B-296

Prenatal Microarray Detection of VCF/DiGeorge syndrome: Impact of Ascertainment and the Importance of the Detection of a Second Microarray Abnormality

Integrated GENETICS
LabCorp Specialty Testing Group

Molinari, Sharon, MS, CGC ; Shafer, Jennifer, MS, CGC ; Riordan, Christine, MS, CGC; Kline, Laura, MS, CGC; Schwartz, Stuart, PhD, FACMG; Rudd, Katie, PhD, FACMG, Schleede, Justin, PhD, FACMG
Integrated Genetics, LabCorp Specialty Testing Group, Laboratory Corporation of America® Holdings, Westborough, MA

I. Introduction

One of the most common structural chromosome abnormalities detected prenatally is a deletion of 22q11.21 associated with DiGeorge/Velo-cardio-Facial (VCF) syndrome. Historically, this deletion has been identified using fluorescent in situ hybridization (FISH) with the TUPLE1 probe in fetuses presenting with a major heart defect detected by ultrasound. With the advent of microarray analysis, this deletion has been identified in fetuses presenting with ultrasound findings other than heart defects, as well as in conjunction with other unexpected microarray copy number variants.

II. Objectives

Demonstrate through case example the importance of prenatal microarray analysis for findings consistent with 22q11.21 deletion syndrome, as well as awareness of identifying the deletion in cases not presenting with typical ultrasound anomalies related to the syndrome. Furthermore, demonstrate the significant chance of finding a second copy number variant in fetuses with 22q11.21 deletions.

III. Methods

Approximately 50,000 prenatal microarrays were reviewed from a period spanning 7 years. Reported ultrasound findings were examined for cases with a 22q11.21 deletion. Cases with a 22q11.21 deletion that showed a second copy number variant were identified.

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 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.

IV. Results

A review of 50,000 prenatal microarray analyses over 7 years revealed 254 cases with 22q11.21 deletion results. Analysis of this data revealed that 7.5% of the samples with the 22q11.21 deletion had a second microarray abnormality detected. Approximately 38% of the fetuses with the 22q11.21 deletion did not have a prenatally detected heart defect. Specifically looking at the trends for the last year, 25% of cases with the 22q11.21 deletion had a second microarray abnormality and >50% with the deletion did not have a heart defect.

CASE PRESENTATIONS

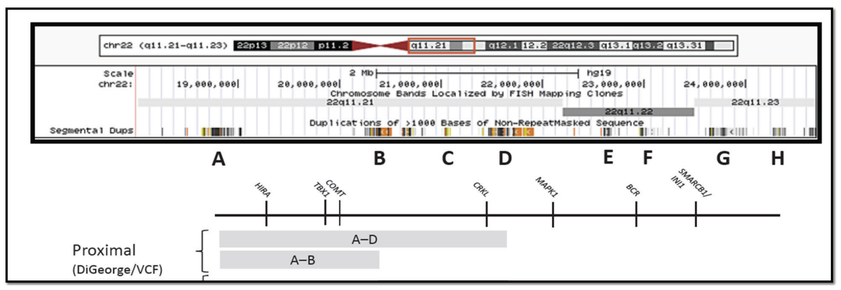
Case 1:

Patient underwent an amniocentesis due to ultrasound findings of bilateral clubfeet and an echogenic intracardiac focus. Microarray analysis revealed a 2.88 Mb deletion of the 22q11.21 region and an 888 kb deletion of the 16p11.2 region associated with autism. Parental studies were not performed; therefore, the inheritance of these two deletions is unknown at this time.

Case 2:

Patient underwent an amniocentesis due to ultrasound findings of Tetralogy of Fallot and a clubfoot. Microarray analysis revealed a 2.88 Mb deletion of the 22q11.21 region and a 498 kb deletion of the 2p16.3 region, including part of the *NRXN1* gene associated with neurodevelopmental problems. The 22q11.21 deletion is *de novo*, and the 2p16.3 deletion is paternally inherited. No clinical information was provided about the paternal phenotype.

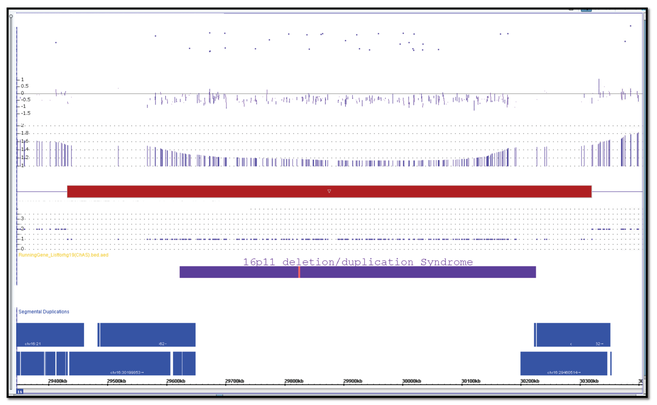
Standard 22q11.21 deletion



SNP Array – 16p11.2 deletion

Deletion interval

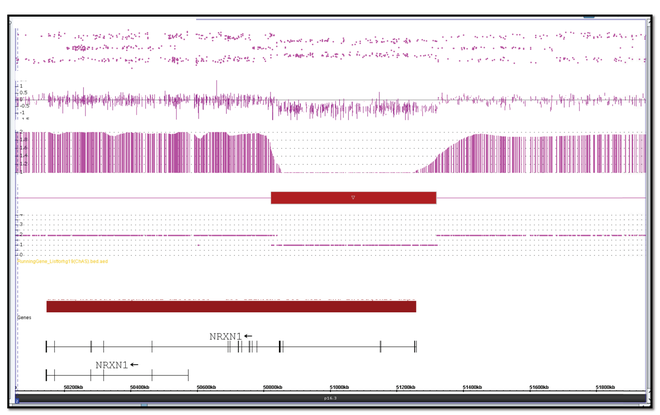
16p11.2 region



SNP Array – 2p16.3 deletion

Deletion interval

NRXN1 gene region



V. Discussion

Counseling related to the option of testing for 22q11.21 deletions has been historically focused on fetuses with congenital heart defects. Previously, testing was by FISH, and therefore, there was no possibility of secondary findings. Since microarray became used in prenatal testing as a standard for fetuses presenting with any ultrasound anomaly, the cases being reported with 22q11.21 deletion have expanded beyond the traditionally associated ultrasound finding of a heart defect. In this database, 38% of 22q11.21 deletion positive fetuses did not present with a heart defect. Following a trend in the data over time, this percentage has grown.

The increase of 7.5% up to 25% of cases with a secondary copy number variant (CNV) demonstrates the importance of counseling patients on the possibility of additional microarray findings which may impact future reproductive risks.

For case 1, the ultrasound findings of bilateral clubfeet and an echogenic intracardiac focus would not have previously led to a targeted discussion of either copy number variant found with testing or their associated phenotype. These findings could leave a patient somewhat blindsided with the results if not counseled about the various possibilities related to both copy number variants and phenotype.

For case 2, counseling regarding the 22q11.21 deletion would have been expected and appropriate given the ultrasound finding of Tetralogy of Fallot. However, the CNV of the 2p16.3 region affecting the *NRXN1* gene associated with neurodevelopmental problems might be completely unexpected if counseling had not included a discussion of the possibility of other findings.

Due to the evidence in our database pointing toward a high incidence of a second CNV, it is important that microarray be considered the standard prenatal diagnostic test for suspected 22q11.21 deletion syndrome. This is true even in the presence of a heart defect that is strongly associated with 22q11.21 deletion syndrome. If case 2 had only 22q11.21 FISH testing, the second CNV would not have been detected.

Overall, these findings suggest that the referral pattern has changed for microarrays. These trends show that there is an increase in the probability of a second microarray anomaly in patients with 22q11.21 deletions and emphasize the importance of counseling patients about the possibility of additional findings. They also highlight the possibility of finding a 22q11.21 deletion in fetuses without any type of heart defect. Over time, this type of data could reshape the prenatal hallmarks for this common deletion.

LabCorp

VI. References

Burnside RD, *Cytogenet Genome Res*. 2015; 146(2):89-99.

Bena et al., (2013) *Am J Med Genet B Neuropsychiatr Genet* 162B(4):388-403. PMID#23533028

Miller DT, Chung W, Nasir R, et al., 16p11.2 Recurrent Microdeletion. 2009 Sep 22 [Updated 2015 Dec 10]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2017. Available from: https://www.ncbi.nlm.nih.gov/books/NBK11167/

McDonald-McGinn DM, Emanuel BS, Zackai EH. 22q11.2 Deletion Syndrome. 1999 Sep 23 [Updated 2013 Feb 28]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1523/

Integrated Genetics is a brand used by Esoterix Genetic Laboratories, LLC, a wholly owned subsidiary of Laboratory Corporation of America® Holdings.
Poster presented at the *NSGC Annual Conference*; 2018 November 14-17; Atlanta, GA.