

I. Introduction

Chromosomal microarray analysis is considered a first tier test for investigation of pediatric patients with intellectual disability and/or congenital anomalies, but is also often employed prenatally when ultrasound findings are detected or there is an increased background risk present based on family history. It is well established that consanguinity increases the general population background risk for both birth defects (and congenital anomalies) along with autosomal recessive (AR) disorders. Microarray analysis can detect consanguinity (or various degrees of relationship) which may assist in narrowing down potential genetic causes for such anomalies and/or recessive disorders. The detection of consanguinity and long continuous regions of homozygosity, from SNP microarray analysis, has been very informative in delineating homozygous deletions associated with recessive diseases in pediatric patients. However, due to the lack of an accompanying phenotype the detection of recessive diseases in prenatal cases is more problematic.

The aim of this analysis is to review a large laboratory's experience with Chorionic Villus (CVS) and amniocentesis samples in which identity by descent has been identified. In this study, we highlight some cases where SNP microarray detected regions of homozygosity (ROH) in which an AR disorder was diagnosed, providing an explanation for the anomalies observed but also vital information for recurrence risk.

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 0.88 kb between each marker. DNA was extracted utilizing standard methods and 250 ng 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.

The SNP array analysis is utilized to detect both copy number changes as well as copy neutral changes. This allows the detection of not only deletion and duplication, but also potential uniparental disomy and identity by descent. The presence of at least two Runs of Homozygosity (ROH) 8 Mb or greater is consistent with identity by descent. The more homozygosity within an individual, the closer the parental relationship.

HOMOZYGOSITY TOOL: The Genomic Oligoarray and SNP array evaluation tool v3.0 is a web-based program that was utilized to retrieve genes (using OMIM, USCS and NCBI databases) and their associated recessive diseases within the homozygous regions detected by the SNP array analysis [Wierenga et al. *Genet Med* (2012) doi:10.1038/gim.2012.136].

IV. Discussion

In this study, SNP microarray analyses of 60,000 prenatal patients have yielded intriguing results with respect to detecting homozygosity and consanguinity. Consanguinity was seen in approximately 1.4% of 8,000 AMA patients, which could be considered a general background finding and not related to an increase risk of a congenital disorder. However, analysis of over 10,000 patients with one identified major anomaly by ultrasound demonstrated consanguinity in ~2.8% of the pregnancies and when there were multiple anomalies, consanguinity was detected in ~4.85%. These findings clearly demonstrate that recessive diseases may be responsible in an increased number of these cases. After revealing homozygosity, molecular analysis has confirmed a number of homozygous mutations, including the genes *ALPL*, *PKHD1*, *SMN1*, *FRAS1*, *WDR34*, *MKS1* and *COQ9*, and well as the detection of homozygous deletions. Several of these are associated with skeletal dysplasia, one type of disorder that may be more amenable to prenatal detection. Additionally, examination of pregnancies studied because of a previous genetic disease within the family, demonstrated consanguinity in ~6.98% of the pregnancies studied suggesting an underlying recessive disease within the family and providing some clues for the delineation of a disorder not previously detected. Overall the findings from this study show the importance of the homozygosity component of the SNP analysis. While only a limited phenotype is usually available prenatally, making correlations difficult, it is noticeably useful in identifying families in which there might be a recessive disease and useful in detecting the specific genes in some cases. This allows for not only establishing a disorder in this pregnancy but provides importance recurrence risk information.

III. Results

Table 1 shows the frequency of the detection of identity by descent (IBD) based on the reason for ascertainment, along with the number of patients studied in each category. The lowest frequency was seen in AMA patients (~1.40% which can be considered a background frequency in populations) while the highest was for patients ascertained because of the presence of a genetic disorder in a previous child (~6.98%). The frequency of IBD was elevated for all groups of patients ascertained prenatally because of abnormal ultrasound findings. **Table 2** gives some examples of disorders that have been detected in prenatal patients because of the elucidation of regions of homozygosity (ROH). The elucidation of a few of these disorders is illustrated in **Figures 1-3**.

Table 1. Frequency of IBD/ascertainment

Ascertainment	Number of Patients	Frequency – IBD
AMA	~10,500	1.40%
US - Major Anomaly	~6,750	2.82%
US - Multiple Anomalies	~1,700	4.85%
US - Multiple (heart)	~1,450	3.12%
US - Minor	~2,300	3.21%
Nuchal Translucency	~3,450	3.05%
Previous Genetic DX	~487	6.98%
Pediatric	>130,000	3.70%

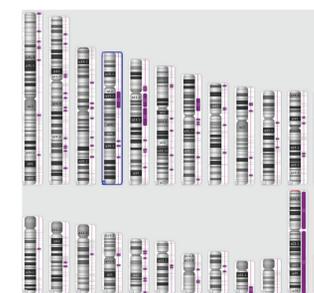
Table 2. Disorders detected – homozygosity

Hypophosphatasia
Polycystic Kidney Disease
Fraser Syndrome
Spinal Muscular Atrophy
Asphyxiating Thoracic Dystrophy
Bardet-Biedl Syndrome
Coenzyme Q10 Deficiency

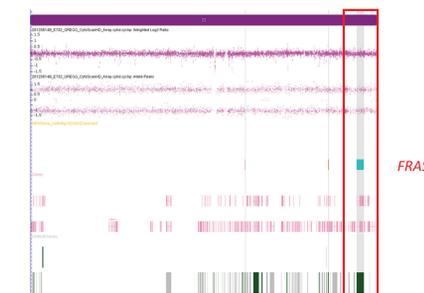
Patient 1:

27 year old female
3rd pregnancy with bilateral renal agenesis.
ROH: 4th-5th degree parental relationship;
Review of ROH: involvement of Fraser syndrome (*FRAS1*)

Array: 4th-5th degree relationship



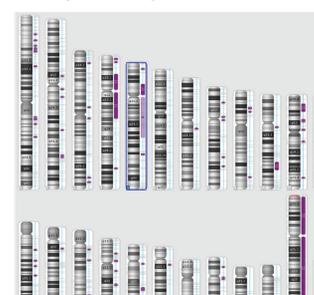
FRAS1 gene on chromosome 4 ROH



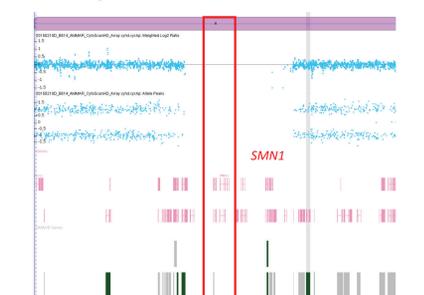
Patient 2:

32 year old female
18.4 weeks gestation
Previous child with *SMN1*
ROH: 3rd degree parental relationship
Review of ROH: *SMN1* gene involvement and confirmed no copies by molecular analysis.

Array: 3rd degree relationship



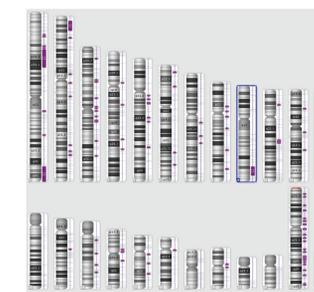
SMN1 gene on chromosome 5 ROH



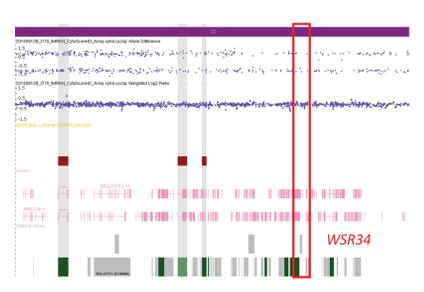
Patient 3:

32 year old female
Ultrasound: limb shortening
Previous child died due to lethal skeletal dysplasia
ROH: 4th-5th degree parental relationship
Review of ROH: involvement of *WDR34* gene (Asphyxiating Thoracic Dystrophy)

Array: 4th-5th degree relationship



WDR34 gene on chromosome 9 ROH



IV. Conclusions

These studies have yielded interesting findings regarding the detection of Runs of Homozygosity in patients studied prenatally and has assisted in providing directions in cases where these homozygous regions can clearly be linked to recessive disorders:

1. It is well established that recessive disorders can be elucidated from the examination of homozygous regions in pediatric patients because of the accompanying phenotypic findings; however this is more problematic in prenatal patients due to limited phenotypic information
2. Consanguinity was seen in approximately 1.3% of the AMA patients studied, which could be considered a general background as there are no known phenotypic anomalies in these patients;

3. Consanguinity in patients with one identified anomaly by ultrasound demonstrated 2 times greater than the background frequency in AMA patients and over 3 times greater when there were multiple anomalies detected by ultrasound suggesting the underlying presence of recessive disorders in these patients;
4. Follow-up of patients with homozygosity with whole exome sequencing has proved effective in elucidating underlying recessive disorders
5. Lastly, based on these studies, it shows the effectiveness and importance in utilizing a high resolution array with adequate SNP coverage to illuminate underlying recessive disorders that are important in both diagnosis and recurrence risk.