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Detection of isodisomy utilizing SNP microarray: Frequency, ascertainment, and implications

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Introduction

Along with being a valuable tool at detecting gains and losses of chromosomal material, SNP microarray has also provided other unexpected information that can assist a patient in resolving the diagnostic odyssey. One such finding is the detection of regions of homozygosity, which occurs when both alleles on a chromosome are identical and can be attributed to multiple causes. When an isolated chromosome demonstrates complete homozygosity, it is known as isodisomy and is associated with uniparental disomy (isoUPD). Isodisomy often is caused by a non-disjunction error in meiosis II followed by a trisomy rescue but could also result from a monosomy rescue event. This large clinical study evaluates cases in which SNP microarray results demonstrated whole chromosome isodisomy. Three populations of specimen categories are analyzed, products of conception (POC), prenatal specimens, and postnatal specimens to identify chromosomes with isodisomy and evaluate chromosome distribution, associated phenotypes and parental origin of isodisomy, highlighting differences between these specimen types.

Methods

Results on over 330,000 patient specimens [200,000 pediatric, 100,000 prenatal, and 30,000 products of conception (POC)] sent in for diagnostic SNP microarray testing over the past 14 years were reviewed to identify results indicating isodisomy of individual chromosomes.

Array methodology:

All studies were done utilizing the Affymetrix® Cytoscan® HD array (Affymetrix® and CytoScan® are Registered Trademarks of Affymetrix, Inc.). This array contains approximately 2.695 million markers across the entire human genome. There are approximately 743,000 single nucleotide polymorphic (SNP) probes and 1,953,000 structural non-polymorphic probes (NPCNs). On the average there is approximately 0.88 kb between each marker. DNA was extracted utilizing standard methods and 250 ng of total genomic DNA extracted was digested with NspI and then ligated to NspI 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. The analysis is based on the GRCh37/hg19 assembly. Data was analyzed using Chromosome Analysis Suite (ChAS 4.0). Isodisomy was evaluated using both the allele difference track and the B-allele frequency track **(Figure 1)**.

Results

Review of over 330,000 diagnostic SNP microarray results yielded 132 cases (~0.04%) overall with isodisomy of an individual chromosome. The frequency of occurrence based on specimen type was 6 POC cases (0.02%), 40 prenatal cases (0.04%), and 86 postnatal cases (0.04%). Three postnatal cases demonstrated two chromosomes with isodisomy. Chromosome 14 was the most common isodisomy finding among the prenatal specimens, while isodisomy 6, 7, and 15 were more frequent in postnatal arrays. There were no cases of isodisomy for chromosomes 13, 16, 17, and 21.

Among the prenatal isodisomy cases, 21 cases (52.5%) involved imprinted chromosomes with 19 (47.5%) involving non-imprinted chromosomes. For postnatal cases, 56 cases (65.1%) involved imprinted chromosomes with 30 (34.9%) involving non-imprinted chromosomes. There were 3 in each category for the POC cases **(Figure 2)**. Of cases with follow-up parent of origin testing (45), 31 cases were found to be of paternal origin while 14 cases were maternal in origin (68.9% vs 31.1%).

There were four cases that demonstrated mosaic aneuploidy cell lines concurrent with an isodisomy cell line (1 POC, 2 prenatal, 1 postnatal). Additionally, one postnatal case resulting in full isodisomy was submitted for microarray with a history of prenatal testing that demonstrated a mosaic trisomy cell line. One case involved testing on amniotic fluid, and then post-delivery testing was completed on cord blood and three placenta samples. The results on these specimens demonstrated a mosaic trisomy 5 cell line, as well as biparental and uniparental isodisomy chromosome 5 cell lines.

Two cases resulted in detection of autosomal recessive conditions associated with the presence of chromosomal isodisomy. In addition, two cases of reported phenotypic sex reversal were among cases with isodisomy of chromosome 2.

There were 5 cases of isodisomy 11 identified (2 POC, 3 prenatal). Within this data set, 1 POC was a first trimester loss. The other was a loss at 25 weeks with ultrasound detection of an omphalocele and macroglossia. One of the prenatal cases showed methylation patterns consistent with Beckwith Wiedemann syndrome (BWS). Ultrasound demonstrated omphalocele and enlarged kidneys. The other two prenatal cases also were reported to have ultrasound findings suggestive of BWS.

Four cases demonstrated mosaic isodisomy for chromosomes with a normal copy number. One prenatal specimen showed 50% isodisomy for chromosome 18 with a normal 18 copy number; however, the microarray testing also demonstrated non-mosaic trisomy 21. There was one postnatal case demonstrating 50-60% isodisomy of chromosome 6. Two POC cases demonstrated 15% of isodisomy of chromosome 2 and 70% of isodisomy of chromosome 3, respectively.

Discussion

The overall frequency of isodisomy is rare with similar frequencies seen in prenatal and postnatal patients. Isodisomy is seen less frequently in POCs within this study, which would support the concept that most cases of lost pregnancies are the result of failure to rescue an aneuploidy. Among prenatal specimens, the number of imprinted chromosomes and non-imprinted chromosomes was similar; unlike postnatal cases where approximately two-thirds of cases are imprinted chromosomes. Given this is a clinical population, it follows that the associated phenotype of an imprinted chromosome would provide a clinical indication for diagnostic testing. This concept is further supported by the detection of more isodisomy 14 cases prenatally, when the phenotype of paternal origin includes abnormalities that can be detected by prenatal ultrasound. Similarly, isodisomy 15 was detected more frequently in the postnatal population. The phenotypes of Prader-Willi syndrome and Angelman syndrome are less likely to involve anomalies that can be detected prenatally. Additionally, the syndrome phenotypes matched the clinical indication in the majority of postnatal cases.

Looking at parent of origin as well as mosaic cases of aneuploidy with isodisomy or mosaic isodisomy with diploid cell lines provides further insight into mechanisms. Of the cases with parent of origin studies in this series, paternal origin was more common than maternal origin.

The cases in which a mosaic aneuploidy line is present support aneuploidy rescue as a mechanism that can result in isodisomy. Four of these cases were mosaic for a trisomy, while one demonstrated mosaic monosomy. While a monosomy rescue would result in two identical chromosomes, the evidence of trisomy rescue supports the division error in meiosis II. Studies have reported cases of trisomy 16 and 21 resulting mainly from errors in meiosis 1. This data provides further support for meiosis I non-disjunction given there were no cases of isodisomy of chromosomes 16 or 21 observed.

In addition to imprinting disorders, isodisomy is associated with other significant clinical implications. One such implication is that isodisomy unmasks deleterious variants in genes associated with autosomal recessive conditions. Within this data set, there are two cases of prenatal detection of autosomal recessive conditions - a homozygous pathogenic sequence variant in the *P3H1* gene and a homozygous deletion in the *TMEM237* gene, both resulting in an abnormal outcome. Additionally, there were 2 cases of isodisomy 2 with sex reversal, suggestive of a candidate gene associated with a recessive sex reversal condition being present on chromosome 2.

Copy-neutral mosaicism of homozygosity of chromosome 11p is due to a post-zygotic derived mitotic recombination event which results in an expanding cell population with segmental uniparental disomy (UPD). There are imprinted genes within this region (11p15.5) that with paternal segmental UPD lead to Beckwith-Wiedemann syndrome (BWS). Within this data, no cases of isodisomy 11 were ascertained past the neonatal period consistent with previous suggestions that non-mosaic isodisomy of chromosome 11 may be associated with a prenatal or neonatal lethality. Although not all pregnancy outcomes were known in this data, the ultrasound findings in our chromosome 11 cases were suggestive of BWS.

There were four cases in the study that demonstrated mosaicism of the isodisomy with no aneuploidy for the same chromosome. The finding of mosaic isodisomy lines suggests multiple repairs of aneuploidy that results in two cell lines, or a trisomy rescue in which the resulting monosomy cell undergoes a chromosome rescue that results in the isodisomy.

Conclusion

In our dataset, the frequency of isodisomy was essentially equivalent in prenatal and postnatal clinical populations, but with clear phenotypic differences between the chromosomes involved in the two populations. The imprinted conditions with a prenatally diagnosable phenotype, such as UPD 14, are more common in the prenatal setting. Phenotypes associated with UPD 6, 7, and 15 are more likely to be ascertained postnatally, as such isodisomy of these chromosomes are more common in the postnatal population **(Figure 3)**. Of the specimens tested, a paternal origin of the isodisomy was more common than maternal origin. Since both chromosomes are identical in cases of isoUPD, the unmasking of an autosomal recessive gene with a deleterious variant is a risk. These data are consistent with meiosis 1 errors being the source of trisomy 16 and 21 since no cases of isodisomy were seen in a rescue. Additionally, no postnatal cases of isodisomy 11 were identified, supporting the previous notion of prenatal or perinatal lethality. Overall, these findings show that SNP microarray analysis, in addition to providing diagnostic information regarding deletions and duplications, can also provide information regarding isodisomy that can have important clinical implications.

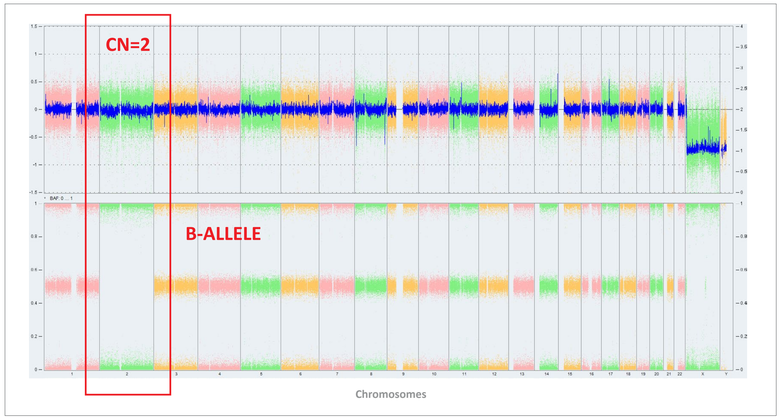

Figure 1. Array data example from case of chromosome 2 isodisomy. Top graph demonstrates Log 2 Ratio of 0, consistent with copy number of 2. Bottom graph demonstrates B-allele track with loss of heterozygosity, loss of the middle line seen on the other chromosomes.

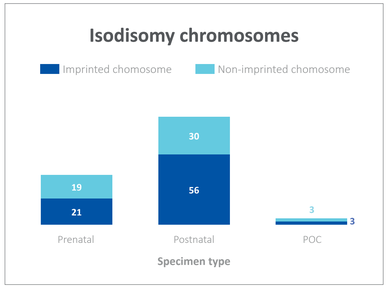

Figure 2. Number of isodisomy chromosomes with parent of origin studies by specimen category.

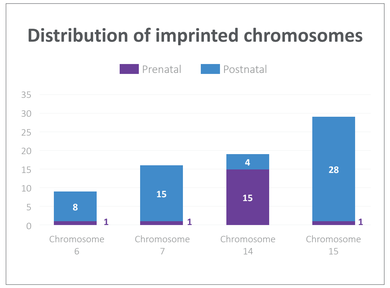

Figure 3. Distribution of the most common imprinted chromosomes within prenatal versus postnatal specimen groups.

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