629/PT

Prenatal detection of WGiUPD in a chimeric cohort: a retrospective review

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I. Introduction

Chimerism, a condition in which a person has not one but two complete genomes in their body, was first conclusively identified in humans in ~1962. Chimerism differs from mosaicism which is a post zygotic mitotic error resulting in two related cell lines. Chimerism is thought to be an error of fertilization and could involve multiple germ cells. Androgenetic chimerism would involve the diploidization of the paternal genome and may involve up to four gametes including fertilization of a single egg or polar body by one or two sperm. Fewer than 50 cases of chimerism have been reported in the literature to date. With the advent of SNP microarray, a specific type of chimerism, involving whole genome uniparental isodisomy (WGiUPD) has been revealed. WGiUPD of paternal origin has been described in over 30 live born infants, most with some features of Beckwith-Wiedeman syndrome (BWS). Individuals can also have features seen in any imprinting disorder caused by paternal uniparental disomy including transient neonatal diabetes and IUGR with chromosome 6, Kagami-Ogata syndrome with chromosome 14, Angelman syndrome with chromosome 15, and Parathyroid hormone (PTH) resistance with chromosome 20. WGiUPD of maternal origin is a much more rare occurrence with a phenotype that has been less defined and only appears rarely in the literature. The presence of GWiUPD also increases the risk for autosomal recessive disorders carried by the parent of origin. Paternal WGiUPD has been recognized in cases of placental mesenchymal dysplasia (PMD) and molar pregnancies. However, there is little information reported about the prenatal detection of this phenomenon.

II. Methods

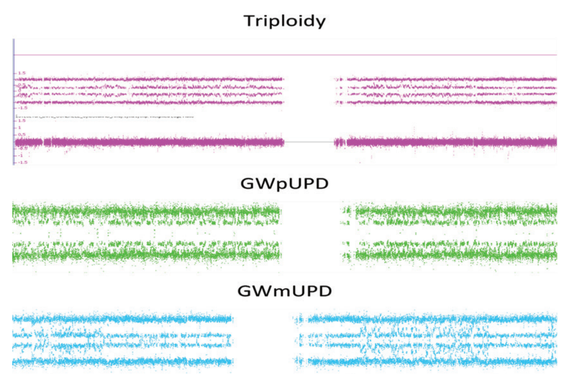
This study retrospectively examined the prenatal detection of WGiUPD in a cohort of ~60,000 prenatal patients and ~20,000 POCs that were studied by microarray analysis utilizing a SNP microarray (Affymetrix® Cytoscan® HD).

ARRAY METHODOLOGY: All studies were done utilizing the Affymetrix® Cytoscan® HD array [Affymetrix® and CytoScan®are registered trademarks of ThermoFisher Scientific]. This array contains approximately 2.695 million markers across the entire human genome. There are approximately 743,000 SNPs and 1,953,000 structural non-polymorphic probes (NPCNs).

On average, there is approximately 0.88kb between each marker. DNA was extracted utilizing standard methods and 250ng of total genomic DNA was digested with NspI, ligated to adaptors, and amplified using Titanium Taq with a GeneAmp PCR System 9700. PCR products were purified using AMPure beads and quantified using NanoDrop 8000.

Purified DNA was fragmented and biotin labeled and hybridized to the Affymetrix Cytoscan® HD GeneChip. Data was analyzed using Chromosome Analysis Suite. The analysis is based on the GRCh37/hg19 assembly.

Figure 1. Examples of allele tracks (allele differences) for triploidy, GWpUPD, and GWmUPD on SNP microarray



III. Results

In total, eight patients were detected prenatally with GWiUPD (~0.017%) and seven POCs revealed WGiUPD (0.035%). In all of the above cases where WGiUPD was detected, the abnormal cell line was present with a frequency ranging from 20-80% of cells. It is not known at this time how the variation in the percentage of WGiUPD cells present effects development or pregnancy outcome. The majority of the seven POCs specimens were submitted for testing because of placental abnormalities including enlarged placentas, mesenchymal dysplasia, and molar pregnancies. The eight prenatal patients were ascertained because of abnormal ultrasound findings. Five of eight had findings that were frequently reported in the literature and consistent with Beckwith-Wiedemann syndrome (e.g. omphalocele, kidney abnormalities) or because of larger placentas. However, two were detected with less common findings including IUGR, oligohydramnios, and clubfoot. These latter two cases were demonstrated to have WGiUPD of maternal origin rather than paternal. Additionally, there was a third maternal WGiUPD with no clinical information available.

Table 1. Prenatal cases

Prenatal	Gestation (Wks)	Specimen	Result	% Abnormal Cell Line	Phenotype Information	Parent of Origin
Case 1	18	amniotic fluid	microsatellite confirmed normal male/teratoma admixture	25%	moderate oligohydramnios	maternal
Case 2	21	amniotic fluid	fetal admixture defined as a chimera of a diploid doubled paternal cell line and a biparental cell line	50%	omphalocele, kidney abnormality, paternal grandmother with multiple losses, and her daughter passed in infancy	paternal
Case 3	34.2	amniotic fluid	apparent chimeric admixture of a diploid doubled maternal cell line and a biparental cell line	25%	IUGR, clubfoot	maternal
Case 4	19	amniotic fluid	female with likely chimera of a diploid homozygous parental cell line and a biparental cell line	40%	abnormal placenta, echogenic bowel, **outcome** – pregnancy delivered stillborn	paternal
Case 5	25.4	amniotic fluid	female with likely chimera of a diploid homozygous parental cell line and a biparental cell line	23% direct – 33% cultured	ventriculomegaly, echogenic bowel, cardiac abnormality, enlarged kidney, shortened long bones, cystic placenta, **outcome** – patient delivered soon after amnio and baby died a week later	unknown
Case 6	>15 weeks	amniotic fluid	apparent female/male chimerism	60%	no clinical	maternal
Case 7	19.6	amniotic fluid	apparent androgenic chimerism	80%	IUGR, large suspected molar placenta, abnormal maternal serum screen for T21	paternal
Case 8	26.5	amniotic fluid	likely chimera of a diploid homozygous cell line and a biparental male cell line	50%	enlarged kidneys bilaterally, multicystic placenta with mesenchymal dysplasia, and positive BWS molecular analysis	paternal

Table 2. POC cases

POC	Gestation (Wks)	Specimen	Result	% Abnormal Cell Line	Phenotype Information	Parent of Origin
Case 9	28.2	POC	female with likely chimera of a diploid homozygous cell line and a biparental cell line of placental origin	33%	cystic placenta, baby born premature between 27-28 weeks with IUGR and still alive 3-4 weeks after delivery	unknown
Case 10	35.1	POC	chimerism associated with placental mesenchymal dysplasia	50%	placental mesenchymal dysplasia, suspected complete hydatiform mole with live fetus, full term baby reportedly normal	paternal
Case 11	9	POC	chimerism associated with PMD	50%	hydatiform mole unspecified	paternal
Case 12	7	POC	chimerism often associated with PMD	50%	fetal loss	paternal
Case 13	9.1	POC	female with apparent chimeric admixture	50%	missed abortion, empty sac	paternal
Case 14	Unk	POC	apparent chimerism with a partial whole genome UPD	25%	missed abortion	unknown
Case 15	33	POC	female with apparent androgenic-biparental chimerism with trisomy 18 in the biparental cell line	30%	later gestation fetal loss, fetal anemia, giant placenta	paternal

Table 3. Ultrasound findings reported on 15 cases of GWiUPD

Ultrasound Findings	1 (Mat)	2 (Pat)	3 (Mat)	4 (Pat)	5 (Unk)	6 (Mat)	7 (Pat)	8 (Pat)	9 (Unk)	10 (Pat)	11 (Pat)	12 (Pat)	13 (Pat)	14 (Unk)	15 (Pat)
Placentomegaly / Abnormal placenta				●											●
Hydatiform mole							●			●	●				
Cystic placenta					●			●	●						
Placental mesenchymal dysplasia								●		●					
Fetal loss												●			
Omphalocele		●													
Urinary anomalies		●			●			●							
Oligohydramnios	●														
IUGR			●				●		●						
Clubfoot			●												
Echogenic bowel				●	●										
Ventriculomegaly					●										
Cardiac anomaly					●										
Short long bones					●										
Empty sac													●		
Fetal anemia															●

IV. Discussion

The advent of SNP microarray analysis has allowed for possibility of identifying chimerism involving whole genome uniparental isodisomy (WGiUPD) thus providing new information previously not available in pregnancy. Based on the results of this retrospective review and a review of the current literature, GWiUPD is a rare occurrence (~0.017% prenatally and 0.035% on POC). That being said, it remains to be determined if GWiUPD is truly rare or under diagnosed due to the associated high rate of pregnancy loss and/or the lack of SNP microarray testing performed on these pregnancies. Additional research and identification of prenatal cases in this WGiUPD chimerism cohort will continue to expand our understanding of the condition and its outcomes in pregnancy. This additional information can result in greater understanding of the immediate implications for prenatal care and counseling. Previous studies have suggested that if WGiUPD is detected in a fetus the pregnancy must be closely monitored for the possibility of premature labor and delivery at ~33 weeks. Couples should also be counseled that GWiUPD can increase the risk of autosomal recessive disorders in the affected pregnancy for any conditions the parent of origin is carrier.

Additionally, since the evidence shows WGiUPD of paternal origin cases are often associated with Beckwith-Wiedemann syndrome, the features and health concerns associated with this condition should be discussed with any couple receiving this diagnosis prenatally while accentuating how varied in outcome these cases can be. Guidelines for Beckwith-Wiedemann syndrome indicate an 8% risk for embryonal tumors when BWS is a result of pUPD11. When the cause is due to paternal GWiUPD, this increases to 50% for a BWS related tumor and as high as 69% for any tumor or mass. Patients with XY/XX chimerism are at risk for germ cell tumors and should be counseled similarly to 45,X/46,XY males with gonadal dysgenesis. Additionally, patients with paternal GWiUPD have presented with phenotypes contrary to BWS (example IUGR) likely due to the percent of abnormal cell line present and in which tissue it is present. Future research may further elucidate if there is a threshold in which GWiUPD is incompatible with life but variability in tissue type, increased AR risk, and other unknown variables may limit the ability to provide this answer.

In this retrospective review of prenatal cases, the data shows that 38% of prenatally detected WGiUPD cases may be maternal in origin and little is known about their predicted outcome accentuating the need to counsel these cases cautiously. Future directions for this research include further study of maternal WGiUPD.

V. References

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