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Prenatal SNP microarray analysis of over 60,000 patients: implications, importance and intriguing findings

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I. Introduction

Microarray analysis has been an important technology in the cytogenetics laboratory over the past decade. This technology has the capability to detect small copy number gains and losses not seen with standard chromosome analysis, as well as, alterations involving homozygosity associated with identity by descent and uniparental disomy. Although microarray testing is now usually recommended as the first tier test for pediatric patients and has been utilized for prenatal samples over the past 8 years, it is still not universally utilized prenatally. In this study, we report on the largest number of prenatal patients utilizing a SNP microarray (Cytoscan® HD – Applied Biosystems™) analysis of over 60,000 specimens. This study not only indicates the efficacy and usefulness of this technology, but also highlights several novel and unusual findings, as noted below.

II. Methods

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 the 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.

III. Results

The frequency of pathogenic abnormalities in AMA patients (not detected by standard chromosome analysis) is ~1.84%; however, ~63% of these abnormalities are associated with neurodevelopmental microdeletion/duplications (e.g. 1q21.1, 16p11.2) and of these ~2/3 are inherited.

Overall the frequency of pathogenic abnormalities (not detected by standard chromosome analysis) in patients referred for ultrasound abnormalities was greater (5.0%) than for AMA patients, and was slightly increased in patients with multiple anomalies (6.1%). However, in patients with a heart defect, the frequency of pathogenic abnormalities was considerably elevated (8.1~8.2%) and while the most frequent single abnormality in this latter group is a 22q deletion, over 66% involved a finding other than a 22q deletion (**Figure 1**).

Figure 1. 3.83Mb deletion of 8p23.1->p23.1

Patient ascertained with ultrasound anomalies (including congenital heart defect, diaphragmatic hernia). Deletion includes loss of the *GATA4* gene

Table 1. Frequency of anomalies based on ascertainment

	Pathogenic	IBD	UPD	Total
Major	4.9%	2.9%	0.4%	8.2%
Major – heart	8.1%	2.4%	0.3%	10.8%
Multiple anomalies	6.1%	5.7%	1.2%	13.0%
Multiple anomalies – heart	8.2%	3.5%	1.0%	12.7%
Nuchal translucency	3.5%	4.4%	0.3%	8.2%
Diaphragmatic hernia	6.2%	2.1%	0.5%	8.8%
Holoprosencephaly	6.9%	2.5%	2.9%	12.3%

The analysis of patients with ultrasound abnormalities has also indicated that for pathogenic abnormalities that were unique (not a specific microdeletion), 18% of patients had microarray anomalies greater than 12Mb (and not detected by standard chromosome studies). Additionally, of all of the pathogenic abnormalities; 11.1% were greater than 7Mb; 75% less than 3Mb; and ~50% less than 1Mb. Examination of unique pathogenic abnormalities, detected in this analysis, revealed ~29.2% of patients had changes that were also detected in one parent.

Overall 542 abnormalities classified as VUS studied in detail

VUS – No pathogenic genes (302 – 56.0%) ~15.2% *de novo*
VUS – Partial duplication of gene (177 – 32.8%) 5.3% *de novo*
VUS – Whole duplication of haploinsufficient gene (33 – 6.1%) 13.0% *de novo*
VUS – Smaller CNV than normally reported (27 - 5.0%) 33.3% *de novo*

By applying strict prenatal reporting criteria, variants of uncertain significance (VUS) are only reported in 1~2 % of prenatal patients (with similar frequencies regardless of ascertainment). The vast majority of these are larger than reporting criteria with no known pathogenic genes and only 11.6% of VUS studied have been shown to be *de novo*.

Utilization of SNPs and detection of homozygosity revealed identity by descent in only 1.1% of AMA patients, but 2~3 times greater in patients with major or multiple anomalies detected prenatally. Follow-up of numerous patients have elucidated underlying recessive genetic disorders delineated within the long homozygous regions.

Long runs of homozygosity associated with uniparental disomy were seen in ~0.6% of patients, while not diagnostic, were confirmed in ~66% of patients

Mosaicism was seen in 1.16% of the samples studied

Copy neutral change – identity by descent

- 3.7% of our postnatal population
- In contrast – ~1.1% in AMA population [background]
- Prenatal – IBD cases detected with abnormal ultrasound [~3.1%]
  - Increased nuchal translucency ~4.4%
  - MCA + heart defects ~3.5%
  - Multiple anomalies ~5.7%

Microarray can only suggest UPD

- Isodisomy – confirmation of UPD
- Molecular testing needed to confirm heterodisomy
- Both imprinted and non-imprinted chromosomes detected
- Most common imprinted chromosomes detected
  - Hypothorax – UPD 14
  - Omphalocele – UPD 11

If UPD is present

- There is a residual risk of early trisomy or monosomy

Is there a potential AR disorder in homozygous region?

- Correlate ultrasound phenotype with genes in ROH whether or not UPD is confirmed
- Follow-up with molecular testing

Mosaicism

- Twice as frequent in CVS as amniotic fluids (AF) (1.89% vs 0.92%)
- Aneuploidy the most commonly seen (~50%)
- 26.2% of cases of mosaicism could not be confirmed
  - Confirmation higher in AF samples than CVS
  - ~33% of AF cultures and 33% of all CVS could not be confirmed
  - However, only 15.1% of AF direct specimens could not be confirmed

Approximately 8.7% of confirmed aneuploidy also showed UPD

Deletions associated with recessive diseases have been reported in 1.0% of cases, which mainly involved 3 different disorders (*NPHP1, CTNS, OTOA*). One homozygous deletion (a deletion involving *TMEM237* in an isodisomy 2 patient) has been detected prenatally. Additionally, parental follow-up has revealed cases where both parents are carriers of a recessive gene deletion and have a 25% risk, of an affected fetus, in the next pregnancy.

IV. Conclusions

The findings in the current study have broad implications of the utilization and interpretation of prenatal microarray analysis including:

1. It is by far the most definitive study to date conclusively showing an increased yield of anomalies both in low-risk and high risk patients;
2. Not all of the findings can be correlated to the reason for referral but will still have broad implications on the pregnancy;
3. There is a higher frequency of neurodevelopmental susceptibility microdeletion/duplications detected than originally anticipated which makes counseling problematic but necessary;
4. The frequency of VUS that are de novo are less than 0.2% indicating that most questions can be resolved with the appropriate parental studies;
5. Homozygosity detection, utilizing a SNP array can detect recessive diseases in an identity by descent family, as well as, have a high predictive value for uniparental disomy;
6. Some pathogenic findings have been shown to be familial, indicating in some cases a parent having a similar phenotype, while in other cases, have definitively shown that 1-2 exon deletions of some genes appear to be benign;
7. Overall all of these findings and conclusions continue to demonstrate that a SNP based microarray analysis should be a first tier test replacing cytogenetic analysis; and should be covered by all insurance carriers.

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