. CONCLUSIONS: Their features of PSS develop from a sequence of deformational changes related to decreased or absent fetal movement.The phenotype is similar to Trisomy 18, but the chromosome of this syndrome is a normal karyotype, so it was the decisive factor in diagnosis. Also, image inspection such as fetal ultrasound and MRI is extremely important for diagnosis of this syndrome. At present the genome of parents and every offspring are subjected to analysis by a next generation DNA sequencer. We expect that the gene(s) responsible for PSS will be identified in the near future.

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Prognostic value of prenatal ultrasound diagnostic features in congenital fetal lung lesions

Choi Wah Kong, Wai Hang Chung, William To

Dept of Obstetrics & Gynaecology, United Christian Hospital, Hong Kong, Hong Kong SAR

OBJECTIVES: To evaluate the prognostic value of prenatal diagnostic features in congenital fetal lung lesions in predicting the perinatal outcome and need for neonatal surgery. METHODS: A retrospective review of all cases with antenatal diagnosis of congenital fetal lung malformations from January 2000 to December 2016 was performed in a single tertiary obstetric unit. Ultrasound records of serial antenatal assessment, outcome of these pregnancies, and neonatal and subsequent follow-up records were reviewed to assess the accuracy of various antenatal ultrasound features in predicting perinatal outcome and the need for surgery. Parameters assessed include gestation at first diagnosis, size and location of lesion, laterality, mediastinal shift, hydropic changes, macro/microcystic, CCAM volume ratio (CVR) and associated abnormalities. RESULTS: Of 77153 deliveries over the study period, 28 congenital lung lesions were diagnosed (incidence of 0.036%). All fetuses had normal karyotype and 3 had concurrent structural abnormalities. Four had pregnancy termination, 2 with concurrent structural defects, 1 with hydrops and 1 with macrocystic cysts. The remaining were carried to term without specific antenatal intervention. Four babies underwent surgical excision of the lesion by 2 years of age. The most reliable prognostic factors for livebirth and without need for subsequent surgery were absence of hydrops, CVR < 1.5 at any gestation, a regressing CVR and macrocysts < 3 cm at any gestation. CONCLUSIONS: Congenital fetal lung lesions were associated with a good general outcome in most cases. The spectrum of severity we observed was apparently milder than that reported in the literature. Antenatal USG predictors can be developed in predicting the need for subsequent surgery in these babies.

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A case of fetal portal vein-hepatic artery malformation with cardiomegaly

Megumi Nakanishi, Takahiro Seyama, Yuzuko Oya, Naomi Ooka, Miyoko Terao, Kota Takei, Kimiyo Takagi, Shiro Yoshida

Nagano Children's Hospital, Nagano Japan, Azumino-shi, Nagano-ken, Japan

OBJECTIVES: Portal vein-hepatic artery malformation is a very rare vascular abnormality. A delay in diagnosis may result in thrombosis, heart failure, and hepatic encephalopathy due to liver dysfunction. We report a case of fetal portal vein-hepatic artery malformation. METHODS: The patient was referred because of fetal cardiomegaly at 32 weeks of gestation. An ultrasound examination revealed that the ductus venosus and the inferior vena cava were absent. Dilation of the portal and hepatic vein and anastomosis of the hepatic artery and vein were confirmed. We suggested polysplenia syndrome. RESULTS: At postnatal ultrasound examination, while the presence of the inferior vena cava was confirmed, we diagnosed a portal vein-hepatic artery malformation. For the hepatic vein aneurysm, anticoagulant therapy was initiated 3 days after birth. No changes were observed in the aneurysm, including thrombus formation. CONCLUSIONS: Prenatal diagnosis will enable the initiation of postnatal treatment, immediately after childbirth, which may lead to the improvement in the prognosis of newborn babies.

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Helical CT pelvimetry visualizes high quality imaging in lower irradiation dose than plain x-ray pelvimetry

Hideaki Sawai, Tomoko Ueda, Mariko Kamihigashi, Yumi Murakami, Hiroaki Shibahara

Hyogo College of Medicine, Nishinomiya-shi, Hyogo, Japan

OBJECTIVES: We evaluated the fetal dose from helical computed tomography (CT) pelvimetry to applicate helical CT pelvimetry in a clinical setting. METHODS: A feto-maternal phantom and bone models were specially designed for dosimetry. Dosimetry was done in plain X-ray and CT. Suitable setting for helical CT pelvimetry was conducted from the phantom study. Practical measurements were done to apply the technique in a clinical setting. RESULTS: The measured dose from plain x-ray pelvimetry was 1.32mGy. Estimated minimum exposure dose for diagnosable helical CT pelvimetry was 0.81mGy. The proper tube voltage for helical CT pelvimetry was 80kVp. In practical measurements, 5 pregnant women underwent helical CT pelvimetry. Computed tomography dose index volumes (CTDIvol) of 5 pregnant women were 1.77mGy, 1.25mGy, 1.05mGy, 0.79mGy and 0.81mGy. CONCLUSIONS: Helical CT pelvimetry enables us to make accurate measurements and reduce the fetal exposure dose compared to conventional plain x-ray pelvimetry.

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Comparison of umbilical cord occlusion methods: Radiofrequency ablation versus laser

Mira Abdel-Sattar[1], Andrew Chon[1], Arlyn Llanes[1], Lisa Korst[2], Joseph Ouzounian[3], Ramen Chmait[1]

[1]*University of Southern California, Pasadena, California, United States*
[2]*Childbirth Research Associates, North Hollywood, California, United States*
[3]*University of Southern California, Los Angeles, California, United States*

OBJECTIVES: To compare outcomes between two different umbilical cord occlusion (UCO) methods: ultrasound-guided radiofrequency ablation (RFA) versus fetoscopic-guided laser photocoagulation. METHODS: A retrospective study was performed on all monochorionic-diamniotic multiple gestations that underwent UCO with RFA or laser from 2006-2016. During this time, there was a shift from laser to RFA due to operator preference and equipment availability. In bivariate analysis, patients treated with RFA were compared to those treated with laser. Patient and operative characteristics associated with intrauterine fetal demise (IUFD) and 30-day survival of the normal co-twin were identified and tested in multiple logistic regression models. Odds ratios (OR) and 95% confidence intervals are reported. RESULTS: Of 60 UCO cases, 18 (30%) underwent RFA and 42 (70%) underwent laser surgery. Use of the RFA method was associated with co-twin IUFD within 24 hours of surgery (3/18 [16.7%] vs 0/42 [0%], p = 0.0238) and at any time after surgery (6/18 [33.3%] vs 1/42 [2.4%], p = 0.0021). In the models, patients who underwent RFA were *more* likely than patients who underwent laser surgery to have an IUFD of the co-twin (OR 13.2, 1.23-142.62, p=0.0331) when controlling for TRAP vs non-TRAP, gestational age at the procedure, and estimated fetal weight percentile of the co-twin. CONCLUSIONS: Despite technical advantages of RFA compared to laser surgery, including ease of surgical access and decreased invasiveness, RFA appeared to be associated with an increased risk of post-procedure fetal demise. Before recommendations can be made regarding the optimal UCO treatment modality, further study is warranted.

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Twin-Twin transfusion syndrome: Controversy regarding the definition of recipient polyhydramnios

Ramen Chmait[1], Andrew Chon[1], Lisa Korst[2], Arlyn Llanes[1], Eftichia Kontopoulos[3], Ruben Quintero[3]

[1]*University of Southern California, Pasadena, California, United States*
[2]*Childbirth Research Associates, North Hollywood, California, United States*
[3]*Florida International University, Miami, Florida, United States*

OBJECTIVES: The definition of twin-twin transfusion syndrome (TTTS) uses a cut-off of the maximum vertical pocket of amniotic fluid in the recipient twin (MVP-R) of ≥ 8 cm. However, some authors have argued that an MVP-R of ≥ 10 cm should be used beyond gestational age (GA) of 20 weeks. The aim of this study was to address whether using a different MVP-R before or after 20 weeks is warranted. METHODS: Consecutive patients with twin pregnancies and a GA ≥ 20 weeks referred for TTTS who elected to undergo laser surgery (n=270) were studied. The patients were divided into two groups: 1) Group I: MVP-R ≥ 8 and < 10 cm; and 2) Group II: MVP-R ≥ 10 cm. All patient characteristics were tested against the outcomes of dual survivorship and the survivorship of at-least-one twin. In addition to the Criteria group and Quintero stage, those characteristics that were statistically significant at a p-value ≤ 0.10 were eligible for inclusion in logistic regression models. RESULTS: 14.1% were in Group I and 85.9% in Group II. Both groups had similar preoperative characteristics, including Quintero Stage (I: 23.7% vs. 25.9%; II 18.4% vs. 18.5%; III 44.7% vs. 44.7%; 13.2% vs. 12.5%; p=0.9928). Of the Stage III/IV patients, 14.5% were in Group I. Group I patients were more likely to have donor intrauterine growth restriction (81.6% vs. 64.7%, p=0.0418) and to have an earlier GA at surgery [21.1 (20.1-24.7) vs. 22.3 (20.0-28.7) weeks, p=0.0007]. Perinatal outcomes were similar between the two groups. Logistic regression models showed no differences in outcomes by Criteria group when adjusted by risk factors. CONCLUSIONS: Restriction of the definition of TTTS to MVP-R ≥ 10 cm beyond 20 weeks is unwarranted. Such decision is arbitrary and results in approximately 15% of TTTS patients to be misdiagnosed or underdiagnosed, including Stage III-IV TTTS patients. A unifying criterion of an MVP-R of ≥ 8 cm eliminates such ascertainment bias and allows recommendation of appropriate management.

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Type II and III congenital pulmonary airway malformation (CPAM) with hydrops treated in utero with percutaneous sclerotherapy

Andrew Chon[1], Lisa Korst[2], Mira Abdel-Sattar[1], Arlyn Llanes[1], Joseph Ouzounian[3], Ramen Chmait[1]

[1]*University of Southern California, Pasadena, California, United States*
[2]*Childbirth Research Associates, North Hollywood, California, United States*
[3]*University of Southern California, Los Angeles, California, United States*

OBJECTIVES: To present the antenatal course and postnatal outcomes of hydropic fetuses with congenital pulmonary airway malformation (CPAM) type II and III treated with sclerotherapy. METHODS: This was a retrospective study of 8 patients with a prenatal diagnosis of CPAM type II or III with secondary hydrops treated *in utero* with percutaneous sclerotherapy using 5% ethanolamine oleate. All patients underwent a detailed ultrasound evaluation including measurement of the CPAM volume ratio (CVR). Patients were divided into those without and with an intrauterine fetal demise (IUFD). Results are expressed as mean (standard deviation) or median (range). RESULTS: Gestational age (GA) at initial sclerotherapy was 22.0 (19.6 - 31.4) weeks; 3 patients underwent 2 procedures. IUFD occurred in 4 cases; 2 died on postoperative day #1; 2 died > 6 weeks after the second sclerotherapy. Preoperative CVR was 3.6 (1.6 – 7.8) in survivors and 2.7 (1.7 – 4.7) in those with IUFD. The mean volume of ethanolamine at the initial sclerotherapy procedure was 3.25 (1.50) (mL) in survivors and 7.50 (1.91) (mL) in IUFD cases. The GA at delivery of the 4 survivors was 38.4 (37.4 – 39.3) weeks; all underwent postnatal resection. CONCLUSIONS: Percutaneous sclerotherapy for CPAM type II and III is technically feasible, but further studies are needed to determine the efficacy, optimal dose of sclerotherapy agent, and safety of this procedure.

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Risk factors for fetomaternal bleeding after laser therapy for twin-twin transfusion syndrome

Andrew Chon[1], Lisa Korst[2], Brendan Grubbs[3], Eftichia Kontopoulos[4], Ruben Quintero[4], Ramen Chmait[1]

[1]*University of Southern California, Pasadena, California, United States*
[2]*Childbirth Research Associates, North Hollywood, California, United States*
[3]*University of Southern California, Los Angeles, California, United States*
[4]*Florida International University, Miami, Florida, United States*

OBJECTIVES: To quantitatively measure and determine risk factors for fetomaternal bleeding (FMB) after laser surgery for TTTS. METHODS: A retrospective study was conducted of Rhesus (Rh)-D negative patients that underwent laser surgery for TTTS and had a postoperative Kleihauer-Betke (KB) test performed. Patients with and without post-operative detectable fetal red blood cells on KB testing were compared to determine risk factors for FMB. RESULTS: Of the 60 Rh(D) negative patients that were eligible for the study, 26 (43%) had a positive KB test. Patients with higher Quintero TTTS stage (III or IV) were more likely to have a positive KB (21/26 [80.8%] vs. 17/34 [50.0%], p = 0.02). Stage III-Recipient and III-Recipient/Donor patients were more likely than all other patients to have a positive KB test (14/21 [66.7%] vs. 12/39 [30.8%], OR = 4.50 [1.27-16.54], p = 0.0162). Gestational age at surgery, placenta location, operative time, total laser energy and total number of vascular communications were not risk factors for a positive KB test. CONCLUSIONS: FMB is relatively common after laser surgery for TTTS, with stage III-Recipient and III-Recipient/Donor patients at highest risk. Further study is required to elucidate the mechanism of FMB in these patients.

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Donor twin head circumference growth after laser surgery for twin-twin transfusion syndrome

Andrew Chon[1], Mary Rose Mamey[2], Douglas Vanderbilt[2], Sheree Schrager[2], Ramen Chmait[1]

[1]*University of Southern California, Pasadena, California, United States*
[2]*Children's Hospital Los Angeles, Los Angeles, California, United States*

OBJECTIVES: To determine the growth pattern between the preoperative and 2 years of age head circumference (HC) among children treated with in utero laser surgery for twin-twin transfusion syndrome (TTTS). METHODS: This was a retrospective analysis of a prospectively enrolled cohort study. Pregnancy and child-level data were collected from children treated between 2007 and 2010 as part a study to assess cognitive performance at 2 years (±6 weeks) corrected age. Prior to laser, donor and recipient twin preoperative HCs were measured by ultrasound using Hadlock percentiles. Repeat HCs were measured at 2 years corrected age and converted into percentiles using WHO reference ranges. A repeated-measures ANOVA was used to examine HC growth between preoperative and two years. The mean differences in HC percentiles and absolute measurements were calculated. RESULTS: 99 children (56 families) were evaluated. The mean recipient and donor twin HC percentiles preoperatively and at 2 years of age were 51st vs. 20th (p =.050) and 60th vs. 49th (p=.676), respectively. Among the 86 dual survivors, the recipients had a mean 13 mm larger HC than their donor siblings at preoperative assessment, which decreased to 5 mm net difference at corrected age of 2 years. Conversion to age-appropriate HC percentiles demonstrated that the net difference between the recipient and donor twin decreased from a 31 percentile increase preoperatively to a 6 percentile increase at 2-years of age. CONCLUSIONS: The mean HC percentiles were significantly smaller for the donor compared to the recipient twin at the preoperative assessment, but not at the 2-year assessment. This finding demonstrates that there is catch-up growth of the donor head circumference after laser surgery.

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Rabbit reproductive toxicology studies of maternal uterine artery Ad.VEGF-DΔNΔC gene therapy for placental insufficiency show no evidence of harm

Dauzat Caroline[1], Carine Bansard[1], Armelle Nguyen[1], Sandrine Bentz[1], Francois Spézia[1], Roy Forster[1], Mark Lees[2], Anna David[3]

[1]*CiToxLAB France, Evreux, France*
[2]*Magnus Growth, London, United Kingdom*
[3]*Institute for Women's Health, University College London, London, United Kingdom*

OBJECTIVES: Placental insufficiency leading to fetal growth restriction (FGR) affects 1:300 pregnancies, is untreatable causing neonatal morbidity and death. Reduced uterine blood flow is a cause. Transduction of uterine arteries in normal and FGR animal models using an adenovirus vector (Ad) encoding VEGF isoforms, increases uterine blood flow and improves fetal growth, suggesting this therapy may benefit human FGR. Using the pregnant rabbit which has a similar placentation to third trimester human placenta, we studied the effect of high and low dose Ad.VEGF-D$^{\Delta N\Delta C}$ vector administration to dams and their offspring. METHODS: Time-mated female primigravid New Zealand white rabbits (18-20 weeks old, 3.3-3.7kg, n=76) underwent X ray guided serial catheterisation and bilateral uterine artery injection under general anaesthesia on day 19 postconception. Dams received intravascular injection (0.8mL) of either Ad.VEGF-D$^{\Delta N\Delta C}$ vector (high or low dose n=26 per group) or formulation buffer (n=24). Dams and fetuses underwent post-mortem analysis at 3 or 10 days post-op (n=4 per time point, per group). The remaining dams delivered and at either 28 days post-op (day 16 postnatal) or day 90 post-op (day 79 postnatal) the pups underwent post-mortem analysis for toxicity, pup survival and biodistribution. RESULTS: Dam post-op survival was good; two dams died, one during surgery and one the day after surgery; two dams miscarried post-op and one dam was killed for breathing problems during recovery. The livebirth and weaning indices were similar in the three groups. There was no vector detectable by RT-PCR in a broad range of fetal or pup tissues at any time point. As expected, vector was detected in falling concentrations in the maternal tissues as the post-op days advanced. Histological analysis of tissues and detection of antibodies by ELISA is underway. CONCLUSIONS: Uterine artery injection of high or low dose Ad.VEGF-D$^{\Delta N\Delta C}$ vector in pregnancy using interventional radiology techniques does not appear to adversely affect rabbit dam or pup survival. There is no evidence of vector spread across the placenta to the pups. These results are encouraging for the next step to the clinic, which is to perform a phase 1 clinical trial of Ad.VEGF-D$^{\Delta N\Delta C}$ for treatment of severe early onset FGR.

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Prenatal treatment of severe fetal hemolytic disease due to anti-M alloimmunization by serial intrauterine transfusions

Lin Li[1], Linhuan Huang[1], Guangping Luo[2], Yan-min Luo[1], Qun Fang[1]

[1]Fetal Medicine Center, Department of Gynaecology and Obstetrics, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China
[2]Guangzhou Blood Center, Guangzhou, China

OBJECTIVES: Fetal hemolytic disease is a common cause of fetal hydrops and fetal morbidity and mortality. Despite its relatively low frequency, the anti-M IgG antibody is one of the causes of severe fetal anemia and intrauterine death; only a few cases have been reported. METHODS: A pregnant woman had a history of three intrauterine deaths due to fetal hydrops. A diagnosis of severe fetal anemia attributed to anti-M alloimmunization was confirmed in her fifth pregnancy. She came to our center for regular monitoring at the beginning of the pregnancy. Five intrauterine transfusions were performed to correct moderate to severe fetal anemia throughout her pregnancy. A male infant, delivered at the 36th gestational week, received two transfusions after birth, and no neurologic abnormalities were observed until the child was six months of age. RESULTS: Our observations show that the characteristics of fetal hemolytic disease secondary to anti-M alloimmunization may be somewhat different from those of disease secondary to anti-D alloimmunization, as shown by the increased rate of hemoglobin decline and the negative DAT result observed in this study. As soon as fetal hydrops or intrauterine death due to anti-M alloimmunization is diagnosed, intensive surveillance involving both antibody titers and Doppler ultrasound measurements should be carried out. IUT is the most effective method of treating fetal anemia due to anti-M alloimmunization. CONCLUSIONS: Anti-M alloimmunization is an important cause of severe fetal hemolytic disease. The characteristics of fetal hemolytic disease due to anti-M alloimmunization may be somewhat different from those of disease due to anti-D alloimmunization.

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Severe fetal anemia due to anti-E and anti-c antibody salvaged through a series of intrauterine transfusions: A case report

Si Li[1], Linhuan Huang[2], Yan-min Luo[2], Qun Fang[2]

[1]Department of Obstetrics and Gynecology, the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China
[2]Fetal Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China

OBJECTIVES: Rh maternal-fetal alloimmunization is one of the major etiology for hemolytic disease of the fetus and newborn. Even though antigenicities of rhesus E and c antigens are relatively weaker than rhesus D (Rh D) antigen, antibodies to these antigens can occasionally result in severe fetal anemia, hydrops, or even intrauterine death. In order to identify the clinical features of Rh E and Rh c alloimmunization, we described a case of severe fetal anemia due to anti-E and anti-c antibody salvaged through a series of intrauterine transfusions. METHODS: This is a case of a pregnant woman with a history of one healthy child and three intrauterine deaths with fetal hydrops. The diagnosis of severe fetal hydrops caused by anti-E and anti-c alloimmunization, was confirmed before her fifth pregnancy in our center. As expected, the fifth fetus suffered from maternal alloimmunization attributed to anti-E and anti-c antibody. The monitoring of fetal anemia was accomplished by the MCA-PSV with ultrasound and cordocentesis for hemoglobin level of the affected fetus. Seven ultrasound-guided intrauterine transfusions were performed to correct moderate to severe fetal anemia during this pregnancy. RESULTS: A female baby, weighing 2.64kg, was finally delivered at the 36th gestational week, with good general condition at birth. She was treated by three blood transfusions, but no blood exchange transfusion after birth. There was no neurologic abnormality observed in the follow-up four months. CONCLUSIONS: Anti-E and anti-c alloimmunization also can cause severe fetal and neonatal hemolytic disease. This case highlights the significance of antenatal antibody screening for women with a history of fetal hydrops even in Rh(D) positive females. Close monitoring for moderate to severe fetal anemia can provide chances of timely intrauterine transfusion and save the anemia fetus.

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Continuous amnioinfusion via a subcutaneously implanted port system with PPROM and anhydramnions <28 + 0 weeks of gestation (first experiences in Russia)

Yuri Naberezhnev[1], Vitaly Tskhay[2], Tatiana Lobanova[2], Anna Dudina[2], Michael Tchirikov[1]

[1] Universitätsklinik und Poliklinik für Geburtshilfe und Pränatalmedizin, Zentrum für Fetalchirurgie Universitätsklinikum Halle, Martin-Luther-Universität Halle-Wittenberg , Halle (Saale), Germany
[2]Voronezh State University, Voronezh, Krasnoyarsk, Russian Federation

OBJECTIVES: Preterm premature rupture of membranes (PPROM) represents one of the main causes of high neonatal mortality and morbidity. Amnitotic fluid loss with oligo/ anhydramnios is associated with extreme preterm birth, pulmonary hypoplasia and the „fetal inflammatory response syndrome“ (FIRS). The aim of the study was to prolong the PPROM-delivery interval without to increase the risk of FIRS using continuous amnioinfusion with artificial amniotic fluid “flush out” therapy, through a subcutaneously implanted port-system. METHODS: Continuous amnioinfusion (100 ml/h, 2,4 L/24h, SDP (4±2 cm) via a subcutaneously implanted port system (Tchirikov Perinatal Port System, PakuMed GmbH, Germany) in patient with PPROM and oligohydramnios on 25 weeks’ gestational using hypoosmotic amniotic fluid like solution . The treatment was conducted according with developed protocol including verification of classical PPROM (PAMG-1, SDP, amnio-dye test), antibiotic therapy with Amoxicillin. The study included bacteriological investigation, leukocytosis, CRP and procalcitonin control. RESULTS: The «flush-out» method decreased leukocytosis from 12.5 to 9.13 x 10^9/l, procalcitonin from 0.5 to 0 ng/ml, a CRP from 0.8 to 0.4 mg/dl. The vaginal biotope was changed (Lactobacillus crispatus to Escherichia coli and Streptococcus salivarius). On the 10th day after PPROM we performed the c-section because of increased leukocytosis (14.68*10^9/L) and procalcitonin greater than 0.5 ng/ml. The concentration of CRP-was normal 0.8 mg/dL The newborn l (girl, weight - 900 g. , length-35 cm, APGAR 1-3-5). The newborn didn’t have any sings of FIRS (leukocytosis – 8.23*10^9, CRP–0,1 mg/dL, procalcitonin–0.6 ng/ml). CONCLUSIONS: The «flush-out» method decreased the PPROM-delivery interval for 10 days. The new method could be certainly use for the treatment of PPROM with oligo/anhydramnion < 28/0 weeks’ gestation. The prospective randomized international trial has been started 2016. Sponsoring: Center of Fetal Surgery, University Hospital Halle (Saale) and Russian Science Foundation, grant no. 15-15-00137.

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Effects of indomethacin therapy for short course tocolysis in the perioperative period on fetuses undergoing neural tube defect repair. A single center experience

Ahmed Nassr[1], William Whitehead[1], Hadi Erfani[1], Jimmy Espinoza[1], Rodrigo Ruano[2], Magdalena Cortes[1], Nancy Ayres[1], Oluyinka Olutoye[3], Darrell Cass[3], Sundeep Keswani[1], Timothy Lee, Christopher Cassady[4], Amy Mehollin-Ray[4], Zhoobin Bateni[1], Stephen Welty[5], Michael Belfort[6], Alireza Shamshirsaz[1]

[1]*Baylor College of Medicine, Houston, Texas, United States*
[2]*Baylor College of Medicine and Texas Children's Hospital Fetal Center, Houston, Texas, United States*
[3]*Michael E. DeBakey Department of Surgery, Texas Children's Fetal Center and Baylor College of Medicine, Houston, Texas, United States*
[4]*Department of Radiology, Texas Children's Fetal Center and Baylor College of Medicine, Houston, Texas, United States*
[5]*Department of Pediatrics, Texas Children's Fetal Center and Baylor College of Medicine, Houston, Texas, United States*
[6]*Department of Obstetrics and Gynecology, Baylor College of Medicine and Texas Children's Hospital Fetal Center, Houston, Texas, United States*

OBJECTIVES: To assess the safety of short course indomethacin therapy used for tocolysis on the fetal heart in fetuses undergoing neural tube defect (NTD) repair. METHODS: A prospective cohort of 50 fetuses with normal preoperative fetal echocardiography (FE) who underwent fetal NTD repair between December 2011 and June 2016 had FE on postop day (POD) 1 and 2 to detect constriction of the ductus arteriosus (DA). All patients received indomethacin for tocolysis (50 mg PO followed by 25 mg PO q 6 hours for 2 days). The POD 2 doses were withheld if ductal constriction was detected on POD 1. Multivariate regression analysis adjusting for various risk factors (estimated fetal weight percentile, maternal weight at surgery, and smoking) was carried out. RESULTS: 50 patients underwent fetal NTD repair. Demographic characteristics are presented in the table. Constriction of DA was detected in 7 fetuses (14%). In 6, this occurred on POD 1, and in 1 on POD 2. Multivariate regression analysis showed that the only independent risk factor for predicting constriction of DA was maternal weight at surgery < 60 kg (p-value 0.02). Constriction was mild in 5, and moderate in 1 fetus. All affected fetuses showed marked improvement within 1 day of stopping indomethacin. In 1 case, constriction was severe with partial reversal after 6 days and complete reversal after 15 days. CONCLUSIONS: Postop maternal indomethacin therapy after fetal surgery requires careful daily monitoring due to the risk of fetal ductal constriction. The association of fetal DA constriction and low maternal body weight warrants further investigation and suggests that lower doses of indomethacin may be recommended for perioperative tocolysis in this subset of population.

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Table: Baseline demographic characteristics

			Ductal constriction	No ductal constriction
Race	White	N (%)	7 (14%)	39 (78%)
	Black		0 (0%)	4 (8%)
Ethnicity	Hispanic		5 (10%)	35 (70%)
	Non-Hispanic		2 (4%)	8 (16%)
smoking	Yes		0 (0%)	10 (20%)
	No		7 (14%)	33 (66%)
Maternal weight	≥60 kg		4 (8%)	39 (78%)
	<60 kg		3 (6%)	4 (8%)
EFW <10 percentile	Yes		0 (0%)	4 (8%)
	No		7 (14%)	39 (78%)
Fetal gender	Male		5 (10%)	19 (38%)
	Female		2 (4%)	24 (48%)
Maternal age		Median (IQR)	29 (22-30)	27 (24-31.3)
Gravidity			2 (2-4)	2 (1-3)
Parity			1 (0-3)	1 (0-1)
GA at surgery			25.4 (24.6-25.6)	24.8 (23.8- 25.4)
BMI			23 (23-24)	29.5 (25-33)
Operative time			235 (196-257.3)	173 (139.8-269)

GA, gestational age; BMI, body mass index; IQR, interquartile range

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The impact of social factors in the use of genetic diagnostic methods

Assel Arginbekova

Association of Kazakhstan MD Genetics, Astana, Akmola Region, Kazakhstan

OBJECTIVES: An environmental and climatic environmental factor negatively affects the reproductive health of the population, and complicates the forecast risk of formation of congenital malformations in the fetus, taking into account characteristics of the region. This is the case in the 3 regions. **Methods**: In the East Region held 339 invasive diagnostic procedures and identified 22 chromosomal aberrations. The maximum concentration of heavy metals exceeds the permissible limits. There were nuclear tests. In Kyzylorda region held 100 and identified 7 anomalies. The region is characterized as an area of the Aral Sea ecological disaster. In South Kazakhstan region held 133, revealed 11 anomalies. In this area, the maximum permissible limits of contaminants in rampant. In the capital of Kazakhstan Astana city held 474, revealed 31 anomalies. Due to large migration from all over the country. Total in Kazakhstan conducted 3197, revealed 236 anomalies (7,4%). RESULTS: Replacing the traditional screening and diagnostics, which are not always safe and reliable, do not always reduce the risk of having a seriously ill child and reduce the level of prenatal mortality by NIPT. In general, Kazakhstan has only one private clinic held NIPT. Kazakhstan hospitals do not apply this method, because of the expensive equipment and low return on investment. The cost of this procedure is defined for the citizens of the $ 600 and more, depending on the selected diagnostic panel. This amount includes not fare well. Medical centers are engaged in a blood transport to foreign countries. CONCLUSIONS: The low level of socio-economic development of regions in the Republic has an average cost of living in $70. This leads to the development of the social vulnerability of the population, and consequently to the low coverage of screening. The complex ecological zone in the Republic complicates the forecast risk of formation of congenital malformations and chromosomal anomalies in the fetus adapted to the region. Deficiency of iodine, calcium, selenium and other vitamins is an essential reason for the development of pathologies in children.

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Trends of genetic prenatal testing in Japan; 1998-2016

Aiko Sasaki[1], Haruhiko Sago[1], Hiroshi Yoshihashi[2], Shigehito Yamada[3], Hidehiko Miyake[4], Nobuhiro Suzumori[5], Fumio Takada[6], Hideaki Masuzaki[7], Fumiki Hirahara[8], Koji Kugu[9], Ikuo Konishi[3]

[1]*National Center for Child Health and Development, Tokyo, Japan*
[2]*Tokyo Metropolitan Children's Medical Center, Fuchu, Tokyo, Japan*
[3]*Kyoto University, Kyoto, Japan*
[4]*Kyoto University Hospital, Kyoto-city, Kyoto, Japan*
[5]*Nagoya City University Hospital, Nagoya, Aichi, Japan*
[6]*Kitasato University, Kanagawa, Japan*
[7]*Nagasaki University, Nagasaki, Japan*
[8]*National Hospital Organization Yokohama Medical Center, Yokohama, Japan*
[9]*Tokyo Metropolitan Bokutoh Hospital, Sumida, Tokyo, Japan*

OBJECTIVES: In Japan, prenatal genetic tests are not covered by the public health care plan and it is difficult to get the number of these tests. With the recent availability of non-invasive prenatal testing (NIPT) and conventional first trimester screening testing, the landscape of prenatal testing in Japan has been changed. To understand the present status of genetic prenatal testing in Japan, we surveyed the number of these tests from 1998 to 2016. METHODS: This study surveyed Japanese main five clinical laboratories. We estimated the total number of genetic prenatal tests; maternal serum markers (including first trimester combined test), amniocentesis (AC), and chronic villus sampling (CVS) from 2009 to 2016 using the annual share of tests of these laboratories in 2008 from the previous nationwide survey. We combined the data from this new 8-year survey with those of the previous survey reported in 2011.This work was supported by funds from grant MHLW-14428175. RESULTS: The share of main five laboratories in 2008 of each testing was about 80-95%. The numbers of each prenatal tests of maternal serum markers (including first trimester combined test) were 20,700 in 2010, 24,100 in 2012, 29,800 in 2014, 35,900 in 2016. The numbers of AC and CVS were 15,200 and 1000 in 2010, 20,000 and 1,700 in 2012, 20,700 and 2,100 in 2014, and 18,600 and 2,000 in 2016, respectively. CONCLUSIONS: The number of maternal serum markers (including first trimester combined test) increased from 20700 to 35900 over last six years. While, the numbers of AC increased until 2014, then decreased in 2015 and 2016. The number of CVS also decreased in 2016. Although the prevalence of genetic prenatal testing is still less than 10% of one million births, the number of invasive prenatal test was reduced in Japan due to the introduction of new non-invasive prenatal testing in 2013.

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The role of traditional serological screening and non-invasive prenatal testing in Down syndrome screening in China

Kaibo Liu, Hongyan Xu, Juan Zhao, Ying Pan, Xue Zhang

Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing, China

OBJECTIVES: We sought to describe the variation trend of coverage rate and detection rate of Down syndrome screening from 2010-2016 in Beijing, China, and tried to find the improving direction of current screening model and the influence of Non-invasive Prenatal Testing (NIPT) to traditional serological screening. METHODS: In Beijing, Down syndrome screening tests are provided to every pregnant woman at antenatal care visits. Descriptive methods was used to describe the coverage of Down syndrome screening with both traditional Serological screening and NIPT. The detection rate and incidence during previous 6 years was analyzed. In October 2015, NIPT was officially introduced into antenatal care visit as first line screening method when the pregnant women ask for or the second line screening method for the pregnant women with result falls into the window between the cut-off value to 1/1000, or for the women who qualified for but refuse prenatal diagnosis. RESULTS: The incidence of Down syndrome has increased from 7.3/100,000 in 2010 to 15.2/100,000 in 2016, the coverage rate of Down syndrome screening increased from 73.5% to 98.1%. The detection rate of traditional serological screening has improved from 74.7% to 84.9%. The detection rate of NIPT in 2016 was 98.93%. For all the Down syndrome in 2016, 52.47% was delectated by NIPT. CONCLUSIONS: The incidence of Down syndrome has increased significantly previous two years, which is considered as the consequence of the increasing proportion of advanced maternal age due to China's relaxing of its more than 3-decade-old one-child family planning policy to a universal two-child policy. NIPT has played a critical role in Down syndrome detection since more than half of the patients was detected by it. The detection rate of traditional serological screening has reached more than 80%, although the rate was not as high as NIPT's, thaditional serological screening will still be an effective screening method in China considering the high cost of NIPT.

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The value of a national infrastructure to facilitate translational research and timely innovation in care pathways for patient benefit

Lyn Chitty[1], Gary Nestor[2], Anna David[3], Andrew Shennan[4], Alex Henderson[5]

[1]Genetics 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
[2]NIHR CRN Coordinating Centre, Newcastle upon Tyne, United Kingdom
[3]Institute for Women's Health, University College London, London, United Kingdom
[4]South London CRN, London, United Kingdom
[5]NIHR Genetics Specialty Group Lead, Newcastle upon Tyne, United Kingdom

OBJECTIVES: England's National Institute of Health Research (NIHR) Clinical Research Network's (CRN) facilitates delivery of high-quality clinical research across all NHS partners (community and acute care) by providing efficient support for the initiation and delivery of funded translational research in the NHS for patient benefit. It supports delivery of studies in as many units across the country as needed to expedite results, ensure good socio-economic inclusion, research in rare diseases and equity of access for patients to research to facilitate timely change in care where appropriate. Here we review the impact of the CRN in translating research in prenatal care. METHODS: A retrospective analysis of research study and participant numbers in the NIHR CRN portfolio in Reproductive Health and Genetics categories was performed, particularly focusing on studies being delivered in the last five years. There are 15 local CRNs (LCRN) forming the National CRN to give total population coverage in England. Studies were subdivided into those delivered in one, or more than one LCRN and outputs from multicentre studies with specific impact on national guidelines for services delivering prenatal diagnosis or screening, or those which delivered therapeutic input reviewed. RESULTS: Since January 2012, NIHR reproductive health and genetics portfolios have supported 444 and 261 studies, recruiting 375,948 and 162,133 participants respectively. 42% and 54% of studies were delivered across multiple CRNs with one recruiting at 72 trusts across all CRNs. These studies have resulted in significant changes in NHS practice including NICE guidance for cffDNA for fetal RHD typing in pregnancy, implementation of aCGH for diagnosis of fetal anomalies, NHS approval for cffDNA for fetal sex determination and monogenic disorders diagnosis, NHS implementation of NIPT for aneuploidy, and improved screening and prevention of preterm birth, fetal growth restriction and pre-eclampsia. CONCLUSIONS: Recently technological and therapeutic advances have enabled the potential for significant changes in prenatal care in pregnancies complicated by fetal anomalies and other adverse outcomes. However, for such changes to be safely implemented into care pathways there must be careful and, where possible/appropriate, large scale evaluation to deliver safe and efficient services that are acceptable to patients. We conclude that the NIHR CRN infrastructure has transformed the delivery of translational research to expedite implementation into NHS practice of these new approaches to prenatal diagnosis,

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screening and treatment for the benefit of patients, and reinforces the value of collaboration in research.

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The first case of canakinumab administration during pregnancy for cryopyrin associated periodic syndrome

Makiko Egawa[1], Naoyuki Miyasaka[1], Ayako Fudono[2], Mayumi Yokota[1], Asuka Hirose[1], Kohsuke Imai[1], Masaaki Mori[1], Tetsuo Kubota[1]

[1]*Tokyo Medical and Dental University, Tokyo, Japan*
[2]*Department of Pediatrics, Perinatal and Maternal Medicine, Tokyo, Japan*

OBJECTIVES: Cryopyrin-associated periodic syndrome (CAPS) is a rare autoinflammatory condition caused by overproduction of interleukin-1β (IL-1β). CAPS is associated with heterozygous mutations in *NLRP3*, the gene encoding cryopyrin, which regulates the production of IL-1β, and canakinumab, a human anti-IL-1β monoclonal antibody that selectively blocks IL-1β has been licensed in Japan since 2011 for CAPS. However, there is no report of its efficacy and safety during pregnancy. So we report here the clinical course of a Muckle-Wells syndrome (MWS) patient administered canakinumab during pregnancy. METHODS: The patient was a 32-year-old Japanese woman in her first pregnancy. At 23 years, she was diagnosed as MWS by NLRP3 mutation (p.H312P) and typical clinical findings, 3 years after the administration of canakinumab, she became pregnant. RESULTS: We thought clinical control of MWS was necessary for the continuation of pregnancy and the benefit exceeded the possible risk of canakinumab for the fetus. And also, given the autosomal-dominant nature of this syndrome, genetic counseling regarding the risk of affected offspring was offered. With her written informed consent, canakinumab was continued during the pregnancy. Her symptoms were stable, and she gave birth to a 2,994-g girl by emergency cesarean delivery. No morphological abnormalities were observed, but her baby was found to have the same *NLRP3* mutation. CONCLUSIONS: This is the first report of the administration of canakinumab during pregnancy in a patient with CAPS. We also measured the concentration of canakinumab in umbilical cord blood and neonatal blood, and its results indicate the active placental transfer of the drug. In addition, if there is a possibility that the use of canakinumab during pregnancy is effective for the affected fetus, prenatal diagnosis by chorionic villus sampling (CVS) might be considered.

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A retrospective analysis of treatment of severe hemolytic disease of the fetus in a single center

Linhuan Huang[1], Zhi-ming He[1], Yi Zhou[1], Yanmin Luo[2], Qun Fang[1]

[1]*Fetal Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*
[2]*The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*

OBJECTIVES: Fetal anemia is caused by Rhesus (RhD) sensitization as a result of RhD incompatibility during pregnancy. This process leads to fetal anemia, and in severe cases can progress to edema, ascites, heart failure, and death. Intrauterine transfusion (IUT) is now well under control and improves the survival of foetuses monitored for fetal anemia. The purpose of this study is to report on the pregnancy and neonatal outcome of IUT for Rh-alloimmunization. METHODS: Retrospective cohort study of all IUT for Rh-alloimmunization in the First Affiliated Hospital of Sun Yat-Sen University, between June 2006 to June 2016. Maternal characteristics, antibody type, antibody titer, the influence of hydrops, gestational age, in addition to perinatal and neonatal outcomes were reviewed. RESULTS: Seventy-one intrauterine intravascular transfusions were performed on 24 patients with severe fetal anemia due to Rh-alloimmunization. The mean period of gestation and hematocrit at first transfusion was 27.2 ± 4.7 weeks and 19.0%±8.8%, respectively. Average number of transfusions was 3 (range 1-6) per patient. Six of the fetuses had hydrops fetalis before the first IUT. Of the 6 hydropic fetuses, 3 survived, 2 intrauterine death during procedures, one termination because of fetal cerebral hemorrhage. Eighteen fetuses without hydrops all survival after IUT and had a good prognosis. Overall survival was 21/24 (87.5%) and mean period of gestation at delivery was 35.3±2.3 (range 31.0-38.5) weeks. CONCLUSIONS: Intrauterine transfusions appear to be an effective mode of therapy improving perinatal survival in fetuses with anemia due to Rh-alloimmunization. Hydropic fetuses had a poor outcome, patients should be referred to specialist centers prior to the development of hydrops.

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Influence of oral probiotic and cranberry capsules on vagina flora in pregnant women with group B streptococcus colonization

Lin Kang[1], Chung-Hwan Chen[2], Meng-Hsing Wu[3]

[1]Department of Obstetrics and Gynecology, National Cheng Kung University Medical College and Hospital, Tainan, Taiwan
[2]Kaohisung Medical University, Kaohisung, Taiwan
[3]Department of Obstetrics and Gynecology, College of Medicine and Hospital, National Cheng Kung University, Tainan, Taiwan

OBJECTIVES: Streptococcus agalactiae (Group B Streptococcus; GBS) infected approximately about 10-30% of pregnant women. Vertical GBS exposure from the vagina or rectum of a colonized woman causes neonatal early-onset GBS disease during the process of delivery. Bacteriocins of some lactobacilli strains can inhibit GBS growth. The aim of this study is to investigate for the influence of oral cranberry and probiotic capsules containing Lactobacillus rhamnosus RF-1 and Lactobacillus acidophilus RF-2 on vagina flora in pregnant women with GBS infection. METHODS: A prospective, randomized, double-blind clinical trial with oral administration of lactic acid bacteria was performed. One hundred and thirty-two healthy pregnant Taiwanese women were orally administrated with (1) daily probiotics with 1 capsule that contained 20 billion colony-forming units (cfu) of Lactobacillus rhamnosus RF-1 and Lactobacillus acidophilus RF-2 (n=44), (2) daily one capsule containing cranberry only (n=43), (3) daily one placebo capsule (n=45). The capsules were used from 35-37 weeks of gestation till delivery. Vaginal and rectal cultures were performed again at admission for delivery. RESULTS: Only 90 pregnant women completed the study (36 in the probiotics group, 28 in the cranberry group, and 26 in the placebo group). The Escherichia coli (E. coli) culture rate significantly decreased both in the probiotics and cranberry group while the GBS culture rate only significantly decreased in the probiotics group but not in the cranberry group. Both cranberry and probiotics can significantly decreased colony count of E. coli and GBS while only probiotics can significantly decreased colony count of Gardnerella. CONCLUSIONS: The combination of probiotic Lactobacillus rhamnosus RF-1 and Lactobacillus acidophilus RF-2 is not only safe for daily use in pregnant women, but also it can alter vaginal flora, especially potential pathogenic bacteria. Besides, cranberry can also reduce the potential pathogenic bacteria of vagina in pregnant women.

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Genomics training among obstetrics and gynecology residents: The missing link

Manesha Putra[1], Jing Dai[2], Gerald Feldman[3]

[1]*Wayne State University/Detroit Medical Center, Detroit, Michigan, United States*
[2]*CS Mott Center for Human Growth and Development, Wayne State University Department Of Obstetrics and Gynecology, Detroit, Michigan, United States*
[3]*Wayne State University School of Medicine, Detroit, Michigan, United States*

OBJECTIVES: Our objective in this study is to investigate the current state of formal genomics education during obstetrics and gynecology (OB/GYN) residency training in the United States. METHODS: We performed a review of curricula/tracks published by all American Council for Graduate Medical Education (ACGME) accredited OB/GYN residency programs' website during the month of December 2016. Information regarding availability and duration of genomics rotation and other non-core OB/GYN rotations were collected (ultrasound, breast health and family planning) along with number of geneticist on faculty and the number of routine genomics conference. Generalized linear model was then used to compare the duration of genomics rotation and other non-core OB/GYN rotations while controlling for number of residents and program type (university-based vs. non-university-based. RESULTS: There were 256 ACGME accredited OB/GYN residency programs, 136 programs (50.8%) were university based. Among these programs, only 1 program (0.4%) had a dedicated genomics rotation and only 33 programs (13.9%) had shared genomics rotations. When compared to other non-core OB/GYN rotations while controlling for number of geneticist in faculty, program type and number of residents, genomics rotation the mean duration of genomics rotation was significantly less than ultrasound (0.08 vs. 0.57 month, $p < 0.05$) and family planning (0.08 vs. 0.41 month, $p<0.05$) but there were no difference compare to breast health (0.08 vs. 0.16 month, $p=0.16$). CONCLUSIONS: In this study, we found that only limited number of ACGME accredited residency programs offer formal genomics rotation regardless of the type of the program. When compared to other non-core OB/GYN rotations such as ultrasound and family planning, genomics rotation was significantly shorter in duration. With the advent of genomics technology, the importance of this field has been increasing significantly in the day-to-day general OB/GYN practice. Despite this, there seems to be a decrease in the emphasis on genomics during OB/GYN residency training. It is possible that similar finding could be observed in other primary care specialties.

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The extent of chromosomal mosaicism influences the clinical outcome of in vitro fertilization treatments

Francesca Spinella[1], Biricik Anil[2], Sara Bono[1], Ettore Cotroneo[1], Marina Baldi[1], Laura Diano[2], Maria Giulia Minasi[3], Ermanno Greco[3], Francesco Fiorentino[1]

[1]*Genoma, Molecular Genetics Laboratories Group, Rome, Italy*
[2]*Genoma Group, Rome, Italy*
[3]*Reproductive Medicine, European Hospital, Rome, Italy*

OBJECTIVES: Embryonic mosaicism is a phenomenon characterized by the presence of two or more genetically distinct cell lineages, typically one with a chromosome abnormality and the other possessing a normal chromosome constitution. In a recent published study, we have demonstrated that mosaic embryos hold the potential to implant and result in the birth of healthy babies. Therefore, the transfer of these embryos is now offered as an option for women who undergo in vitro fertilization (IVF) resulting in mosaic embryos but no euploid embryos. Here we investigated whether the extent of chromosomal mosaicism might influence the development potential of mosaic embryos. METHODS: The transfer of mosaic embryos at different aneuploidy percentage was offered to 73 women for whom IVF had resulted in no euploid embryos between May 2013-March 2016. The comparison of the clinical outcome obtained after transfer of mosaic embryos with low (<50%) and high (≥50%) aneuploidy percentage, was performed in order to assess a statistically significant difference in the development potential between the two groups. To obtain reference curves for determination of mosaicism percentage we assessed 114 diploid/aneuploid mosaic reconstructed samples (10–90% mosaicism) using both next generation sequencing (NGS) and array-comparative genomic hybridization (array-CGH) techniques. RESULTS: Transfers of mosaic embryos with ≥50% of chromosomally abnormal cells resulted in a live birth rate of 16.7% and involved a miscarriage rate of 10%. In contrast, mosaic embryos with<50% mosaicism resulted in a live birth rate of 39.5%, with a miscarriage occurring in 7.0% of the transfers. All pregnancies that went to term have a normal karyotype. A comparison of the clinical outcomes between the groups showed a significantly higher ongoing clinical pregnancy rate/embryo transfer (39.5% vs 16.7%; p=0.036), and baby born rate (41% vs 17%;P=0.027) in embryos with aneuploidy percentage <50% compared to embryos with a mosaicism level>50%. CONCLUSIONS: Mosaic embryos with low aneuploidy percentage (<50%) have higher chances to result in healthy babies born compared to embryos with higher mosaicism levels (≥50%). The results of this study further confirm that mosaic embryos can develop into healthy euploid newborns. We demonstrated that the extent of mosaicism affects the IVF success rate. Priority for transfer should be given to mosaic embryos with low mosaicism levels.

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Implantation properties of endometrial mesenchymal stem cells isolated from menstrual fluid are modulated by menstrual cycle hormones

Sebastian Venegas[1], Stephanie Acuña[1], Pía Venegas[1], Manuel Varas[2], Sebastian Illanes[2]

[1]Department of Obstetric and Gynaecology and Laboratory of Reproductive Biology, Faculty of Medicine, Universidad de los Andes, Santiago, Chile
[2]Universidad de los Andes, Santiago, Chile

OBJECTIVES: Mesenchymal stem cell population derived from endometrial tissue have been identified in the menstrual blood (MenSCs). Current research have been focused on their properties as stem cells, however, the response of this cells to hormones involved in the menstrual cycle, and how these hormones could modulate processes occurring in the endometrial niche such as implantation are still unclear. The objective of this study was evaluate the properties of MenSC under estrogen (E2) and progesterone (P4) hormones mimicking menstrual cycle, and evluate markers of the window of implantation. METHODS: Menstrual fluids were obtained from 4 healthy women, and MenSCs were isolated and cultured. Three different condition were evaluated, 1) MenSCs without hormones stimulation (control), 2) MenSCs stimulated with estrogen and progesterone (E2+P4), 3) MenSCs pre-aconditionated with E2 24 hours before the stimulation with E2+P4 (E2-E2+P4). Migration and angiogenesis were evaluated by scratch assay and endothelial cell tube formation assay respectively. Genes involved in the implantation process (LIF, VEGFa, TGF-β, and HOXA-10), and angiogenesis (FGF2 and Endoglin) were measured by quantitative RT-PCR in each group. RESULTS: The functional assays showed an increase in the angiogenesis capacity of the MenSCs pre-aconditionated with E2 and stimulated with E2+P4 in comparison with de control group. Migration assay didn´t show any difference among the different groups. On the other hand, MenSCs stimulated with E2-E2+P4 showed an increase in the gene expression of LIF, FGF2, and Endoglin, but not in VEGFa, HOXA-10, and TGF-β in comparison with de control group. CONCLUSIONS: The hormones involved in the menstrual cycle modulated the expression of the angiogenic factors FGF2 and Endoglin, and correlate with an increase in the angiogenic properties of MenSCs. Also the stimulation with the hormones induces the expression of LIF. The modulation of MenSCs with hormones could highlight their potential role in the implantation process.

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Comprehensive chromosomal analysis of blastomeres with developmental arrest

Takema Kato[1], Sachie Nishiyama[2], Yukio Nishiyama, Kaori Yoshikai[3], Yukino Matsuda[3], Tomio Sawada[3], Hikari Yoshizawa[4], Hiroshi Furukawa[4], Haruki Nishizawa[4], Maki Kato[4], Asuka Kato[4], Hiroki Kurahashi[5]

[1]*Fujita Health University, Toyoake-shi, Aichi, Japan*
[2]*Nishiyama Clinic, Tsu, Japan*
[3]*The Sawada Women's Clinic Fertility Center, Nagoya, Japan*
[4]*Fujita Health University, Toyoake, Japan*
[5]*Division of Molecular Genetics, Institute for Comprehensive Medical Science, Fujita Medical University, Nagoya, Japan*

OBJECTIVES: Approximately 50% of human embryos produced by *in vitro* fertilization (IVF) cannot develop into blastocyst and arrest during early embryo development. Previous studies have shown the high rate of chromosomal abnormality in arrest embryos by chromosome microarray or FISH analyses. Hence chromosomal abnormality has been considered to be one of the important factors causing developmental arrest of embryo. Here, we examined what type of chromosomal abnormalities influence embryo development. METHODS: Embryos were produced by routine ICSI procedures. The embryos that had not reached the blastocyst stage at day 7 post insemination were defined as arrested embryos. After obtaining the informed consent, a total of 24 arrested embryos were collected for chromosomal analysis. Patients' average age was 37.5 years (30-45). Embryos were subjected to whole genome amplification (WGA) using SurePlex DNA Amplification System. Nextera libraries for next-generation sequencing were prepared from WGA and subsequently sequenced with a VeriSeq PGS system by MiSeq. The sequencing data were analyzed by a BlueFuse Multi analysis software and evaluated the chromosomal contents. RESULTS: We found that 23 out of 24 blastomeres with developmental arrest (95.8%) showed aneuploidy. Euploidy was observed only in one blastomere (4.2%). Simple constitutional trisomy was observed in one blastomere (1/23), while the remaining blastomeres showed multiple complex chromosomal aneuploidy with mosaicism (22/23). We also found that the different mosaic ratio among chromosomes and speculate that the chromosomal aneuploidy might occur at different cleavage stages. CONCLUSIONS: Most of the developmental arrest was due to aneuploidy of mitotic origins. Defective mitotic checkpoint, a surveillance system to ensure accurate chromosome segregation, might contribute to error-prone segregation leading to chromosomal instability that induces developmental arrest during early embryonic stage.

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Successful use of preimplantation diagnosis for a rare de novo chromosomal rearrangement involving a ring chromosome 11 with a neocentromere

Diane Myles Reid[1,2], David Chitayat[1], Elena Kolomietz[2]

[1]*Mount Sinai Hospital, Toronto, Ontario, Canada*
[2]*University of Toronto, Toronto, Ontario, Canada*

OBJECTIVES: Neocentromere formation can occur following interstitial deletions, providing stability to the broken chromosome fragments. In cases involving paracentric deletions, this occurs through formation of a marker ring chromosome. Neocentromere formation has been associated with most chromosomes, but only once previously reported with 11p. We report (1) the first case of neocentromere formation in a ring chromosome formed following a paracentric interstitial deletion of 11p11.2p14.3, involving both the WAGR and PSS critical regions and (2) the successful use of PGD for this rare chromosomal rearrangement. METHODS: Prenatal findings were assessed with detailed ultrasound and amniotic fluid chromosomal microarray and karyotype analysis. Postnatal investigations included fetal autopsy as well as parental microarray and karyotype analysis. Preimplantation genetic diagnosis was completed with microarray analysis (BlueGnome 24sure+) on trophectoderm cells biopsied from Day 5 or 6 blastocysts. RESULTS: The proband presented at 19 weeks gestation with fetal anomalies on ultrasound, including ACC, colpocephaly and bilateral parietal foramina. Her medical history included bilateral congenital cataracts, bilateral iris coloboma and hypothyroidism. ArrayCGH revealed an 11p11.2p14.3 deletion in the fetus including the genes EXT2, ALX4, WT1 and PAX6. The same deletion, plus mosaicism for a ring chromosome 11 with a neocentromere, was detected in the mother. The proband subsequently had IVF/ICSI/PGD. Transfer of a euploid/balanced embryo resulted in a clinical pregnancy. Amniocentesis confirmed normal chromosomal microarray and a male karyotype with the 11p11.2p14.3 deletion plus ring chromosome 11 with a neocentromere. CONCLUSIONS: To our best knowledge, this is the first reported case of neocentromere formation in a ring chromosome formed following an interstitial deletion of 11p11.2p14.3. PGD was successful in identifying euploid/balanced embryos suitable for transfer as well as embryos that were unbalanced and had inherited the interstitial deletion on chromosome 11. PGD with appropriate genetic counselling should be considered as an option for families diagnosed with rare chromosomal rearrangements.

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Caveats to transfer of embryos with autosomal monosomy by preimplantation genetic screening: Implications for embryo selection of a systematic literature review of autosomal monosomy survivors

Rosemary Reiss[1], Megan Bunnell[2], Louise Wilkins-Haug[1]

[1]*Brigham and Women's Hospital, Boston, Massachusetts, United States*
[2]*Geisel School of Medicine, Dartmouth College, Hanover, New Hampshire, United States*

OBJECTIVES: Successful pregnancies have occurred following transfer of IVF-derived embryos reported as monosomic on preimplantation genetic screening (PGS) when no euploid embryos were identified. Such transfers are made on the assumption that these embryos, or at least their inner cell mass, may in fact be euploid, but if truly monosomic will lead to early miscarriage. We reviewed published case reports of liveborns with full or mosaic autosomal monosomies to anticipate risks of such embryo transfers. METHODS: PubMed and Ovid-Medline were systematically searched using the following terms: Full monosomy, Complete monosomy, Mosaic monosomy, Monosomy mosaicism and related MeSH subheadings to identify case reports of live-born individuals with autosomal monosomy for a whole chromosome, whether fully monosomic or mosaic. Identified cases were analyzed for autosomal monosomy type, testing methodologies and live-born phenotype. RESULTS: Forty-six reports describing 50 individuals with autosomal monosomy met the selection criteria: one case each of monosomy 14 and 16, three each for monosomy 15 and 18, five for monosomy 20, seven for monosomy 22, and 30 for monosomy 21. Apparently non-mosaic monosomy was reported only for chromosome 21. There were no reports with monosomy for the larger chromosomes 1 through 13, nor for chromosomes 17 or 19, autosomes with highest gene density. Most reported individuals had severe handicaps and died in infancy. Several survived into childhood or adolescence. Mosaic monosomy 20 was identified in one adult with recurrent stillbirth. CONCLUSIONS: Given potential for survival of handicapped individuals with monosomy for chromosomes 15, 18, 20, 21, and 22, and, albeit rarely, for chromosomes 14 and 16, transfer of embryos with PGS results apparently monosomic for these chromosomes should be undertaken only with great caution. Couples considering such transfers should receive extensive counseling. If ongoing pregnancies result from such transfers, cytogenetic studies sensitive enough to detect low frequency mosaicism should be performed on amniotic fluid samples to confirm euploidy.

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Reproductive management for a Chinese family with MUSD

Honghui Zhou[1], Yuanqing Yao[1], Yanqin You[1], Hui Wang[1], Fang Yang[2], Wei Dong[2], Zhiying Gao[3]

[1]*Department of Ggynaecology and Obstetrics, Chinese PLA General Hospital, Beijing, China*
[2]*BGI Shenzhen, Beijing, China*
[3]*Department of Obstetrics and Gynecology in General Hospital of People's Liberation Army, Beijing, China*

OBJECTIVES: Maple syrup urine disease (MSUD, OMIM #248600) is a rare autosomal recessive disorder with mental and physical retardation, feeding problems, and featured urinal odor. A couple who had a female proband child of MSUD came to our hospital for having a healthy child. This study aimed to develop a customized reproductive solution to fulfill this clinical demand by integrating preconception diagnosis, preimplantation genetic diagnosis (PGD) and prenatal testing into the management of preconception-to-neonate care. METHODS: We initially performed targeted sequencing in the couple and the proband child using a customer-designed panel to identify the MSUD causing mutations. After post-test counseling, the couple decided to receive in-vitro-fertilization (IVF) treatment and PGD. In each blastocyst, whole genome amplification of trophectoderm cells was performed, and single nucleotide polymorphism-based linkage analysis was used to identify the embryo free of disease. Amniocentesis was performed at 22^{+2} weeks of gestation, followed by Sanger sequencing and real-time PCR confirmation. After delivery, Sanger sequencing and tandem mass spectrometry were used to confirm PGD results using neonatal heel blood. RESULTS: Preconception diagnosis showed that the proband contained the compound heterozygous mutations of maternal p.Tyr131Cys (c. 392 a > G) and paternal dupEX2-4 in *BCKDHA* gene. During IVF, ten embryos were obtained for day 5 biopsy and PGD analysis showed, one blastocyst contained compound heterozygous mutations, three were carriers of paternal dupEX2-4 in *BCKDHA*, another one were suspected trisomy 16, and one was unconfirmed whether be a paternal mutation carrier. Two of the remaining four normal blastocysts were chosen to transplant. Eight weeks after transfer, a monochorionic diamniotic twin and an empty embryo sac were confirmed by ultrasound. At the 37th gestational week, a healthy twin was born, presenting normal results of metabolic examination and genetic confirmations. CONCLUSIONS: We successful provided a trajectory of reproductive testing for this couple, which led to the healthy birth of a twin. The integrated management strategy in this study demonstrates valuable experience in treating similar cases and potentially other monogenic disorders.

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Complimentary role of karyotype and microarray in assessing mosaic trisomy 5

Marie Discenza, Stephanie Guseh, Kathryn Gray, Louise Wilkins-Haug

Brigham and Women's Hospital, Boston, Massachusetts, United States

OBJECTIVES: To review a case of prenatal diagnosis of mosaic Trisomy 5, and identify an instance where karyotype and microarray were of clinical utility together. METHODS: A 38 year old G5P3013 pregnancy via IVF with preimplantation genetic screening (PGS) for aneuploidy due to advanced maternal age was seen for genetic counseling at 20 weeks gestation after fetal survey and echocardiogram showed double-outlet right ventricle, pulmonary atresia and complete AV canal defect, femur length 2.8 SD's below the mean, and overall growth lagging 2 weeks behind IVF dating. SNP Microarray (Affymetrix Cytoscan) on uncultured amniocytes was normal. Trio whole exome sequencing on cultured amniocytes and was normal. At 25 weeks gestation fetal MRI showed frontal bossing, truncated sacrum and prominent fetal clitoris and labia were observed. RESULTS: After review of prenatal testing and ultrasound findings, karyotype analysis was performed on cultured amniocytes to investigate whether a de novo balanced translocation would help direct specific areas of the genome for deeper sequencing. Trisomy 5 was observed in 4/20 metaphases across 2 separate cultures. Prenatal karyotype was mos 47,XX,+5[4]/46,XX[16]. Re-review of microarray studies identified low-level mosaicism which fell below the reporting threshold for this assay. Trisomy 5 was not observed upon re-review of PGS data. Blood karyotype at delivery was 46,XX with 40 cells scored. Placental karyotype was not available to address potential CPM. CONCLUSIONS: In the setting of any genetic test that is reported as normal, residual risks exist. At least 2 pregnancies with trisomy 5 and ultrasound abnormalities have been reported via karyotype on amnioctyes (Penchaszadeh et al., 1988 and Sciorra et al., 1992) which included IUGR, facial dysmorphism and congenital heart defects. Thorough pretest counseling about a test's purpose and limitations are important for informed consent. In the setting of an abnormal ultrasound, careful consideration of the information provided by the testing chosen should be considered as karyotype analysis; microarray and whole exome sequencing each have their strengths and weakness.

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Mosaic tetrasomy 18p leading to discordant cell-free DNA and invasive testing results: A case report

Stephanie Guseh, Kathryn Gray, Marie Discenza, Louise Wilkins-Haug

Brigham and Women's Hospital, Boston, Massachusetts, United States

OBJECTIVES: To review a case of apparently discordant prenatal testing results in a 39-year old G3P1012, who presented for consultation regarding a recent fetal diagnosis of mosaic tetrasomy 18p. METHODS: The patient had cell-free fetal DNA (cfDNA) testing performed at 11 weeks gestation, which was reported as high risk for trisomy 18. She subsequently had a normal nuchal translucency at 12 weeks and underwent chorionic villus sampling (CVS). Fluorescent in situ hybridization on the direct CVS confirmed trisomy 18, but the results from the cultured CVS two weeks later showed a normal karyotype of 46,XX. Given this discordance, the patient had a follow-up ultrasound at 17 weeks, which showed normal fetal anatomy. Cultured cells from the amniocentesis at that time demonstrated mosaic tetrasomy 18p, with 47,XX,i18p[4]/46,XX[22]. RESULTS: The patient was counseled that cfDNA testing is based on massive parallel sequencing, in which the additional material from a triplicated p-arm of chromosome 18 was at a level sufficient to cross the threshold for reporting an increased risk for trisomy 18. Because the FISH probes are pericentric, FISH could not distinguish between an additional chromosome 18 and the isochromosome of 18p. The abnormal cells with tetrasomy 18p were likely lost in culture, and the final CVS culture resulted in a normal karyotype. In the amniocentesis, the fetal fibroblasts were tested and allowed for the detection of mosaic iso(18p). CONCLUSIONS: The patient was counseled regarding the nuances of each technology that may have led to her seemingly discordant results. She was also counseled that the tests target different embryologic cell lines, including: trophoblasts for cfDNA and the direct sample from the CVS, mesenchymal core cells for the culture from CVS, and the inner cell mass for fibroblasts in the amniotic fluid. Though this pregnancy is ongoing and the outcome is yet uncertain, this case highlights the subtleties of commonly utilized tests and emphasizes the importance of understanding each technology when interpreting test results and counseling patients.

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Application of chromosomal microarray analysis in prenatal diagnosis for specific abnormalities detected by ultrasound

Ting Hu[1], Zhu Zhang[2], Jianmin Wang[2], Liu Hongqian[1], He Wang[2], Liu Shanling[1]

[1]*Prenatal Diagnosis Center, West China Second University Hospital, Sichuan University, Chengdu, China*
[2]*West China Second University Hospital, Sichuan University, Chengdu, China*

OBJECTIVES: Chromosomal microarray analysis (CMA) has recently been recommended as a primary prenatal diagnostic tool for a fetus with one or more major structural abnormalities identified on ultrasonographic examination. The aim of this study is to investigate the clinical outcome of CMA in prenatal diagnosis for specific abnormalities detected by ultrasound. METHODS: From September 2014 to January 2017, amniotic fluid samples from 1429 pregnancies with abnormal ultrasound results and without common aneuploidies were detected by CMA with Affymetrix® CytoScan™ 750K arrays. The gestational ages of the pregnancies were from 18 to 33^{+6} weeks. The pregnancies with gestational age between 18 and 28 weeks were simultaneously evaluated by traditional karyotyping, and for those whose gestational were above 28 weeks, rapid aneuploidy testing-- QF-PCR was performed at the same time. Abnormal ultrasound findings were stratified according to organ system involvement, including abnormalities in multiple organ systems, single structural ultrasound anomaly and soft markers. RESULTS: Among the 1429 samples, 81 cases (5.67%) were detected with pathogenic copy number variations (pCNVs) by CMA, including 6 Wolf-Hirschhorn syndrome, 5 Williams-Beuren syndrome, 5 22q11 deletion syndrome (Velocardiofacial / DiGeorge syndrome), 5 1q21.1 recurrent microdeletion, 6 16p13.11 recurrent microduplication and 5 16p13.11 recurrent microdeletion. Twenty-nine cases (10.55%) were detected with pCNVs in 275 cases with structural ultrasound abnormalities in multiple ogran systems, 33 cases (8.46%) were detected with pCNVs in 390 cases with a single structural ultrasound anomaly, and 19 cases (2.49%) were detected with pCNVs in 764 cases with soft markers. CONCLUSIONS: CMA could be considered as an effectively prenatal diagnostic tool, because it has prominent advantage over chromosomal karyotyping in the detection of chromosomal abnormalities in fetus with ultrasound detected abnormalities. However, it should be noted that CMA was unabled to identify balanced translocations.

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Processing and storage affects the measurement of cell free placental microRNAs (cffmiRNA) in maternal plasma

Luisa Olaya[1], Jonathan Hyett[2], Susan McLennan[1]

[1]*The University of Sydney, Sydney, New South Wales, Australia*
[2]*Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia*

OBJECTIVES: There is limited data about the effect of sampling and storage processes on the measurement of cffmiRNAs. The aim of this study was to investigate the stability of placental specific cffmiRNAs in maternal plasma. METHODS: Blood was collected from 12 pregnant women into two types of collection tubes (EDTA and RNA BCT) and kept at 4^{O}C or room temperature for up to 72 hours. Circulating cffmiRNA levels of seven placental specific miRNAs (reported previously) were measured using qRT-PCR. Expression of these cffmiRNAs was also assessed in samples from 9 non-pregnant women. Samples were spiked with cel-miR-39 to enable normalisation. RESULTS: Samples were collected at 18±4 weeks' gestation. Quantities of cffmiRNAs varied more as the time interval between sampling and processing extended. Levels of variability would be minimised by restricting this interval to a maximum of 48 hours. No significant difference in test performance was noted between the two types of tubes. Expression of three of the seven cffmiRNAs (miR-518b, miR-525 and miR-526a*) appeared to be specific to pregnancy; miR-518b was not identified in all pregnant samples. The remaining four miRNAs were not placental specific – although they were only expressed in low levels in non-pregnant samples. CONCLUSIONS: Samples transported in EDTA or RNA BCT tubes appear to be robust allowing reliable processing and measurement up to 48 hours after sampling. Some miRNAs that were previously described as being placental specific were also found in the plasma of non-pregnant women – albeit in low concentrations. This work has established an appropriate matrix for cffmiRNA sampling and processing for standardisation of future work.

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Beyond Down syndrome phenotype: Paternally derived isodicentric chromosome 21 with partial monosomy 21q22.3

Manesha Putra[1], Urvashi Surti[2], Jie Hu[2], Deanna Steele[3], Michele Clemens[2], Devereux Saller[2], Svetlana Yatsenko[2], Aleksandar Rajkovic[2]

[1]Wayne State University/Detroit Medical Center, Detroit, Michigan, United States
[2]Department of Obstetrics, Gynecology, and Reproductive Sciences, Magee-Womens Hospital, University of Pittsburgh, Pittsburgh, Pennsylvania, United States
[3]Genescreen, Bernardsville, New Jersey, United States

OBJECTIVES: Isodicentric chromosome 21 is a rare form of chromosomal rearrangement that may result in trisomy 21 as well as segmental monosomy for the terminal long arm of chromosome 21. In this report we describe prenatal diagnosis and neonatal follow-up of a fetus with a paternally derived de novo isodicentric chromosome 21 with a concurrent ~1.2 Mb deletion of 21q22.3 region, [46,XX,idic(21)(q22.3)]. METHODS: Fluorescence in situ hybridization (FISH) analysis for chromosomes 13,18, 21, X, and Y was performed on uncultured amniotic fluid cells. G-banded karyotypes were studied on cultured amniotic fluid cells and peripheral blood samples of both parents following routine protocols. DNA sample was extracted from cord blood for microarray analysis. Chromosomal microarray analysis (CMA) was performed using the 180K CGH+SNP whole genome oligonucleotide comparative genomic hybridization (aCGH) platform (ISCA design, Agilent, Santa Clara, CA) following manufacture's protocol. Array results were displayed by Cytogenomics v2.5.8 software using the human genome build GRCh37/hg19. RESULTS: Interphase FISH performed on uncultured amniotic fluid cells suggested the presence of trisomy 21. Chromosome analysis performed on the cultured amniotic fluid cells detected a female karyotype with an isodicentric chromosome resulting in trisomy 21 in all 20 cells analyzed, 46,XX,idic(21)(q22.3). This isodicentric chromosome showed two copies of the long arm of chromosome 21, joined at q22.3, along with both centromeres and the short arms. Microarray analysis performed on DNA extracted from the cord blood revealed a gain of copy number in the 21p11.2-q22.3 region of chromosome 21 and a loss of copy number in the terminal (21q22.3-qter). CONCLUSIONS: In this report, the infant presents with unusual phenotype of Down syndrome and additional defects such as esophageal atresia and tethered cord syndrome. The resulting phenotype in this infant might be a coalescence of the partial trisomy and monosomy 21, as well as homozygosity for idic(21). The utilization of chromosomal microarray in this case has enabled accurate characterization of a rare chromosome abnormality, facilitated further phenotype-genotype correlation, provided bases for clinical implications and possible future consequences of idic(21) in this child, and produced evidence for proposed molecular mechanism underlying this rearrangement.

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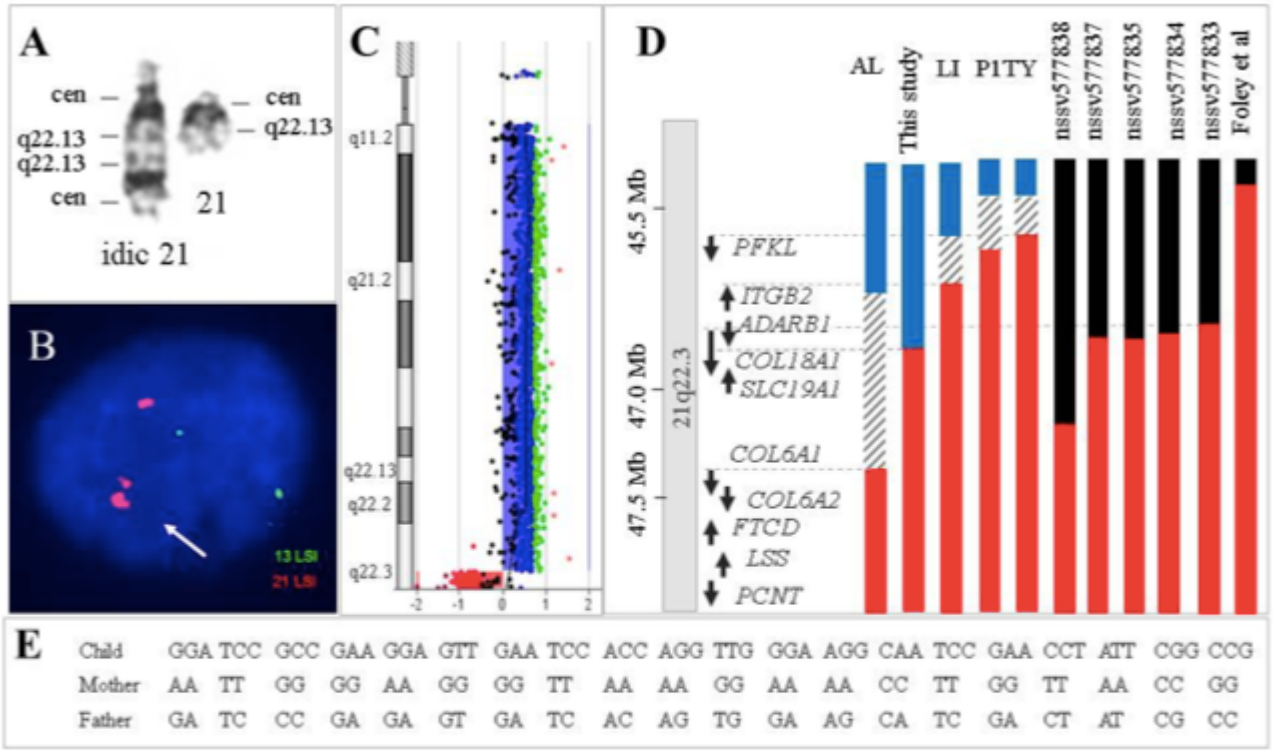


Figure 1. A. A partial G-banded karyotype showing a bisatellited isodicentric chromosome 21 (idic21) **B.** Fluorescence in situ hybridization (FISH) analysis **C.** Array CGH analysis detected a gain for the 21q11.2-q22.3 region **D.** Schematic view of the 21q22.3 deletion regions in five patients with isochromosome 21 including the current study, three patient (TY, AL, and LI) reported by Pangalos et al, (1992) and Egashira et al, (2008). We also included five cases with the 21q22.3 terminal deletions listed in the ClinGen CNVs database. **E.** Informative chromosome 21 SNPs are displayed for the child and both parents

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Fetal growth in multiple gestations: Effects of CVS followed by selective reduction at a single institution

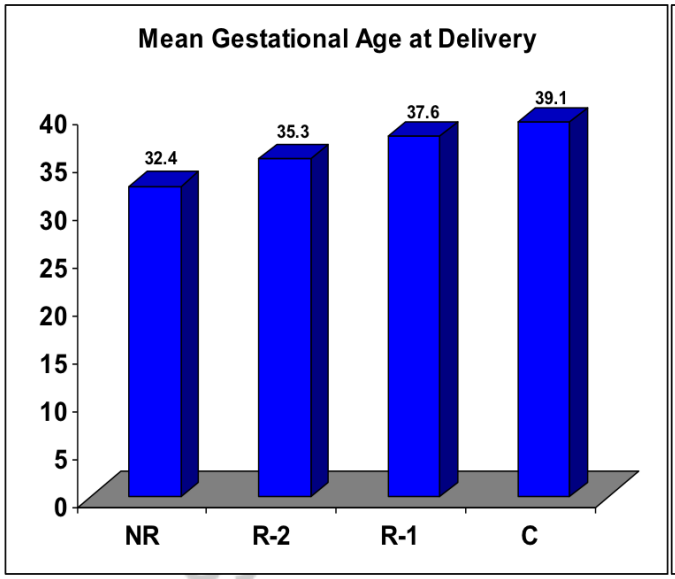
Steve Rad[1], Megha Gupta[2], Margareta Pisarska[2], John Williams III[2]

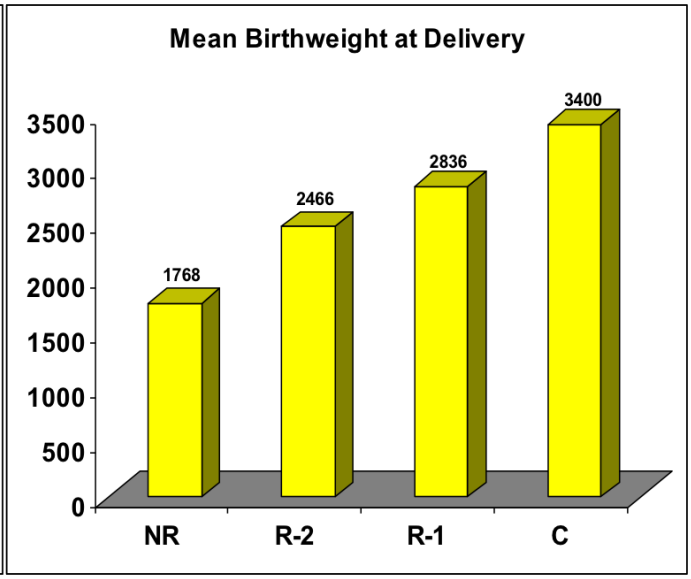
[1]*International Fetal Center at CMFM, and Cedars-Sinai Medical Center, Beverly Hills, California, United States*
[2]*Cedars Sinai Medical Center, Los Angeles, California, United States*

OBJECTIVES: To determine whether selective reduction in a cohort of triplet gestations undergoing CVS affects birth outcome. METHODS: 120 women with triplets and 278 singleton controls (C) who underwent CVS at 10–13 weeks at a single institution were studied. Patients were offered serial detailed ultrasound examinations. Patients were categorized based on the number of selective fetal reductions and compared to the main outcomes of gestational age (GA) at delivery, birth weight (BW) and fetal growth assessed by ultrasound measurement of CRL, BPD, HC, AC, FL & estimated fetal weight (EFW). ANOVA was used for statistical analysis. RESULTS: Of 120 patients with triplets, 13 elected to continue (NR), 69 reduced to twins (R-2), 38 reduced to singleton (R-1). Mean (± SEM) maternal age for C, NR, R-2 and R-1 groups were 38.6 ± 0.20, 38.1 ± 0.36, 37.7 ± 0.26, 38.1 ± 0.56 years (not significant). Mean GA at delivery for C, NR, R-2, R-1 groups were 39.1 ± 0.12, 32.4 ± 0.83, 35.3 ± 0.31, 37.6 ± 0.36 weeks ($p<0.05$). Mean BW in C, NR, R-2, and R-1 groups were 3400 ± 33.0, 1768 ± 131.2, 2466 ± 153.5, 2836 ± 87.2 grams ($p<0.05$). CONCLUSIONS: Selective reduction of triplets (singletons > twins) resulted in later delivery and larger BW than women continuing with triplets. Singleton controls had significantly later delivery and larger BW compared to triplets reduced to singletons. This suggests that multiple gestations may be pre-programmed for growth by the end of the first trimester. Further studies are in progress to evaluate maternal, fetal, and neonatal outcomes in these pregnancies.

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Clinical indications and diagnostic yield of prenatal testing for skeletal dysplasias

Elizabeth Kramer Dugan, Tara Funari, Gabriele Richard, Jeanne Meck, Lisa Vincent

GeneDx, Gaithersburg, Maryland, United States

OBJECTIVES: Using a multi-gene panel designed for detection of 29 skeletal dysplasias expected to present in the prenatal period, we aimed to determine (1) the diagnostic rate of the panel and (2) the ultrasound abnormalities that were associated with the highest diagnostic rate. METHODS: Fetal specimens from 116 pregnancies referred for prenatal diagnosis for suspected skeletal dysplasia following abnormal ultrasound were tested. A custom designed NextGen sequencing panel interrogating the complete coding regions of 22 genes and 1 known pathogenic variant in IFITM5 was used. Of these tested genes, 7 are associated with autosomal dominant inheritance (AD), 12 with autosomal recessive (AR), 2 with AD/AR, 1 X-linked dominant and 1 X-linked recessive. Identification of pathogenic or likely pathogenic variant(s) (P/LP) consistent with gene and disease mechanisms was considered diagnostic. Physician-provided clinical indications were retrospectively reviewed and compared to the outcome of genetic testing. RESULTS: Prenatal testing provided diagnostic results in more than half of fetuses (65/116; 56%) with causative variants identified in 10 genes associated with fetal skeletal dysplasias (FGFR3: n=27, COL1A1: n=12, COL1A2: n=7, COL2A1: n=6, SOX9: n=5, SLC26A2: n=4, DYNC2H1: n=1, EVC2: n=1, LEPRE1: n=1, FGFR2: n=1). The most common clinical test indications include short limbs (85%), abnormal ribs/small chest circumference (44%), bowed/fractured bones (34%), and upper limb abnormalities (13%). A diagnostic rate >80% was achieved when at least two indications were either: abnormal ribs/small chest, bowed/ fractured bones, or upper limb abnormalities. 22% of cases with isolated short limbs tested positive. CONCLUSIONS: The diagnostic yield of our prenatal skeletal dysplasia panel was 56%. Among fetuses with a positive test outcome, 58 of 65 (89%) had an autosomal dominant disorder with 46% (27/58) of these in *FGFR3* alone, and 7 of 65 (11%) an autosomal recessive disorder. Although these findings demonstrate genetic heterogeneity, only a few ultrasound findings (abnormal ribs/small chest circumference, bowed/ fractured bones, upper limb abnormalities) highly correlate with positive results for these distinct disorders. Our findings underscore the clinical utility of a comprehensive, multi-gene sequencing approach in the prenatal diagnosis of skeletal dysplasias.

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Smith-Magenis syndrome resulting from a 2.9 Mb deletions at 17p11.2 in a fetus inherited from its mother: First report from China

Li Wang, Nan Bai, Lina Liu, Ren Shu Min, Xiangdong Kong

The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

OBJECTIVES: Smith-Magenis syndrome (SMS) is a rare developmental disorder that affects many parts of the body, and it is caused by a deletion at the 17p11.2 that includes the *RAI1* gene or by *RAI1* gene point mutation. Virtually all occurrences are de novo, and are not associated with advanced maternal age or advanced paternal age. The purpose of this case report is to present the prenatal diagnosis and molecular cytogenetic characterization of SMS by single nucleotide polymorphism (SNP) microarray using uncultured amniotic fluid cells, and to explore the cause of 17p11.2 microdeletions on the fetus. METHODS: Amniocentesis was performed because of an expended posterior cranial fossa (13 mm) by sonographic examination and magnetic resonance imaging (MRI) of the fetal brain and skull. Uncultured amniotic fluid cells were analyzed by the whole-genome and high-resolution SNP microarray analysis. RESULTS: The results of SNP microarray using uncultured amniotic fluid cells showed that a 2.9 Mb deletions at 17p11.2, and the deletions were inherited from its mother which was identified by SNP microarray. Meanwhile, SNP microarray provided accurate information on the breakpoint regions and the size of 17p deletion. The deletions at 17p11.2 encompass the SMS "critical region", which includes many genes including *RAI1*. CONCLUSIONS: This case illustrates that SNP microarray is a rapid, precise and sensitive technology to identify small unbalanced chromosomal abnormalities and can be applied in prenatal diagnosis. To the best of our knowledge, this is the first report of an SMS resulting from deletions at 17p11.2 in a fetus inherited from its mother in mainland China.

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Clinical application of SNP array analysis in prenatal diagnosis of congenital heart disease

Wang Yan, Ping Hu, Zhengfeng Xu

Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University, Nanjing, China

OBJECTIVES: The objective of this study was to evaluate the clinical utility of prenatal microarray testing for chromosomal abnormalities in fetuses with congenital heart disease (CHD). METHODS: In this study, we conducted a prospective study to evaluate the feasibility of CMA for prenatal diagnosis of fetuses with CHD in clinical practice. We successfully analyzed 571 fetal specimens of amniocytes using SNP array. RESULTS: Clinically significant chromosomal abnormalities were identified in 127 (22.2%) cases, including 65 (11.4%) with aneuploidy, 20 (3.5%) with partial aneuploidy, and 42 (7.4%) with pathogenic microdeletion/microduplication. Variants of uncertain significance were obtained in 25 cases (4.4%). The frequency of chromosomal abnormalities in CHD plus extra cardiac anomalies group was significantly higher than isolated CHD group (48.0% vs. 15.8%, $P<0.05$). There was no significant difference between the detection rates in isolated CHD group and CHD plus soft marker group (15.8% vs. 22.7%). CONCLUSIONS: Our study suggests SNP array is a reliable, robust, and high-resolution technology for prenatal diagnosis of CHD in clinical practice. In addition, the presence of extra cardiac anomalies increases the risk of chromosomal abnormalities for fetuses with CHD, while the presence of soft marker is not related to the frequency of chromosomal abnormalities for fetuses with CHD.

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Incidence of soft markers at the time of "routine" second trimester ultrasound at a tertiary care centre in Canada

Marie Buitendyk[1], Stephanie Winsor[2], Michelle Tran[1]

[1]*McMaster University, Hamilton, Ontario, Canada*
[2]*McMaster University Medical Centre, Hamilton, Ontario, Canada*

OBJECTIVES: The goal of this study was to determine the current incidence of soft markers in our patient population undergoing “routine” ultrasound as compared to historical published rates. We anticipated an increased incidence of soft markers because of improved ultrasound resolution and increased diagnostic expertise, thus decreasing their significance in terms of risk for aneuploidy. The hope is for this data to be used as a counseling tool for our patients, to provide reassurance, and to help triage which patients need formal genetic counseling. METHODS: All patients undergoing routine anatomy ultrasound (18-24 weeks) at McMaster University Medical Centre from May 2 – July 18, 2016 were included in the study. The list of “Soft Markers” was predetermined. All scans were reported by Maternal Fetal Medicine or Radiology subspecialists. Scans for multiple gestations were analyzed per fetus. If ultrasounds were incomplete, these were tracked accordingly once completed. Patients were considered “high risk” if they were over 35, had a history of aneuploidy or had suspicious findings on a community ultrasound. Descriptive statistics were performed. RESULTS: A total of 255 fetuses/295 scans were included. There were 67 soft markers identified in 55 fetuses. 27.3% of fetuses with soft markers had more than one. The number of choroid plexus cysts identified was 17, giving an incidence of 6.7%. Incidence was also calculated for newer soft markers including linear insertion of the AV valves (0.4 %) and echogenic foci lateral to the stomach (0.8 %). An unexpected finding was a 41% incidence of “missed views”. Overall the incidence of soft markers in an identified "high risk" population was 27.0% and 9.9% in an identified "low risk" population. CONCLUSIONS: The incidence of “Soft Markers” in this study is higher than previously published rates. A common but anxiety provoking example is choroid plexus cysts. The incidence in our study was 6.7 % compared to the 1% cited in the SOGC Committee Opinion (2005). Data was collected on newer soft markers. The finding of a high proportion of incomplete scans has prompted an inquiry into work flow and quality assurance at our center. This data has provided updated information for our caregivers and patients and is guiding our referral triage process to the prenatal diagnosis clinic.

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Web-based education portal to increase women's understanding of non-invasive prenatal screening; A model to enhance future services?

Katie Ellis[1], Michael Bonifacio[2], Elizabeth Gosch, Karen Sivieng, Steven McArthur[2]

[1]*Genea, Deakin, Australian Capital Territory, Australia*
[2]*Genea, Sydney, New South Wales, Australia*

OBJECTIVES: In many cases, women are not adequately educated about NIPS prior to consenting to testing for a variety of reasons. Further, concerns have been raised that women are less able or less likely to refuse testing as there is no risk to the fetus. Genea designed an education portal to increase patient understanding of the technology as well as a Counselling Decision Aid to see if this form of education may assist women make the decision that was right for them. In addition, an education portal was also designed for General Practioners to aid understanding of the technology. METHODS: The Education portal was advertised through print articles and online, including using social media forums. Completed pre and post-video questionnaires were forwarded to the Genetic Counsellor for analysis. Analysis also included visits to those accessing the webpage but leaving the page before completing all questionnaires to help understand where and why women were leaving the page. RESULTS: Initial analysis at the time of abstract submission, indicates there is a position for web-based information particularly by women accessing services. Much greater awareness through various advertising mediums is needed to reach the target audience. Social media forums, such as Facebook, are very helpful in raising the profile and awareness of new education portals. CONCLUSIONS: Web-based education will be a useful resource to women with limited access to appropriately trained Clinicians and Genetic Counsellors and may help women engage with their health provider/s more readily. Further analysis is needed to elucidate if this method is as effective as face to face consultation and to understand if other methods of education are more helpful in this area.

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Genetic counseling with NIPT: Necessity of considering congenital diseases not targeted by the tests and false positives

Akimune Fukushima, Kayono Yamamoto, Tomoharu Tokutomi

Department of Clinical Genetics, School of Medicine, Iwate Medical University, Morioka, Iwate, Japan

OBJECTIVES: Non-invasive prenatal testing (NIPT) has a high negative predictive value for trisomy 21, 18, and 13. However, there is concern that clients will mistakenly assume that the characteristic of a high negative predictive value also holds for a positive predictive value, or will forget the possibility that fetuses may have congenital abnormalities other than those tested for in NIPT. It is therefore essential that these points be clearly explained in genetic counseling with NIPT. Here we report two cases with congenital disease other than those tested for in NIPT and a case that was false positive. METHODS: 1) NIPT negative cases: Of the pregnant women who underwent NIPT in our hospital from June 2015 to May 2016, an investigation was conducted of 141 patients. One case (partial tetrasomy of chromosome 15) of two of 108 reported neonate cases that had congenital abnormalities was further investigated. 2) NIPT false-positive case: Of 121 women who underwent NIPT in our hospital from February 2016 to January 2017, an investigation was conducted of one case that was false-positive for trisomy 13. The mother had received a full explanation during the first genetic counseling meeting, and provided consent. RESULTS: 1) NIPT negative cases: The rarity of partial tetrasomy of chromosome 15 was explained to the mother by the primary doctor of NICU. She stated that the connection with the medical genetics department she had had since the early stage of pregnancy was reassuring. We remain engaged with this family. 2) NIPT false-positive case: Despite previous counseling and further explanations of the positive NIPT results, the mother could not understand sensitivity, specificity, and negative/positive predictive values. Further genetic counseling alleviated her anxiety regarding the amniocentesis required for a definitive diagnosis. The baby had a normal karyotype. CONCLUSIONS: Although NIPT has a high negative predictive value, it is ultimately a screening test. It should always be kept in mind that NIPT results are not certain and that some cases cannot be foreseen from NIPT results. At the same time, arranging it so that clients can easily make contact with the genetic medicine department even after testing is important for genetic counseling with NIPT.

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Phenotypic heterogeneity in three families with typical microdeletion of chromosome 22q11.2 involving TBX1

Linhuan Huang[1], Yanmin Luo[2], Yi Zhou[1], Zhi-ming He[1], Qun Fang[1], Shaobin Lin[1]

[1]*Fetal Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*
[2]*The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*

OBJECTIVES: 22q11.2 deletion syndrome (22q11.2DS) is caused by a 1.5-3 Mb microdeletion of chromosome 22q11.2, typically characterised by conotruncal heart defects, cleft palate, thymic and parathyroid dysplasia with resulting immune defects, hypocalcaemia, and learning disabilities. The phenotypic variability associated with 22q11.2DS is well known. Here, we report on three unrelated adult patients with 22q11.2DS in three families. METHODS: All patients had nearly normal features and had a history of two foetuses/infants with congenital heart defects. Clinical examination, chromosomal analysis with FISH and genomic DNA analysis via microarrays were performed. We also searched for possible correlations between phenotypes, inheritance, and genotypes. RESULTS: The three patients demonstrated a de novo 22q11.2 deletion by high-density whole-genome single nucleotide polymorphism (SNP) microarray analysis. The deletions were approximately 2.5 Mb, with the identical breakpoint from 22:18,916,842 to 22:21,465,659. The molecular details and phenotypic features of the three patients with 22q11.2 deletion are compared. Moreover, additional CNVs were found in some patients and we try to make a genotype-phenotype correlation. CONCLUSIONS: We discuss underlying aetiology for the clinical heterogeneity in the phenotype in these patients and comment on the complexity of genetic counseling for microdeletion 22q11.2. In particular, we assessed the co-existence of additional CNVs and their contribution to phenotypic variations in 22q11.2DS.

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Prenatal diagnosis of a 11q deletion associated with abnormal ultrasound findings by single nucleotide polymorphisms microarrays

Linhuan Huang[1], Shaobin Lin[1], Yanmin Luo[2], Zhi-ming He[1], Yi Zhou[1], Qun Fang[1], Jianzhu Wu[1]

[1]*Fetal Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*
[2]*The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*

OBJECTIVES: The 11q deletion syndrome (Jacobsen syndrome) is a rare genetic disorder associated with numerous clinical features. To describe the prenatal phenotype of the 11q deletion syndrome and present the molecular characterization of the deletion in the case presented. METHODS: A 36-year-old primigravid underwent cordocentesis at 28 weeks gestation due to the presence of severe congenital heart disease at the ultrasound scan, including mitral atresia, tricuspid atresia, hypoplastic left heart, ventricular septal defect, and persistent upper venous cavity. Chromosomal analysis and genomic DNA analysis via single nucleotide polymorphisms (SNP) microarrays (CytoScan HD arrays, Affymetrix, California, USA) were performed. RESULTS: Prenatal karyotyping of cultured cord blood cells revealed a derivative chromosome 11, or der(11), with a deletion on the region of 11q24 and an addendum of a small chromosomal segment of unknown origin. SNP-Array has detected a 14.6 Mb duplication at 9p and a 14.7 Mb deletion at 11q. The maternal karyotype was subsequently found to be 46,XX,t(9;11)(p23;q24). Therefore, the fetus has inherited a derivative chromosome 11 derived from the maternal translocation, and her karyotype was 46, XX, der(11) t(9;11) (p23;q24) mat. Prenatal SNP-Array and karyotyping revealed an 11q deletion (Jacobsen syndrome). CONCLUSIONS: The 11q deletion syndrome (Jacobsen syndrome) probably underlie the complex heart defects detected in the fetus. Compared with routine karyotype analysis, aberrant regions can be identified with SNP-Array with greater resolution and accuracy. This has provided useful information for prenatal diagnosis and genetic counseling.

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Clinical application of SNP array analysis in spontaneously discharged products of conception: A prospective study

Linhuan Huang[1], Shaobin Lin[1], Jianzhu Wu[1], Zhi-ming He[1], Yanmin Luo[2], Yi Zhou[1], Qun Fang[1]

[1]*Fetal Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*
[2]*The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China*

OBJECTIVES: Cytogenetic analysis of products of conception is essential for the management of pregnancy loss, but the currently performed G-banding method is not necessarily applicable to spontaneously discharged products of conception because of poor quality for culture. Single nucleotide polymorphism (SNP) array, which has advantages over karyotyping, including higher resolution and dispensing with cell culture, it has been used routinely in pediatric and prenatal genetic diagnosis in clinical practice, but it has rarely been applied to miscarriage analysis. Therefore, the purpose of this study was to evaluate the advantage of high-resolution SNP array in identifying genetic aberrations in products of conception. METHODS: A total of 155 cases diagnosed as missed abortion underwent the dilation and curettage procedure. All 155 products of conception specimens, including 139 from first-trimester miscarriage and 16 from second-trimester miscarriage were collected consecutively. SNP array (CytoScan 750K arrays, Affymetrix, California, USA) was performed on these samples. RESULTS: SNP array was successfully analyzed in 152 cases (152/155, 98.1%) and failed in three cases because of substandard QC metrics probably resulted from poor DNA quality. SNP array yielded a 63.8% (97/152) abnormality rate. Trisomy was the major abnormality, accounting for 64.9% (63/97) of all abnormalities, including trisomy 16 (23.7%), trisomy 22 (10.3%), trisomy 13, trisomy 21, trisomy18, and trisomy 4. Monosomy accounted for 12.4% (12/97), including monosomy X and monosomy 21. Other chromosome abnormalities accounted for 18.6% (18/97), including triploidy, double trisomy, mosaicism, deletion/duplication, monosomy plus trisomy. Whole-genome UPD and likely pathogenic CNVs accounted for 3.1% and 1.0%, respectively. CONCLUSIONS: Our study demonstrated that SNP array had certain advantages including a higher success rate, detection of copy number variations and uniparental disomy. It is a reliable, robust, and high-resolution technology for genetic diagnosis of miscarriage in clinical practice, in particular, with a history of pregnancy loss who desire to investigate the cause for recurrent miscarriage.

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Changing indications for prenatal genetic services over the past decade: Implications for genetic counselling practice in a tertiary centre

Joanne Kelley, Melissa Graetz, Lisa Hui

Mercy Hospital for Women, Heidelberg, Victoria, Australia

OBJECTIVES: Prenatal genetic testing has been transformed by technological advances, including noninvasive prenatal testing (NIPT), chromosomal microarrays and specialised fetal imaging. We have previously reported an increase in major chromosome abnormalities detected in our unit between 2011-2015. Described as a balance of both science and art, the art of genetic counselling lies in exploring the psychosocial elements of genetic information, and often intensely emotional decision-making that ensues. Our aim was to analyse the indications for referral for genetic counselling in our tertiary maternity hospital over a 10 year period, and reflect on implications for genetic counsellors and service provision. METHODS: The numbers and indications for genetic counselling referrals in a tertiary maternity centre in Melbourne, Australia from 2006 to 2016 were retrieved from hospital databases, medical records, genetic files and procedural logbooks. Referrals were categorized into a single major indication for referral, including fetal abnormality on ultrasound, increased risk screening test result (including combined first trimester screening, NIPT, second trimester serum screening), advanced maternal age, family history of a genetic condition and prior history of pregnancy with a genetic abnormality. Statistical significance was analysed using the chi-squared test for proportions or trend as appropriate. RESULTS: There were significant changes in the most common indications for referrals over the study period, with recent years showing a significant increase in the number of referrals for fetal structural abnormalities from 20% (118/580) in 2011 to 34% (174/452) in 2016 ($p<0.0001$). In contrast, referrals for an increased risk screening result decreased from 32% (187/580) in 2011 to 23% (104/452) in 2016 ($p = 0.001$). Significantly, the annual rate of abnormal results from invasive prenatal diagnosis increased steadily from 4% (13/294) in 2006, to 25% (47/187) in 2016 ($p <0.0001$). CONCLUSIONS: The patient population referred to our genetic counselling service has increased in acuity and complexity over the past decade. The decrease in referrals for an increased risk from aneuploidy screening likely reflects the availability of NIPT, however a higher total number of women receive abnormal chromosome results after diagnostic testing. The increase in the number of women receiving a diagnosis of a fetal/chromosome abnormality has important implications for service provision to ensure that this vulnerable patient group receive appropriate care and psychological support. Supervision and self-care is essential for genetic counsellors to manage the increasingly demanding nature of the role.

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Remember the rare: A case report of hypophosphatasia

Jennifer Kussmann, Nicole Safina

Children's Mercy Hospital, Kansas City, Missouri, United States

OBJECTIVES: To remind providers to include rare conditions in their counseling and diagnostic work up of babies with fetal anomalies. Families benefit from through counseling prenatally that clearly outlines the diagnostic process and potential outcomes for each diagnosis. METHODS: HH presented at 21 weeks gestation due to concerns for a fetal skeletal dysplasia. Ultrasound revealed short, curved, fractured long bones, rib fractures, hypomineralization of the skull and bilateral club feet. The patient was followed with serial ultrasound at the high risk center. She had a negative maternal serum screen and declined amniocentesis. Although the differential diagnoses at the time included osteogenesis imperfecta (OI), hypophosphatasia, campomelic dysplasia or a chromosome disorder, the bulk of the conversations with the family focused on OI. The family met with members of the Bone Mineral team to discuss the possibility of postnatal bisphosphonate therapy. RESULTS: RH was born at 35 weeks gestation. She required respiratory support and intubation. She was noted to have bowed, dimpled extremities, a large fontanelle and a soft, globular cranium. Skeletal survey revealed demineralized bones, bowing of the long bows with possible fractures and wide skull sutures. She was given a presumptive diagnosis of OI. Initial labs indicated a low alkaline phosphatase. Serum and ionized calcium were mildly elevated. Molecular testing confirmed the diagnosis of hypophosphatasia. RH was transferred to Cincinnati Children's Hospital for enrollment in a clinical trial of asfotase alfa for enzyme replacement therapy (ERT). CONCLUSIONS: Hypophosphatasia is a rare inherited metabolic bone disorder. Although many features overlap with OI, the treatments, outcomes and inheritance patterns are different. Providers needs to keep an open mind and include rare disorders when counseling families. Our family had met other families and were well versed in the treatments and outcomes for OI. Hypophosphatasia was briefly mentioned as a possibility in the prenatal counseling but no details were provided. The family was unprepared for this diagnosis, her complex medical needs including a tracheostomy, gastrostomy, cranial vault reconstruction and the need to move out of state for 14 months for ERT.

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Short fetal femur during the third trimester: Clinical feature analysis and etiology exploration

Yuan Ren, Yanping Lu

Department of Obstetrics and Gynecology, Chinese PLA General Hospital, Beijing, China

OBJECTIVES: To study the clinical features and to explore the etiology of short fetal femur during the third trimester. METHODS: From January 2015 to December 2016, 24 singleton pregnancies with short fetal femur detected by ultrasonography during the third trimester were referred to the Chinese PLA General Hospital. Clinical data were collected, karyotype or SNP array， FGFR3 c.1138 mutation detection were carried out via invasive procedure to detect chromosomal abnormalities and achondroplasia (ACH), respectively. The deviation of femur length from the mean value of the gestational age in ultrasonography was expressed as the *Z*-score. The difference between ACH and isolated short femur (ISF, in the absence of associated structure abnormality or genetic abnormality) was then explored. RESULTS: Among the 24 fetuses, 11 had abnormal genetic test results (54.2%, 13/24), including 10 cases of ACH, 1 case of Ellis-van Creveld Syndrome, 1 case of Pallister-Killian Syndrome，and 1 case of Xq28 Duplication Syndrome. In the 11 ISF fetuses (45.8%, 11/24), 3 cases were fetal growth restriction, 1 was constitutional small fetus and 7 cases were unexplained. The median Z-scores(interquartile range) for ACH and ISF during the third trimester were -5.19（-6.31- -4.77）, -2.89（-4.68- -1.83）, respectively. The short femur in ACH was more severe than in ISF (P=0.002). CONCLUSIONS: The etiology of short fetal femur is complicated, including skeletal dysplasia, chromosomal abnormality, fetal growth restriction, as well as normal variants during fetal development. Genetic test should be considered during the antenatal consultation.

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Fetus as a key to genetic diagnosis

Rosalba Sevilla-Montoya, Alejandro Martínez Juárez, Irma Monroy Muñóz, Alfonso de Jesús Martínez García, Guadalupe Razo, Monica Aguinaga

Instituto Nacional de Perinatología IER, Mexico City, Mexico

OBJECTIVES: The aim of this work is to focus on some specific fetal anomalies (Cardiac rhabdomyomas, cystic hygroma and radial ray anomalies) as an important part of a family medical history. Gathering all the family background, phenotype examination, previous pregnancies and siblings can lead us to detect some genetic diseases and avoid as far as possible, some of their complications. Seeing the fetus as our main source of information can help us reach an otherwise unsuspected family disease. METHODS: We report our experience focusing on the lack of a previous family genetic disease diagnosis but with clinical criteria for Tuberous Sclerosis Complex (TSC) in the cases of cardiac rhabdomyomas, Noonan syndrome in fetuses with cystic hygroma and Holt Oram syndrome in fetuses with radial ray anomalies (RRA), from January 2012 to December 2016. Chromosomes anomalies were ruled out with karyotype and MLPA analysis. All the patients' medical charts were reviewed; also a complete physical examination, and family pedigree were performed by a Perinatal Geneticist. RESULTS: Rhabdomyomas were prenatally identified in 7 cases, from these TSC was definitively diagnosed in 6 fetuses and as possible in one. One parent had a definitive diagnosis in 3 cases(42.85%) and possible in 2(28.57%); 2 (28.57%) of our cases appear to be *de novo*. Cystic hygroma was identified in 18 fetuses and Noonan syndrome in 3(16.6%) patients; all of them had an affected mother not previously diagnosed. Radial ray anomalies occurring in association with family history of congenital heart disease, normal chromosomes, no teratogenic exposure and an affected mother were found in 2 patients. Holt Oram was diagnosed in both. CONCLUSIONS: Some fetal anomalies such as rhabdomyomas, cystic hygroma and radial ray anomalies can indicate the need of searching for other defects in the family to discard some monogenic diseases. Even when molecular analysis can reach the diagnosis; this is a multistage approach which could be long, expensive and not always available. A careful physical examination and a complete family history can lead us to detect a mild or subtle phenotype of a specific genetic disease and help provide a most accurate genetic counselling regarding their reproductive risks in each entity.

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More than meets the OI: Expanding the prenatal phenotype of Osteogenesis Imperfecta

Samantha Stover[1], Tamara Solomon[1], Sarah Huguenard[2], Melissa Russo[1], Mildred Ramirez[1], Sina Haeri[3], Karolina Adam[4], Amy Mehollin-Ray[1], V. Reid Sutton[1]

[1]*Baylor College of Medicine/Texas Children's Hospital, Houston, Texas, United States*
[2]*Baylor College of Medicine, Houston, Texas, United States*
[3]*Austin Maternal-Fetal Medicine, Austin, Texas, United States*
[4]*Houston Perinatal Associates, Houston, Texas, United States*

OBJECTIVES: Osteogenesis imperfecta (OI) is the most common antenatally diagnosed skeletal dysplasia. Lethal or severe forms are often detected by ultrasonography with micromelia, hypomineralization, fractures, deformed extremities, and a restricted ribcage. However, advancements in ultrasonography and molecular diagnosis suggest a more variable prenatal phenotype. METHODS: Three OI cases of varying severity were referred for skeletal dysplasia evaluations after abnormal anatomy surveys with maternal-fetal medicine, and recommendations for future evaluations were extrapolated from their molecular diagnoses. RESULTS: Detailed ultrasonography revealed micromelia with seemingly normal mineralization and no appreciable fractures in all cases. Specific imaging findings, genetic testing results, and outcomes are summarized in Table 1. Selective reduction in case 1 and optimal planning of postnatal treatment in case 3 were practicable due to their prenatally obtained molecular diagnoses. CONCLUSIONS: The prenatal phenotypes of many genetic conditions have expanded due to advancements in ultrasonography and molecular diagnosis. Each case described did not present with typical findings suggestive of lethal/severe forms of OI but were later confirmed within this spectrum based on specialized molecular testing and/or postnatal evaluations. Furthermore, prenatal diagnosis defined prognosis in two cases and facilitated pregnancy management decisions. Our series illustrates the importance of considering OI in the differential for prenatal skeletal dysplasias with an atypical presentation of micromelia and bowed extremities without obvious hypomineralization or fractures, and the utility of performing specialized molecular testing in such cases.

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	Case 1 (Twin B)*	Case 2	Case 3
Gestational age	19w6d	28w1d	24w6d
Imaging			
Prenatal	Micromelia, bowed femurs, irregular distal femoral metaphyses	Micromelia, bowed femurs, flared hypoplastic ribs, enlarged heart, oligohydramnios	Micromelia, bilateral bowed femurs and tibias, ventricular septal defect, persistent right umbilical vein
Postnatal	None	Severe micromelia with fractures and severely decreased mineralization, angular deformities, macrocephaly, platyspondyly, short ribs, depressed nasal bridge, low-set ears	Micromelia with multiple fractures of varying age, bowing deformity of the long bones, wormian bones, irregular proximal humeri metaphyses
Cytogenetics			
Karyotype	46,XX	None	47,XXY
CMA	Normal		Consistent with karyotype
Molecular testing			
Platform	Prenatal WES	Postnatal low bone mass panel**	Prenatal WES
Results	De novo heterozygous c.1378G>A (p.G460S) variant in *COL1A2*	Heterozygous novel intronic c.2350-10T>A VUS in *COL1A2*	De novo heterozygous c.2146G>A (p.G722S) variant in *COL1A1*
Analysis	Consistent with OI type III	Effect unclear; severe phenotype not expected from VUS alone	Consistent with OI type III/IV
Outcome	Selective reduction of twin B; full term delivery of twin A	Palliative care was elected and the infant died shortly after birth	Postnatal treatment with intravenous bisphosphonates and testosterone

*Dichorionic twins; twin A with normal anatomy and prenatal diagnostic testing (46,XX; normal CMA).
**Autosomal dominant/recessive forms of OI included.
CMA: Chromosomal microarray.
WES: Whole exome sequencing.
VUS: Variant of uncertain significance.

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Choroid plexus cyst in prenatal diagnosis

Masatoshi Yamaguchi, Kazumi Kusumoto, Masanao Ohhashi, Hiroshi Sameshima

Department of Obstetrics and Gynecology, University of Miyazaki, Miyazaki, Japan

OBJECTIVES: Prenatal untrasound observation is common procedure in obstetric and gynecological clinic in Japan. Fetal choroid plexus cyst (CPC) is one of the soft marker. Originally, CPC was reported as marker of trisomy 18. However, recent reports suggest incidence of trisomy 18 is not so high when CPC without another abnormal finding (isolated CPC). We recently experienced genetic counseling of case of isolated CPC. METHODS: Case report; Client was 37 years old pregnant women (G2P1). At 14 weeks of gestation, she was pointed out bilateral CPC. She was informed more than 20% risk of trisomy 18 and referred to our out clinic. During genetic counseling, she strongly wishes to verify with amniocentesis. Fetal karyotype was confirmed as normal karyotype. We experienced several similar cases. Threfore we reviewed the literature about CPC. RESULTS: In the literature, since 1990's, incidence of the trisomy 18 in isolated CPC may not associated with trisomy 18. If we summarized 7 papers described isolated CPC and CPC with other abnormal findings, only 0.9% of the isolated CPC was trisomy 18. CONCLUSIONS: We need reavaluate significance of CPC as a soft maeker of fetal diagnosis.

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Functional analysis of cell-free RNA using mid-trimester amniotic fluid supernatant in pregnancy with the intrauterine growth restriction

Yeonkyung Cho[1], Hee Young Cho[2], Sungshim Shin[1], Dong Hyun Cha[3], Yun-jeong Shin[4]

[1]*Obstetrics and Gynecology, CHA Gangnam Medical Center, Seoul, South Korea*
[2]*Obstetrics and Gynecology, CHA Bundang Medical Center, CHA University, Seongnam, South Korea*
[3]*CHA Gangnam Medical Center, CHA University, Seoul, South Korea*
[4]*3Genetic laboratory, CHA Gangnam Medical Center, Seoul, South Korea*

OBJECTIVES: The purpose of this study is to identify the differentially expressed genes and to investigate the important biological pathways in pregnancy by using cell-free fetal RNA of amniotic fluid supernatant. The intrauterine growth restriction (IUGR) fetuses are compared to the normal growth fetuses in the second trimester as control. METHODS: Amniotic fluid samples were obtained from pregnant women for fetal karyotyping and genetic diagnosis at 16–19 weeks of gestation. For this study, 15 IUGR samples and 9 control samples were selected, and cell-free fetal RNA was isolated from each supernatant of the amniotic fluid for microarray experiments. Gene expression profiles were identified and analyzed with the Affymetrix default analysis settings. The significant differential gene expressions for the Affymetrix data were defined by t-test. In addition, gene lists that satisfied the threshold criteria for p-values by multiple testing corrections were selected and gene sets were analyzed with the DAVID and PANTHER. RESULTS: In this study, 411 genes were differentially expressed between the intrauterine growth restriction group and the normal growth fetuses group. Of these genes, 316 genes were up-regulated, while 95 genes were down-regulated. In terms of gene ontology, the up-regulated genes were highly related to metabolic process as well as protein synthesis, while the down-regulated genes were related to receptor activity and biological adhesion. In terms of tissue-specific expression, the up-regulated genes were involved in various organs while down-regulated genes were involved only in the brain. CONCLUSIONS: This study identified the differentially expressed genes in amniotic fluid supernatant from the intrauterine growth restriction fetuses compared to the normal growth fetuses. The results show that the important brain-related genes are predominantly down-regulated in the intrauterine growth restriction fetuses during the second trimester of pregnancy. This study also suggested possible genes related to fetal development such as bcl-2, LRP10 and IGF-2. To monitor the fetal development, further study may be needed to elucidate the role of the genes identified.

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Screening for placenta-specific miRNA (miR-518b) target genes in the placenta-derived mesenchymal stem cells

Naoki Fuchi, Kiyonori Miura, Hideaki Masuzaki, Tao-Sheng Li

Nagasaki University, Nagasaki, Japan

OBJECTIVES: Increased levels of circulating placenta-specific C19MC miRNAs has been demonstrated to be associated with the development of pregnancy-related disorders such as preeclampsia and fetal growth restriction. However, the target genes of C19MC miRNAs in the placenta remain not fully understood. We screened the target gens of miR-518b associated with pregnancy-related disorders using the microarray analysis in placenta-derived mesenchymal stem cells (MSCs). METHODS: This study protocol was approved by the IRB for Ethical, Legal and Social Issues. Uncomplicated term placentas were obtained following written informed consent. We expanded MSCs primarily from chorionic villi (CV-MSCs). Using CV-MSCs transfected with a miR-518b mimic, we screened for miR-518b target genes by microarray analysis. RESULTS: Microarray analysis indicated a number of genes that were up- or down-regulated by the miR-518b mimic. Among the 124 target genes down-regulated more than 2-fold, two genes (*TH* and *HSD3B1*) were previously demonstrated to be involved in the pathogenesis of preeclampsia, and five genes (*EDNRA, AGER, WNT2, C9*, and *TRPM2*) play roles in preeclampsia with fetal growth restriction. Likewise, among the 112 target genes up-regulated over 2-fold by the miR-518b mimic, four genes (*HPX, SERPINB2, LPA*, and *TNFSF10*) show involvement in preeclampsia, and two genes (*CD69* and *STC1*) are involved in preeclampsia with fetal growth restriction. CONCLUSIONS: We screened a number of target genes of miR-518b, and demonstrated that some of them were associated with the pathogenesis of preeclampsia and/or fetal growth restriction.

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First-Trimester screening for adverse pregnancy outcomes based on cell-free fetal DNA, cell-free total DNA, and biochemical markers

Shin Young Kim[1], Hyun Jin Kim[1], Ji Hyae Lim[1], So Yeon Park[1], Dong Wook Kwak[2], Hyun Mee Ryu[2]

[1]*Laboratory of Medical Genetics, Medical Research Institute, Cheil General Hospital and Women's Healthcare Center, Seoul, South Korea*
[2]*Department of Obstetrics and Gynecology, Cheil General Hospital and Women's Healthcare Center, Dankook University College of Medicine, Seoul, South Korea*

OBJECTIVES: To develop the best first-trimester screening model for adverse pregnancy outcomes based on cell-free fetal DNA (cffDNA), cell-free total DNA (cfDNA), and biochemical markers. METHODS: A nested case-control study was conducted with 219 singleton pregnancies including 18 subsequently developed preeclampsia (PE), 15 gestational hypertension (GH), 55 small for gestational age (SGA) and 131 uncomplicated pregnancies. First-trimester cell-free DNA was extracted and measured by real-time quantitative PCR using *DSCR3* and *HYP2* as markers of cffDNA and cfDNA, respectively. First-trimester [pregnancy-associated plasma protein A (PAPP-A), placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1), soluble endoglin, and a disintegrin and metalloproteinase 12 (ADAM12)], and second-trimester [alpha-fetoprotein, β-human chorionic gonadotropin, unconjugated estriol, inhibin-A] biochemical markers were also analyzed. RESULTS: The best combined model included the first-trimester *DSCR3*, *HYP2*, PAPP-A, sFlt-1/PlGF ratio, ADAM12 for PE, GH, and SGA achieved an area under the ROC curve of 0.995 (95% CI 0.947-1.000), 0.991 (95% CI 0.937-1.000), and 0.988 (0.944-0.999), respectively. Using the best combined model, screening at a fixed 5% false-positive rate, the detection rate of PE, GH, and SGA was 91.7%, 87.5%, and 86.5%, respectively. CONCLUSIONS: Effective first-trimester screening for adverse pregnancy outcomes can be provided by a combination of cffDNA, cfDNA, PAPP-A, sFlt-1/PlGF ratio, and ADAM12.

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Pneumocephalus prior to epidural injection during labor

Katherine Jane Chua[1], Helen Ma[2], Tricia Yusaf[2], Dongping Zhang, MD[2], Aleksandr Fuks, MD[2]

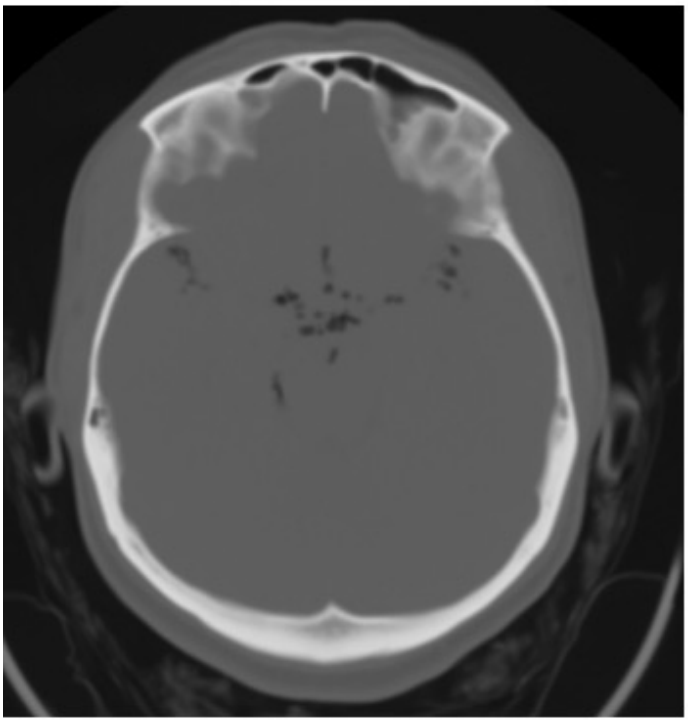
[1]*Saint George's University School of Medicine, St. George, Grenada*
[2]*Queens Hospital Center Icahn School of Medicine at Mount Sinai, Jamaica, New York, United States*

OBJECTIVES: We present a case study of a 31 year old female (G4P0030) at 39 weeks 6 days who presented with a sudden severe headache within minutes after the introduction of local lidocaine into the subcutaneous tissue prior to her epidural. METHODS: There was initial concern for subarachnoid hemorrhage and the patient received a head CT scan. The CT scan revealed diffuse air (pneumocephalus) along the distribution of the intracranial arteries. Attempt of epidural placement was discontinued. The patient's headache resolved following administration of Tylenol and Reglan. RESULTS: Our patient continued into labor and delivered a term baby. Approximately 12 hours after the lidocaine administration, repeat head CT scan revealed 50% resolution of the intracranial air. During her 6 week follow up, she denied any residual symptoms or complications of intracranial air during childbirth. CONCLUSIONS: Our case demonstrates that neurological complications may occur after lidocaine injection in the area overlying the spinal column as a complication during pregnancy. Such complications, including pneumocephalus, should be quickly identified as a possible etiology of severe headaches in pregnant patients.

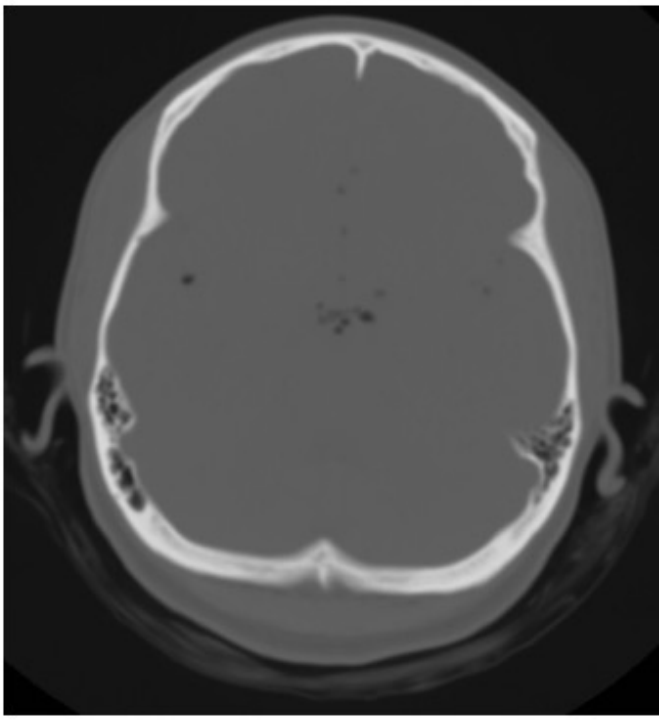
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Initial Head CT



Head CT 12 hours after Lidocaine Injection



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Prediction of unscheduled delivery in patients with morbidly adherent placenta: A cohort study

Ahmed Nassr[1], Karin fox[1], Hadi Erfani[1], Zhoobin Bateni[1], Amir Shamshirsaz[1], Bahram Salmanian[1], Elias Kassir[1], Amirhossein Moaddab[1], Jerasimos Ballas[1], Martha Rac[1], Jimmy Espinoza[1], Concepcion Diaz-Arrastia[1], B Wycke Baker III[1], Christopher Cassady[2], Shiu-Ki Rocky Hui[1], Jun Teruya[1], Venkata Bandi[1], Michael Coburn[1], Wesley Lee[3], Edwina Popek[1], Steven Clark[1], Gary Dildy[1], Michael Belfort[3], Alireza Shamshirsaz[1]

[1]Baylor College of Medicine, Houston, Texas, United States
[2]Department of Radiology, Texas Children's Fetal Center and Baylor College of Medicine, Houston, Texas, United States
[3]Department of Obstetrics and Gynecology, Baylor College of Medicine and Texas Children's Hospital Fetal Center, Houston, Texas, United States

OBJECTIVES: To identify predictors for unscheduled (emergent) delivery in patients with morbidly adherent placenta (MAP) METHODS: This is a retrospective cohort study including patients with pathologically confirmed MAP who received care and delivered in two of our affiliated tertiary centers between 2011 and 2016. All ultrasound scans were reviewed, and relevant demographic characteristics and clinical data were abstracted. Only patients with documented cervical length measurements according to AIUM guidelines at time of presentation at our institutes were included in the study. A multivariate logistic regression was conducted to identify predictors of outcome and adjust for potential confounders. Patients were classified into scheduled and unscheduled.Outcome was defined as need for unscheduled delivery in patients with MAP. RESULTS: Of 164 patients with pathologically confirmed MAP during the study period, 58 patients met our study criteria. Baseline demographic characteristics are presented in table 1. On multivariate regression analysis (table 2), only number of previous cesarean section (CS) ≥2 was independent predictor of outcome (p-value 0.009). History of preterm birth approached significance (p-value 0.05). The final model for prediction of unscheduled delivery in patients with MAP included history of preterm birth, CL < 25 mm, Gravidity ≥ 3, Maternal age ≥35 years, BMI ≥ 35, Number of previous CS ≥ 2 and adjusted for GA at time of CL measurement. CONCLUSIONS: Number of previous CS ≥ 2 is an independent predictor for unscheduled delivery in patients with MAP. History of preterm birth is also associated with increased risk for unscheduled delivery (borderline significance). These findings are valuable for counselling and planning care for this group of potentially critically ill patients as selected cases may benefit from earlier scheduled delivery at 32-34 weeks to avoid risk of unscheduled delivery.

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Table (1): Demographic characteristics

Maternal age (years)	Median and IQR	34 (28-37)
BMI (kg/m²)		30.1 (26.1-35)
Number of previous CS (N)		2 (1-3)
CL at time of presentation		37.8 (28.7-45)
Parity (N)		2 (1-3)
GA at time of CL measurement		24.3 (20.7-27.9)
GA at delivery		33 (31-35)
History of preterm birth	N (%)	13 (22.4%)
Unscheduled delivery		33 (56.9%)

BMI, body mass index; CS, cesarean section; CL, cervical length; GA, gestational age; IQR, interquartile range

Table (2): Multivariate logistic regression for predictors of unscheduled delivery in MAP patients:

	OR (95% CI)	**P-value**
Maternal age ≥35 years	0.6 (0.16 – 2.2)	0.418
BMI ≥ 35	1.6 (0.5 – 5.8)	0.441
Number of previous CS ≥ 2	11.4 (1.8- 71.1)	0.009
Cervical length < 25 mm	6.2 (0.5 – 86.0)	0.171
History of preterm birth	8.1 (1.00 – 65.9)	0.050

OR, odds ratio; CI, confidence interval; BMI, body mass index

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Prediction of small-for-gestational-age neonates by angiogenic factors: A case control study

So Hyun Shim[1], Dong Hyun Cha[2], Hee Jin Park[1], Young Joo Jeong[3]

[1]Department of Obstetrics and Gynecology, CHA Gangnam Medical Center, CHA University, Seoul, South Korea
[2]CHA Gangnam Medical Center, CHA University, Seoul, South Korea
[3]Department of Obstetrics and Gynecology, Chonbuk National University Hospital, Chonbuk National University, Jeonju, South Korea

OBJECTIVES: Not only preeclampsia neonates but also small-for-gestational-age (SGA) neonates in the absence of preeclampsia are at increased risk of morbidity and mortality. Early recognition of fetuses at increased risk of being growth-restricted enables more appropriate surveillance and optimization of management for reduced risk of adverse fetal outcomes. We investigated the potential value of the maternal serum levels of placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) for prediction of SGA neonates. METHODS: Included in the study were 530 singleton pregnant women who had attended prenatal screening program. The maternal serum levels of PAPP-A and fetal nuchal translucency were measured at 10 – 13+6 weeks, and quadraple markers at 15 – 20+6 weeks. Uterine-artery doppler ultrasonography was performed at 20 – 24+6 weeks. Additionally, we measured the plasma levels of sFlt-1 and PlGF at 24 – 28+6 weeks and 29 – 36+6 weeks, respectively, and calculated the sFlt-1/PlGF ratio. After excluding 22 preeclampsia singletons, 47 SGA singletons and 461 control-group subjects were compared to identify the relationship between each of markers and customized birth weight. RESULTS: The MoM values of the PAPPA, hCG and uE3 levels were significantly lower in the SGA group (1.25 vs. 1.00, p=0.036; 1.16 vs. 0.93, p=0.001; 1.09 vs. 0.99, p=0.041). Among the serum levels measured at 24 – 28+6 weeks, the sFlt-1 level and sFlt-1/PlGF ratio were higher and the PlGF level was lower in the SGA group, but did not attain statistical significance. The sFlt-1/PlGF ratio as determined at 29 – 36+6 weeks, however, was significantly higher in the SGA group (14.42 vs. 28.62, p=0.037). There were also significant differences in the uterine-artery doppler ultrasonography measurements between the two groups. CONCLUSIONS: At the third trimester, dissimilarly to the first and second trimesters, SGA and placental insufficiency might not be noticed, because SGA determination is made only by ultrasound biometry. Thus, besides ultrasound biometry measurement for prediction of SGA, this study explored the feasibility of the early-third-trimester sFlt/PlGF ratio as an objective measurement and possibly useful SGA predictor in combination with doppler ultrasonography.

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Factors associated with success after emergency cervical cerclage

Ga-Hyun Son, Keun-Young Lee

Hallym University College of Medicine, Kangnam Sacred Heart Hospital, Seoul, South Korea

OBJECTIVES: To assess the factors associated with success after emergency cervical cerclage. METHODS: A total of 103 women who underwent an emergency cerclage between March 2013 and Oct 2016 were included. We analyzed pregnancy outcomes after emergency cerclage, and univariate and multivariate analyses were used to determine factors associated with achieving more than 32 weeks' gestation. RESULTS: Of the 103 women, 26 (25.2%) delivered at <24 weeks' gestation, 40 (38.8%) delivered at ≥32 weeks' gestation. We compared patients with delivery at <32 weeks' gestation and those with delivery at ≥32 weeks' gestation. A previous preterm birth history, cervical dilatation at cerclage, and maternal serum CRP levels on admission were siginificantly different between the two groups (p=0.002, 0.015, and 0.003, repectively). In multivariate analysis, the presence of preterm birth history, prolapsed membranes beyond the external cervical os, and high preoperative maternal serum CRP levels were independently associated with reduced likelihood of achieving 32 weeks' gestation after emergency cerclage. CONCLUSIONS: The presence of preterm birth history, prolapsed membranes beyond the external cervical os, and high preoperative maternal serum CRP levels were independently associated with reduced likelihood of achieving 32 weeks' gestation after emergency cerclage.

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P-180

Angiogenic markers associated with preeclampsia in pregnancies complicated by polycystic ovary syndrome

Taylor Weilnau[1], David Cantonwine[2], Thomas McElrath[2], Shruthi Mahalingaiah[3], Louise Wilkins-Haug[4]

[1]*Brigham and Women's Hospital/Harvard Medical School, Brookline, Massachusetts, United States*
[2]*Brigham and Women's Hospital/Harvard Medical School, Boston, Massachusetts, United States*
[3]*Boston University Medical School, Boston, Massachusetts, United States*
[4]*Brigham & Women's Hospital, Boston, Massachusetts, United States*

OBJECTIVES: Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder among reproductive age women, with an incidence in the population of 6-10%. Women with PCOS who achieve pregnancy are at increased risk of pregnancy complications, especially those related to aberrant placentation such as preeclampsia. Work by others has shown significantly different microscopic placental characteristics, such as reduced placental volume and weight, as well as more chronic villitis and intervillositis, in pregnancies complicated by PCOS compared to unaffected pregnancies. We hypothesized that PCOS pregnancies, when compared to control pregnancies, are associated with longitudinal alterations in angiogenic markers. METHODS: This is a secondary retrospective cohort analysis of singleton pregnancies from Brigham and Women's Hospital in Boston, MA, between 2006 and 2008. Pregnancy outcomes, that included diagnosis of preeclampsia or other obstetric complications, were validated by a panel of physicians. PCOS cases were initially identified by self-report, ICD-9 codes, and altered androgen testing. Potential cases were then validated by medical record extraction utilizing a specific diagnostic criterion. Placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) were measured at 4 time points (median 10, 18, 26, and 35 weeks gestation) during pregnancy. RESULTS: Of the included N=1,235 singleton pregnancies, N=40 (3.02%) were complicated by PCOS. PCOS pregnancies were at a slight increased risk, but are not significantly more likely to be complicated by preeclampsia (12.5% vs. 9.3%). PCOS pregnancies had comparable levels of sFlt-1 and PlGF to control pregnancies at 10, 18, 26, and 35 weeks of gestation. These values remained comparable even after being controlled for preeclampsia. CONCLUSIONS: Pregnancies affected by PCOS were found to have comparable angiogenic profiles at multiple time points throughout gestation when compared with control pregnancies. These levels of angiogenic factors persisted even after we controlled for preeclampsia, a disorder often attributed to aberrant placentation. This finding suggests that the aberrant placentation and the associated clinical phenotypes of preeclampsia found in previous studies of PCOS pregnancies may be driven by other parameters of PCOS beyond angiogenic abnormalities.

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Asymptomatic liver hemorrhage as complication of HELLP syndrome

Katherine Jane Chua[1], Helen Ma[2], Tricia Yusaf[2], Dongping Zhang, MD[2], Aleksandr Fuks, MD[2]

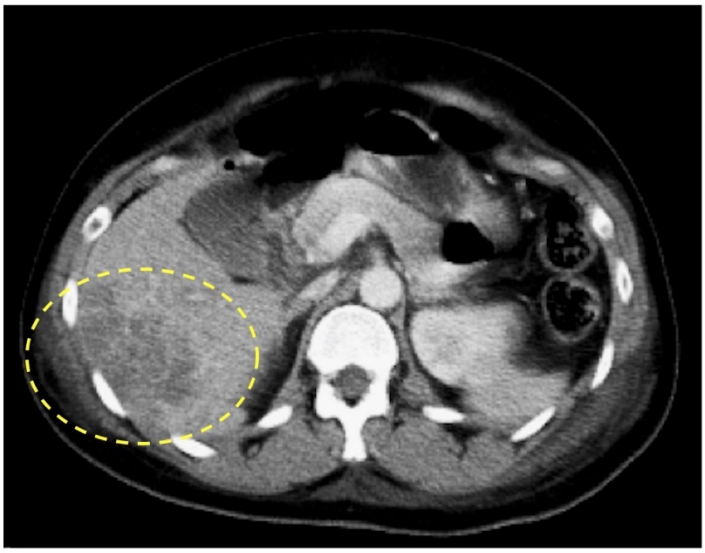
[1]Saint George's University School of Medicine, St. George, Grenada
[2]Queens Hospital Center Icahn School of Medicine at Mount Sinai, Jamaica, New York, United States

OBJECTIVES: We present a case study on a 33 year old, Gravida 2 Para 1, at 35 weeks gestation with a liver hemorrhage noted after Cesarean delivery, which was performed for severe preeclampsia with HELLP syndrome. METHODS: The patient underwent Cesarean delivery via low transverse uterine incision and delivered a live female neonate with APGAR of 9/9 and weight of 1950g. She was asymptomatic but her elevated blood pressures could only be controlled with multiple anti-hypertensive medications. Her liver function tests were trending upward and the platelet counts were decreasing. RESULTS: The patient was eventually transferred to another facility and was successfully treated with conservative management. Repeat abdominal CT scan revealed a decrease in liver involvement subsequent to the patient's transfer. At her 2 month post partum follow up, the patient was completely resolved of all complications from HELLP syndrome. Her blood pressure and laboratory values returned to their normal ranges. CONCLUSIONS: Non-operative management for liver hemorrhage due to HELLP syndrome was shown to be successful. Although the patient was asymptomatic, physicians must suspect liver hemorrhage when liver function tests progressively worsen to prevent a liver rupture and need for liver transplant.

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Abdominal CT Before Transfer to Other Facility



Abdominal CT After Transfer to 0ther Facility



Article

P-182

Assessing the carrier rate of junctional epidermolysis bullosa, LAMB3-related

Catherine Terhaar[1], Andrea Harbison[2], Tessa Niemchak[2], Betsy Swope[2], Ryan Longman[3], Marie Sharpe[3]

[1]*Progenity, Rockford, Michigan, United States*
[2]*Progenity, Ann Arbor, Michigan, United States*
[3]*University of Miami Miller School of Medicine, Miami, Florida, United States*

OBJECTIVES: Junctional Epidermolysis Bullosa (JEB) is a severe blistering skin condition with onset at birth or during the neonatal period, sometimes resulting in death within the first year of life. Carrier testing is critical to identifying at-risk couples. This information is important for pre-conception and prenatal planning along with postnatal care. Carrier frequency of JEB has been calculated as 1/350. Four known genes are associated with JEB. *LAMB3* accounts for 70% of cases, for a calculated 1/500 carrier frequency. Our goal is to assess the carrier rate of JEB, *LAMB3*-related, in the general population and in various ethnic groups. METHODS: We retrospectively reviewed genotyping results for JEB, *LAMB3*-related for 11,135 individuals tested with a commercially-available pan-ethnic expanded carrier panel.

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All positive test results for JEB, *LAMB3*-related were identified. To adjust for the genotyping panel's detection rate of 48%, a corrective factor of 2.1 (100/48) was applied. The carrier rate for JEB, *LAMB3*-related was calculated overall and for the following ethnic groups: American/Black, Ashkenazi/Jewish, Asian, Caucasian/White, Hispanic, Native American, Other or Mixed, and Sephardic Jewish. RESULTS: The overall calculated carrier rate for JEB, *LAMB3*-related in the general population was 1/475 (11/11135 tests). Positive tests were identified in the Caucasian/White (6/4901) and Other or Mixed (5/2785) groups. With the correction for detection rate, the calculated carrier rate was: 1/384 in the Caucasian/White and 1/263 in the Other or Mixed. No carriers were identified in the African American/Black (785 tests), Ashkenazi / Jewish (361 tests), Asian (1091 tests), Hispanic (1137 tests), Native American (16 tests), or Sephardic Jewish participants (58 tests). CONCLUSIONS: This is the first report to document JEB, *LAMB3*-related, carrier rate in the general population and individual ethnic groups. We report a general population carrier frequency of 1/475, which is similar to the 1/500 assumed frequency based on overall JEB carrier frequency and *LAMB3*-related disease incidence. As more patients and providers pursue expanded carrier screening, it will be important to continue to reassess carrier and disease frequencies for these rare conditions based on larger datasets and more diverse patient populations. Knowledge of carrier status gained from carrier testing can guide preconception and prenatal counseling.

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Identifying unexpected carriers with expanded pan-ethnic carrier testing

Andrea Harbison[1], Catherine Terhaar[2], Betsy Swope[1], Christina Settler[1], Lindsay Dohany[1], Carol Holland[1], Jeana DaRe[1], Ryan Longman[3], Tessa Niemchak[1]

[1]*Progenity, Ann Arbor, Michigan, United States*
[2]*Progenity, Rockford, Michigan, United States*
[3]*University of Miami Miller School of Medicine, Miami, Florida, United States*

OBJECTIVES: Ethnicity-based carrier testing for single gene conditions is the current standard of prenatal care. Technological advances have allowed for rapid expanded carrier testing for hundreds of genetic mutations across a significant number of conditions, improving detection of at risk carrier couples in non-targeted ethnic groups. Our current study assesses how many individuals screened positive for a condition outside of the expected ethnicity group. METHODS: A retrospective review of over 30,000 carrier test results was performed. We identified positive carriers of 23 conditions currently part of ACOG/ACMG recommendations including cystic fibrosis (CF), Fragile X, spinal muscular atrophy (SMA), hemoglobinopathies, and Ashkenazi Jewish disorders. Ethnicity was self-reported. For each condition and ethnicity, the expected numbers of positive carriers were calculated and compared to observed rates. A Chi-square analysis was performed to calculate statistical significance ($p \leq .01$). RESULTS: When evaluating for Ashkenazi Jewish diseases, a higher than expected number of patients who identify as Caucasian screened positive for Tay-Sachs disease (46 individuals observed as carriers vs 21.7 expected) and Fanconi anemia type C (15 individuals observed as carriers vs 1.4 expected). Patients who identified as Hispanic screened positive for CF and SMA more often than expected as well. All of these observations were statistically significant. A select data set of findings is represented in Table 1. CONCLUSIONS: In this cohort, we identified unexpected carriers based on patient reported ethnicity. Testing patients with an expanded pan-ethnic panel will identify more carriers of conditions that would be missed by current ethnicity-based screening conventions. In addition, outcome data from these tests will allow for more knowledge and accurate assessment of carrier frequencies of genetic conditions. Expanded carrier testing will identify more carriers, allowing for more complete risk assessments and consideration of planning options.

Table 1: * indicates $p \leq 0.01$

	Cystic fibrosis		Spinal muscular atrophy		Fragile X		Fanconi anemia, type C		Tay-Sachs disease	
	Expected positiv	Observed positiv	Expected positiv	Observed positiv	Expected positiv	Observed positiv	Expected positiv	Observed positiv	Expected positiv	Observed positiv

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	es	es	es	es	es	es	es	es	es	es
African American	58.3	57	46.5	48	16.7	16	0.5	0	8.5	2
Ashkenazi Jewish	15.67	7	8.1	3	1.46	1	4.1	4	11.3	14
Asian	9.75	4	28.6	28	6.28	4	0.2	0	3.2	1
Caucasian	420	483	297.9	230*	42.35	55	1.4	15*	21.7	46*
Hispanic	123	204*	65.7	133*	32.39	21	1	2	16.7	5

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Copy number variant calling on a 177 gene expanded carrier screening panel including DMD

Kyle Beauchamp, Peter Grauman, Greg Hogan, Kevin Haas, Genevieve Gould, Kenny Wong, Gabriel Lazarin, Eric Evans, Imran Haque, Dale Muzzey

Counsyl, South San Francisco, California, United States

OBJECTIVES: Expanded carrier screening (ECS) identifies couples whose future children are at high risk of Mendelian conditions. Historically, ECS has been performed with limited or no copy number variant (CNV) calling, often restricted to calling a handful of founder deletions. The lack of broad CNV calling reduces the detection rate of ECS in less well-studied ethnicities and increases residual disease risk among patients testing negative. For these reasons, we investigated panel-wide CNV calling on an ECS cohort of more than 17,000 patients. METHODS: Here we report pre-validation CNV data for a new 177-gene ECS panel on a cohort of 17,114 anonymized patient samples. We performed CNV deletion and duplication calling on 161 autosomal genes and 10 genes on chromosome X (calls for six genes, such as *SMN1,* are treated as special cases and were not included in this CNV analysis). Copy number calling was performed using a Hidden Markov Model on next generation sequencing depths at targeted positions. Since this retrospective analysis was performed for the purpose of research and development, calls were aggregated without performing variant interpretation for clinical impact. RESULTS: Overall, 4% of patients have at least one CNV in the autosomal regions of interest. Among the 161 autosomal genes tested, we observed 420 deletions and 315 duplications in 113 of the genes. In *DMD*, we observed 23 deletions and 22 duplications. Although the genes with the most observed deletions (*NEB, CLN3, GALC, CTNS)* contain known founder mutations, 67% of called CNVs are located outside of the six genes for which we called deletions in our previous 112-gene panel (*CLN3*, *CTNS*, *GALC*, *HEXA*, *MCOLN1*, and *NEB)*, highlighting the importance of expanding CNV analysis beyond a handful of founder variants. CONCLUSIONS: Using a 17,114 patient cohort, we have retrospectively evaluated the impact of CNVs on a 177 gene ECS panel. Most of the observed CNVs are outside the six genes for which we called founder deletions in previous work, indicating that broader use of CNV calling will improve detection rates in ECS and minimize residual disease risk when a patient is negative. The presented findings have motivated the inclusion of panel-wide deletion calling in our clinical ECS panel, where novel deletions undergo real-time variant interpretation to assess clinical impact.

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Relationship between self-perception and social factors in people with Down syndrome

Hidehiko Miyake[1], Shigehito Yamada[2], Yosuke Fujii[2], Shingo Io[2], Mariko Taniguchi-Ikeda[3], Mari Urano[4], Yuka Ozasa[5], Makoto Kanai[6], Akimune Fukushima[7], Yoichi Matsubara[8], Kayoko Saito[4], Ikuo Konishi[2]

[1]*Kyoto University Hospital, Kyoto, Japan*
[2]*Kyoto University, Kyoto, Japan*
[3]*Kobe University, Kobe, Japan*
[4]*Tokyo Women's Medical University, Tokyo, Japan*
[5]*Tokyo Medical and Dental University, Tokyo, Japan*
[6]*Shinshu University, Nagano, Japan*
[7]*Department of Clinical Genetics, School of Medicine, Iwate Medical University, Morioka, Iwate, Japan*
[8]*National Research Institute for Child Health and Development, Tokyo, Japan*

OBJECTIVES: Down syndrome (DS) is one of the most common conditions identified in prenatal genetic testing. In genetic counseling about DS, genetic counselors are advised to provide information that includes lifestyle and life course. Previously published studies showed that the majority of people with DS had positive feelings about their surroundings and themselves. On the other hand, some people with DS experienced social difficulties. The aim of this study was to investigate the social factors affecting self-perception in people with Down syndrome. METHODS: We conducted a nationwide survey with all 5,025 members of the Japanese Down Syndrome Society in 2015. Our study consisted of two questionnaires. Questionnaire 1 was for parents, family members, or guardians. It included questions seeking basic information on the proband, as well as the educational status, occupational status, public assistance, welfare services, daily life, and disclosure status of the diagnosis for the person with DS. Questionnaire 2 was for people with DS who were over 12 years old. It aimed to measure self-perception, self-esteem, and attitudes towards people around them. This study was approved by the ethics committee. RESULTS: In total, 1,571 responses and 866 responses were received from Questionnaire 1 and Questionnaire 2, respectively. The number of valid responses of Questionnaire 2 was 852. Eleven of 852 responses had no response from parents, family members, or guardians. We examined the correlation between the two sets of data. Residential area and the disclosure status of the diagnosis did not affect positive feelings. In working people, employment style (open employment or supported employment) and annual salary affected daily happy feelings. Although employment style did not influence job satisfaction, having a low salary resulted in decreased levels of satisfaction. CONCLUSIONS: Occupational status affected self-perceptions of people with DS. The present study highlights the challenges of young people and adults with DS in employment. This data will contribute not only to genetic counseling but also to the improvement of the welfare system.

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Should umbilical cord puncture be stepped down from the stage of history in invasive prenatal diagnosis?

Yanlin Wang

Prenatal Diagnosis Center, International Peace Maternity and Child Health Hospital, Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China

OBJECTIVES: It's seems that amniotic fluid is enough to do any genetic testing, but amniotic mosaicism is a eternity problem .This study: 1. To investigate the differences between true and false chimeras from karyotypes of amniocytes and chorionic villus cells during prenatal diagnosis, to provide a clinical basis for genetic counseling. 2. To discuss the value of umbilical cord blood in chromsome mosaicism validation. METHODS: Pregnant women who have undergone amniocentesis or CVS for karyotype diagnosis due to various high risk factors were selected between January 2013 and June 2016. 7027 amniotic fluid samples and 251 chorionic villus samples were included in this study. Results with mosaic were collected based on the classification guidelines by Hsu for recording of fluorescence in situ hybridization (FISH) and umbilical cord blood validation results and follow-up records. RESULTS: 1. 13 cases were detected to level Ⅱ chromosomal mosaic, with a detection rate of 0.14% and 1.2% in amniotic fluid and chorionic villus, respectively. All of cases were validated to be pseudo mosaic by cord blood and neonatal follow-up normal. 2. There were 35 cases level Ⅲ chromosomal mosaic, with a detection rate of 0.37% and 3.58% in amniotic fluid and chorionic villus, respectively. These cases were retestd by FISH or Umbilical cord blood, accounting for up to 90% of the total. 3. There are also 24% other aneuploid mosaicism besides sex and 21/18/13.One chr-20 aneuploid mosaicism case in amniotic ffluid was confirmed normal karyotype in umbilical cord blood. CONCLUSIONS: 1. The level Ⅱ chromosomal mosaic is false and no need to be testified by cord blood. 2. Karyotype analysis combined with pre-culture FISH results or cord blood validation is an effective method to distinguish between true and pseudo mosaic. FISH results from uncultured cells were more representative of the true situation of mosaic in the sample. 3. The validation of umbilical cord blood still have a certain value in the case of the scarce genetic technical resources in china which will save many babies from termination of pregnancy by anxiety parents.

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Noninvasive methods for predicting congenital cytomegalovirus infection in high-risk pregnant women

Tanimura Kenji[1], Shinya Tairaku[1], Yasuhiko Ebina[1], Morioka Ichiro[2], Satoshi Nagamata[1], Kana Ozaki[1], Morizane Mayumi[1], Masashi Deguchi[1], Toshio Minematsu[3], Hideto Yamada[1]

[1]Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, Kobe, Japan
[2]Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan
[3]Research Center for Disease Control, Aisenkai Nichinan Hospital, Nichinan, Japan

OBJECTIVES: Human cytomegalovirus (CMV) is the most common cause of congenital viral infection in humans. It has been recently suggested that early diagnosis and antiviral therapy may improve neurological outcomes of infants with symptomatic congenital CMV infection (CCI). However, universal screening of newborns for CCI is not recommended. Therefore, the prenatal detection of mothers and newborns at high risk for CCI is important. The aim of this prospective cohort study was to determine maternal clinical, laboratory, and ultrasound findings that effectively and noninvasivelypredict the occurrence of CCI in high-risk pregnant women. METHODS: Pregnant women who visited the Kobe University Hospital underwent CMV IgG and IgM tests, and women who had positive tests for both CMV IgG and IgM received a series of examinations after informed consent was obtained. Three hundred CMV IgM–positive women were enrolled. The maternal clinical and laboratory findings, including serum CMV IgM and IgG; IgG avidity index (AI); antigenemia assay (C7-HRP); PCR for the detection of CMV-DNA in the maternal serum, urine, and uterine cervical secretion; and ultrasound findings, were evaluated. To determine predictive factors for CCI, logistic regression analyses were performed. RESULTS**:** In 22 of the 300 women, CCI was confirmed using PCR for CMV-DNA in newborn urine. Univariate analyses demonstrated that the presence of maternal flu-like symptoms, presence of ultrasound fetal abnormalities, serum titers of IgM, positive results for C7-HRP, AI<40%, and positive PCR results in the uterine cervical secretion were statistically associated with the occurrence of CCI. Multivariable analysis revealed that the presence of ultrasound fetal abnormalities (OR, 31.9; 95% CI, 8.5–120.3; p<0.001) and positive PCR results in the uterine cervical secretion (OR, 16.4; 95% CI, 5.0–54.1; p<0.001) were independent predictive factors for CCI in IgM-positive women. CONCLUSIONS: This is the first prospective cohort study to suggest that the presence of CMV-DNA in the maternal uterine cervical secretion and ultrasound fetal abnormalities are predictive of the occurrence of CCI in high-risk pregnant women. These results allow the clients to get more information by performing noninvasive procedure before they give consent for invasive procedures, i.e. amniocentesis. Furthermore, neonatologists can identify high-risk neonates who need workup and antiviral therapy for CCI.

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Prenatal detection of fetal growth restriction in twins: The TWIG study

Marwan Ali[1], Jena Miller[2], Catherine Chan[1], Jessica Fields[1], Laura Houston[3], Kiley Bernhard[4], Angela Hawk[5], Shiraz Sunderji[6], Danish Siddiqui[7], Eugene Chang[8], Adam Sandlin[9], Everett Magann[9], Suneet Chauhan[10], Stephen Chasen[11]

[1]Weill Cornell Medical College, New York, New York, United States
[2]Johns Hopkins University, Baltimore, Maryland, United States
[3]MAHEC Ob/Gyn Specialists, Asheville, North Carolina, United States
[4]Center for Urban Population Health, Milwaukee, Wisconsin, United States
[5]Regional Obstetrical Consultants, Chattanooga, Tennessee, United States
[6]University of Toledo, Phoenix, Arizona, United States
[7]Aurora Sinai Medical Center, Milwaukee, Wisconsin, United States
[8]Medical University of South Carolina, Charleston, South Carolina, United States
[9]University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States
[10]University of Texas Health Science Center at Houston, Houston, Texas, United States
[11]Weill Cornell Medical College, New York, New York, United States

OBJECTIVES: Serial ultrasound, using composite assessment of fetal weight, is believed to be the most accurate method to assess fetal growth in twin gestation. The objectives of this review are to: 1. Determine the likelihood of identifying IUGR in twin gestations. 2. Compare the risk of composite neonatal morbidity (CNM) and mortality among twins with detected IUGR vs. those with undetected IUGR. METHODS: This is a multicenter, retrospective chart review, examining non-anomalous twins, delivered at 5 academic tertiary centers. We examined the likelihood of detecting small for gestational age (SGA; birth weight < 10%) as IUGR prenatally. The risks of CNM among detected versus undetected were also determined. CNM was defined as the presence of any of the following: RDS, BPS, IVH III/IV, necrotizing enterocolitis, proven sepsis, hypoglycemia, thrombocytopenia, hyperbilirubinemia, hypothermia, neonatal seizures, umbilical artery pH <7.00, or neonatal death. Data was compared using Chi-square analysis and Fisher's exact test. RESULTS: A total of 919 twin pregnancies were included in our study, 455 (50%) of which were found out to have at least 1 SGA neonate at birth. Twin A was found to be SGA in 264 pregnancies (58%), while twin B was found to be SGA in 333 pregnancies (73%). Both twins were found to be SGA in 142 pregnancies (31%). Of the 455 pregnancies complicated by SGA, 132 (29%) were prenatally detected as IUGR, while 323 (71%) were undetected, with a sensitivity of 29% [95% CI = (24.8%, 33.2%)] and a specificity of 95.7% [95% CI = (93.8%, 97.5%)]. CONCLUSIONS: Our data demonstrates that when used for twin pregnancies, ultrasound has relatively low sensitivity for predicting SGA newborns. CNM in those pregnancies with undetected IUGR is less than those in whom IUGR is suspected, and similar to pregnancies without IUGR, however. Thus, it is not clear that a higher detection rate would lead to improved outcomes. As common interventions in pregnancies with

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suspected IUGR, including earlier delivery, may themselves increase CNM, future studies should seek to identify factors aside from estimated weight that are related to adverse neonatal outcomes.

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X-chromosome inactivation patterns do not show unbalanced division of blastomere was the etiology of twinning in female selective intrauterine growth restriction

Xuan Huang[1], Qi Wang[2], Ye Wang[3], Qiang Zhao[4], Jun Yang[5], Qun Fang[6]

[1]*Fetal Medicine Center, Department of Obstetrics and Gynecology, First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China*
[2]*Zhongshan School of medicine,SYSU, GUANGZHOU, China*
[3]*Fetal Medicine Center, OB&GYN, the 1st affiliated hospital of SYSU, guangzhou, China*
[4]*department of OB&GYN, Jiangmen Central Hospital, Jiangmen, China*
[5]*Nanchang Public Security Bureau, Nanchang, China*
[6]*Fetal Medicine Center, Deparment of Gynaecology and Obstetrics, The First Affilicated Hospital, Sun Yat-sen University, Guangzhou, Guangdong Province, China*

OBJECTIVES: The aetiology of selective intrauterine growth restriction (sIUGR) is unclear. In order to investigate if unbalanced division of blastomere is involved in twinning of female sIUGR, X-chromosome inactivation patterns in monochorionic twins complicated with sIUGR were compared to those without sIUGR. METHODS: DNA was extracted from the placenta of 40 female MC twins, 20 with and 20 without sIUGR. X-inactivation patterns were determined by DNA digestion with MspI and HpaII followed by microsatellites analysis of a polymorphic trinucleotide repeat in the androgen receptor gene. RESULTS: The percentage of individuals with skewed (⩾25/75%) inactivation patterns was no different in MC twins with sIUGR compared to those without sIUGR. Skewed X chromosome inactivation(SXCI) rate in small fetus of female sIUGR was 30%, higher than the large fetus whose rate was 20%. But there was not statistically different between them. SXCI rate in small fetus of sIUGR was 20%, the same as that in normal twins. CONCLUSIONS: We found no difference in the frequency of skewed X-inactivation patterns in sIUGR. X-inactivation patterns do not show unbalanced division of blastomere was the etiology of twinning in female sIUGR.

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Two cases of prenatal genetic diagnosis for neonatal alloimmune thrombocytopenia for women with the history of neonatal alloimmune thrombocytopenia in the prior pregnancy

Masashi Deguchi, Tanimura Kenji, Uenaka Mizuki, Morizane Mayumi, Morioka Ichiro, Hideto Yamada

Department of OB/GYN, Kobe University Graduate School of Medicine, Kobe, Hytogo, Japan

OBJECTIVES: Neonatal Alloimmune Thrombocytopenia (NAIT) is blood-related disease caused by maternal antibodies raised against alloantigens on fetal platelets. NAIT can be a cause of perinatal intracranial hemorrhage (ICH) in full-term neonates and death or severe neurologic sequelae. Early elective C-section can be chosen to minimize the risk of bleeding at delivery for affected fetus. We experienced two cases of prenatal genetic diagnosis and perinatal management of women with the past pregnant history of NAIT. METHODS: Case 1: The 29-year-old Russian woman with Human platelet antigens (HPA)-1a antibody married again and get pregnant with another Japanese. Her first child suffered ICH and thrombocytopenia (15,000 /μl) due to HPA-1a antibody and diagnosed as NAIT. Genetic test of HPA-1 of parents was HPA-1a/a (patient) and HPA-1b/b (spouse). Fetal genotype was presumed as HPA-1a/b and suspected increased risk for NAIT. C-section was chosen to minimize the ICH risk. Newborn suffered thrombocytopenia (67,000/μl) but not ICH. He was discharged at day7 without any problem (Plt 286,000/μl), and was not found any problem at 1-month checkup (Plt 442,000/μl). RESULTS: Case 2: The 32year-old Japanese mother with HLA-A2 antibody conceive the second child. Her first child was diagnosed as NAIT due to thrombocytopenia (47,000 /μl). Serological test shows positive HLA-A2 antibody, then an amniocentesis was performed at 32 GW to determine fetal HLA type. The fetus did not carry HLA-A2, and was assumed to have no risk of NAIT, so vaginal delivery was chosen. Newborn did not suffered thrombocytopenia (250,000/μl) nor ICH, and the pediatric histories indicated normal development at 18 months of age. CONCLUSIONS: Although a screening for HPA/HLA antigens to avoid NAIT is not currently recommended in the world, perinatal genetic diagnosis of the NAIT risk for women with past history of NAIT by amniocentesis might be effective to decrease ICH by caesarian delivery, prepare antigen negative donor platelets for the neonates and avoid unnecessary cord blood sampling or caesarian delivery.

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Noninvasive prenatal diagnosis of fetal severe α-thalassemia at first and and early second trimester in Li nationality China

Yi Ling[1,3], Yanxin Wei[2], Chen Zejun[3], Ning Zhang[3], Jin Song[3], Yu Yanhong[2]

[1]*Nanfang Hospital, Affiliated Hospital of Southern Medical University, Guangzhou, China*
[2]*Southern Hospital, Affiliated Hospital of Southern Medical University, Guangzhou, China*
[3]*The First Affiliated Hospital of Hainan Medical University, Haikou, Hainan, China*

OBJECTIVES: Fetal middle cerebral artery peak systolic velocity is used for the diagnosis of fetal anemia most from 18 gestational weeks using Mari's stand and population. The purpose of our study was to establish reference of first and and early second trimester fetal middle cerebral artery peak systolic velocity in Li nationality China and compare it which commonly Han nationality. To determine the value for the noninvasive diagnosis of fetal severe α-thalassemia at first and and early second trimester in the high prevalence populatuin of Li nationality in Chiana. METHODS: 100 singleton Pregnaneies(15 thalassemia fetues and 85 normal fetues) at 11-18 gestational weeks were performed.with ultrasound and color Doppler imaging of GE E8 system .Fetal middle cerebral artery peak systolic velocity (MCA-PSV) were measured. CVS or aminiocentesis were carried on when there was risk for severe αthalassemia. Using MCA-PSV>1.55MOM as cut off value, the sensitivity and specificity wasnanalyzed in fetuses with homozygous alpha thalassaemia. RESULTS: All MCV-PS were measured during 11-18weeks. MCV-PS was increased with gestational week.There was no difference between Li and Han population. The sensitivity and specificity was 100% (7/7) and 90% (9/10) respectively in fetuses with homozygous alpha thalassaemia using MCA-PSV>1.55MOM as cut off value. Conclusion: Fetal MCA-PSV can be measured from first trimester. There was no difference between Li and Han population. It is an an effective tool in prenatal screen and diagnosis of severe αthalassemia. CONCLUSIONS: Fetal MCA-PSV can be measured from first trimester. There was no difference between Li and Han population. It is an an effective tool in prenatal screen and diagnosis of severe α-thalassemia in Li nationality.

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Veracity of Automatic Measurement by NT Location and Thickness Measurement Software

Yi Ling[1,2], Wang Yuefeng[2]

[1]*Nanfang Hospital, Affiliated Hospital of Southern Medical University, Guangzhou, China*
[2]*The First Affiliated Hospital of Hainan Medical University, Haikou, Hainan, China*

OBJECTIVES: To study the possibility of clinical application of NT Location and Thichness Measurement Software. METHODS: Randomised choose 100 cases of NT scan pictures(CRL 45mm-84mm),each NT picture includes mark1 picture with NT measuremen and mark2 picture without.Mark1 picture was manual measured by skillful expert,which is contral group.Mark2 pictures were automatically identified and measured by the NT Location and Thichness Measurement Software and was regardes as study group. RESULTS: There was no significant difference between the contral group and study group. The study group is more close to the contral group by the increase of the gestrational age. CONCLUSIONS: There is no difference between the result from the NT Location and Thichness Measurement Software and the result by manual measurement.

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The Belgian Microarray Prenatal Consortium (BEMAPRE): national prenatal microarray results and postnatal follow-up

Joke Muys[1], Katrien Janssens[2], Yves Jacquemyn[3], J.M. Kerstjens[4], The Belgian Microarray Prenatal Consortium (BEMAPRE)[5], Bettina Blaumeiser[2]

[1]*University Hospital Antwerp, Edegem, Belgium*
[2]*Center for Medical Genetics, Universiteit Antwerpen, Antwerpen, Belgium*
[3]*University Hospital Antwerp, Edegem, Belgium*
[4]*University of Groningen, Groningen, Netherlands*
[5]*Belgian Society for Human Genetics, Edegem, Belgium*

OBJECTIVES: In Belgium, samples for prenatal genetic diagnosis (amniotic fluid/chorion villi) are analyzed by Chromosomal Microarray Analysis (CMA) instead of karyotyping since 2013. The main challenge herein lies in the interpretation of copy number variants (CNVs) for which knowledge about postnatal outcome is limited. All Belgian genetic centers have agreed on prenatal CNV classification and reporting. The goal of our research is to investigate 1) recurrence and incidence of non-benign CNVs (variants of unknown significance (VOUS), pathogenic variants and susceptibility variants) in our Belgian population and 2) investigate genotype-phenotype correlations using clinical data of children with prenatally registered non-benign CNVs. METHODS: A national database was established containing all fetuses in whom a non-benign CNV (variant of unknown significance, a pathogenic subchromosomal CNV or susceptibility variant) was found following an invasive prenatal analysis. A panel of experts studied these included variants and looked for overlap and recurrence in our Belgian population. From January 2017 onwards, parents of children included in the database were sent questions on pregnancy outcome and approved for parental-completion standardized developmental questionnaires (Ages and Stages Questionnaire 3 and SE-2) when their child reached the age of 36 months. RESULTS: From 2013 to 2016, more than 13000 prenatal CMAs have been performed in Belgium. Of these samples, 10.2% were aneuploidies. 11.5% of samples showed the presence of a non-benign CNV (1523/13266 children) and were included in the database. Presuming a karyotype resolution of 10Mb, microarray showed an overall added value of 2.8%. Presented data will be recurrent pathogenic CNVs, susceptibility CNVs and VOUS in our Belgian population, their distribution of indications for invasive diagnosis and the added value of using microarray per indication. Furthermore, an overview of our questionnaires and the current status of our postnatal follow-up will be presented. CONCLUSIONS: Ameliorating genotype–phenotype knowledge of prenatally registered CNVs is necessary to improve parental counseling in a prenatal setting. Assessing development of children in whom a non-benign CNV was found by invasive prenatal diagnosis has started and will be of undeniable importance to clinical decision-making.

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LB-3

Cytogenetic analysis of 1372 mental retardation children

Jiemei Zheng

Chengdu, Sichuansheng, China

OBJECTIVES: To analyse the relationship between mental retardation and chromosomal abnormalities in children, and evaluate the significance of prenatal screening and diagnosis in preventing the birth of mental retardation children. METHODS: 1372 mental retardation children under 14 years old . Their peripheral blood was cultured through routine method and chromosome G band. RESULTS: 1018 cases of abnormal karyotypes were detected in 1372 mental retardation children, with a rate of 74.20%(1018/1372). In abnormal karyotypes, abnormal chromosome number was mainly trisomy 21 syndrome, 953 cases were detected with a rate of 93.61% (953/1018). In chromosomal structural abnormalities, 42 cases of unbalanced chromosomal structural abnormalities and 6 cases of balanced chromosomal structural abnormalities were detected, with a rate of 4.13% (42/1018) and 0.59% (6/1018). 1 case of sex chromosome number abnormalities（47, XXY） and 1 case of abnormal sex chromosome structure（ 46,X,der(Y)(p?)） were found. 10 cases of Marker chromosome karyotype with a rate of 0.98% (10/1018) were detected. CONCLUSIONS: Mental retardation of children is closely related with chromosome abnormal karyotypes. The prenatal diagnosis before birth and the propagation of healthy birth and child care are efficient method for preventing the birth of mental retarded children with heredity risks

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Tracheal agenesis with broncho-esophageal fistula: A rare and unexpected finding in a newborn

Sihem Darouich[1], Nadia Boujelbène[2], Souhir Bouzguenda[3], Dhikra Kacem[2], Radhia Ben Ghorbel[2], Samia Kacem[4], Aida Masmoudi[3]

[1]*Fetopathology Unit, Bizerte, Tunisia*
[2]*Pathology Department, Tunis, Tunisia*
[3]*Embryo-Foetopathology Department, Tunis, Tunisia*
[4]*Department of Neonatology, Tunis, Tunisia*

OBJECTIVES: Tracheal agenesis (TA) is an extremely rare malformation which results inevitably in immediate respiratory distress after delivery. Our aim was to describe a case of TA in a premature neonate and to discuss its prenatal ultrasound diagnosis difficulty and etiopathogenesis. METHODS: We report the autopsy findings in a premature male neonate presenting with TA. He was delivered at 32 weeks by a 39-year-old woman with three previous healthy children and admitted in neonatal resuscitation unit because of respiratory distress syndrome. Attempts at endotracheal intubation and tracheostomy were unsuccessful. The baby died within 2 hours of birth. The antenatal ultrasound was unremarkable. Karyotype was not performed. RESULTS: Keeping the diagnosis of TA in mind, barium esophagography was performed and showed TA with bronchoesophageal fistula of Floyd type III. A complete autopsy was performed. On dissection, the two main bronchi were opening separately in the lower part of the esophagus. The lungs appeared solid with right lung hypolobulation. On microscopic examination, the pulmonary alveoli were filled with fibrinous exudate and erythrocytes. The associated malformations included characteristic facial dysmorphism, double superior vena cava, ostium secundum atrial septal defect, patent arterial duct and horseshoe kidney. The postmortem radiography didn't show vertebral anomalies. CONCLUSIONS: The prenatal sonographic diagnosis of TA is difficult. It is possible with MRI, but the really challenge is to think about this pathology. The association of TA with other congenital malformations has been the subject of particular interest. It may be a component feature of the VACTERL/VATER association. The constellation of malformations presented in our case have overlapping features with VACTERL association. However, the typical facial dysmorphism and the absence of caudal anomalies suggest trisomy 18. A different pattern of association known as TACRD association was described. Our case has all the characteristic features of this association except duodenal atresia. Unfortunately, the etiopathogenesis of both associations remains

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LB-5

Rapid, efficient, non-invasive fetal sex determination direct from maternal plasma

Lucy Jenkins[1], Louisa Steel[1], Michael Parks[2], Fiona McKay[1], Chris Sale[2]

[1]*NE Thames Regional Genetics Laboratory, London, United Kingdom*
[2]*Nonacus Ltd, Birmingham, United Kingdom*

OBJECTIVES: Many European countries determine fetal sex by analysis of cell free DNA (cfDNA) in maternal plasma to triage pregnancies at high risk of sex-linked disorders for invasive testing. This has significantly reduced the invasive testing rate in this high risk group by targeting male bearing pregnancies for X-linked disorders. In the UK this test was approved in our public sector National Health Service (NHS) in 2011 and is now delivered by five NHS Regional Genetics laboratories. Current approaches require cfDNA extraction from plasma prior to analysis. Here we present validation of a method analysing cfDNA direct from maternal plasma. METHODS: Our current protocol involves duplicate cfDNA extractions (QIAsymphony SP) from 2ml of double spun plasma followed by detection of the *SRY* and *CCR5* genes by quantitative real-time PCR (Q/RT-PCR) (Taqman, ABI 7300). We identified 100 stored plasma samples from high risk pregnancies of 7-10 weeks gestation with fetal sex tested previously and clinically confirmed. Up to thirteen 80ul plasma samples with controls were tested simultaneously using the Cell3™Direct: Fetal Sex Determination kit (Nonacus, UK) which detects the *SRY*, *TSPY*, *DAZ* and *CCR5* genes by multiplexed Q/RT-PCR. Thresholds for classification of male or female were established. RESULTS: Results will be presented on the cohort of 100 stored samples tested. This cohort includes both male and female bearing pregnancy samples previously inconclusive using the standard protocol and several samples below 9 weeks gestation. This technique has been shown to be more sensitive than the current protocol. Hands-on time in the laboratory was reduced from three hours for extraction and set-up for a single sample with the current protocol to two hours for 13 samples. CONCLUSIONS: This is the first direct from plasma, non-invasive prenatal test to determine fetal sex. Removal of the extraction step streamlines the protocol and reduces consumables and labour costs. The pre-plated assay allows a rapid turnaround of approximately 3 hours from sample receipt, allowing same-day reporting and improving the current turnaround time of 3 days. 1- 13 samples can be tested per run, increasing throughput and reducing hands-on time. The reduced plasma volume required allows for a smaller blood collection or for further testing if required. Further validation on a larger cohort is required to define precise sensitivity with confidence.

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LB-6

Interleukins and cervical length in prediction of preterm delivery in symptomatic patient

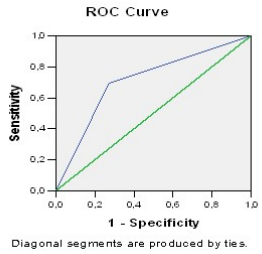
Marija Hadzi Lega, Ana Daneva Markova, Elena Dzikova, Mile Tanturovski, Vlatko Girevski

University Clinic for Gynecology and Obstetrics, Skopje, Macedonia

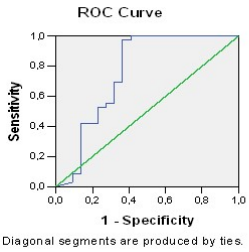
OBJECTIVES: To assess the use of cervical length, IL-2R and IL-6 in the prediction of preterm delivery in symptomatic patient. METHODS: The study was conducted as a prospective study at the University Clinic of Gynecology and Obstetrics, University "Ss. Cyril and Methodius" in Skopje Macedonia. The study included 58 women with singleton pregnancies with a menstrual age between 24 and 36+6 gestational weeks, admitted at the Clinic with a diagnosis of preterm labor defined as uterine contractions and/or changes in the consistency of the cervix. Cervical length was prospectively measured, and we also assessed IL2R and IL-6 from cervicovaginal fluid and serum in them. The obtained data was digitized, and all statistical tests were performed. RESULTS: Cervical length with optimal cut-off value 21,5 mm was significant predictor for preterm birth, which gave a sensitivity of 69.4%, specificity of 72.7%, PPV 75%, and NPV of 54%. The univariate analysis of cervico-vaginal secretion IL-6 gave a sensitivity of 69.4%, specificity of 68.2%, PPV 78.1%, NPV 57.69%, LR+ 2.18, LR- 0.45 and AUC of 0.759. Serum concentratios of IL-6, had no diagnostic use in the prediction of preterm delivery. We also found significant diffrences in the serum concentrations of IL-2R with sensitivity of 69.4%, specificity of 68.2%, PPV 78.12%, NPV 57.7%, LR+ 2.18, LR- 0.45 and AUC of 0.688. CONCLUSIONS: Cervical length < 21,5 mm, IL-6 in cervicovaginal secretion witn sensitivity of 69.4%, specificity of 68.2%, PPV 78.1%, NPV 57.69%, LR+ 2.18, LR- 0.45, AUC of 0.759 and IL-2R in serum with sensitivity of 69.4%, specificity of 68.2%, PPV 78.12%, NPV 57.7%, LR+ 2.18, LR- 0.45 and AUC of 0.688 may significantly improve the risk assessment for preterm delivery, help to plan subsequent pregnancy management and reduction of unnecessary hospitalization. However, the study is only the begining of this type of research in our population. Further research is required in terms of the evaluation of cost-benefit.

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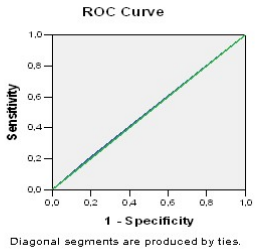
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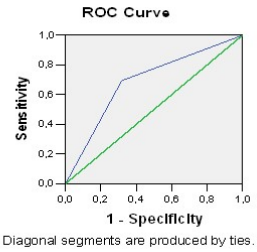
ROC curve for the performance of cervical length as a predictor of preterm delivery



ROC curve for the performance of IL – 6 in cervix as a predictor of preterm delivery



ROC curve for the performance of IL – 6 in serum as a predictor of preterm delivery



ROC curve for the performance of IL – 2R in serum as a predictor of preterm delivery

Author Mar

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