Structural Variant Analysis Unveils Loss-of-Function Promoter Deletion in Candidate Gene *WDR44*

Poster Number: P735

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Introduction

The University of North Carolina clinical genomic analysis (GENYSIS) core facility assists clinical researchers with the bioinformatic analysis, variant prioritization and classification, and clinical reporting of germline variants identified in samples from participants of IRB-approved exome and genome studies[1]. As part of the GENYSIS bioinformatics pipeline development, we investigated the utility of short-read structural variant (SV) detection algorithms to identify SVs in trio genome datasets previously determined negative after standard sequence variant analysis.

Methods

Trio genome sequence data of fetal samples from patients enrolled in the Prenatal Genetic Diagnosis by Genomic Sequencing (PrenatalSEQ) multicenter study was made available to GENYSIS for reanalysis. SVs were genotyped using Delly[2] and annotated using AnnotSV[3]. The resulting data was filtered to include only deletion or duplication variants < 50 kb that overlapped at least one exon of a RefSeq transcript. Manual review of the SVs was performed using the Integrative Genomics Viewer (IGV)[4] to visualize and evaluate the short-read alignments spanning the SV regions. SVs of potential significance were confirmed by Sanger sequencing in a clinical lab.

Results

A 994 bp maternally inherited hemizygous deletion of the 5' UTR and predicted upstream promoter region of the *WDR44* gene on chromosome X was identified in the genome data from a male fetus (Figure 1).

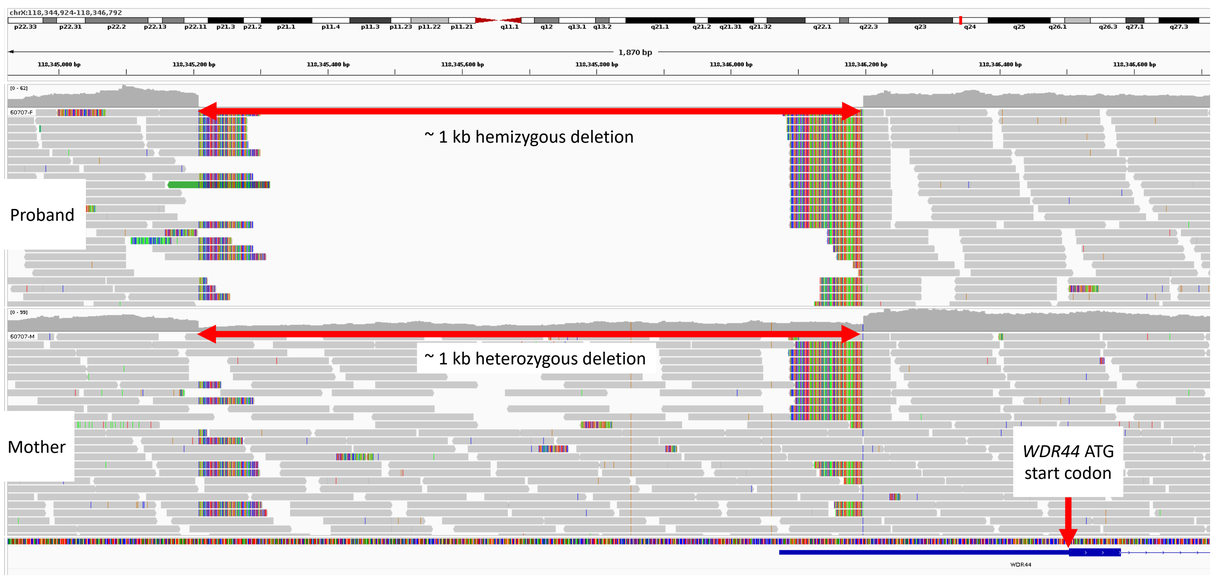

Figure 1: IGV[4] displaying aligned sequence reads for the *WDR44* gene 5'UTR

The *WDR44* gene encodes a member of the WDR family of proteins that have been implicated in multiple neurological disorders, ciliopathies, and endocrine disorders[5]. However, there is currently no established gene-disease relationship for *WDR44* reported in OMIM[6] or ClinGen[7]. A recent publication reported *WDR44* gain-of-function variants associated with a ciliopathy-related developmental phenotype[8]. Only one other patient with a large deletion of several genes including *WDR44* has been reported with a ciliopathy-related phenotype[9]. Thus, the ~1 kb deletion was clinically reported as a variant of uncertain significance (NC_000023.11:g.118,345,209-118,346,202del).

Case Presentation

Prenatally, the fetus was identified via ultrasound to have:

- a cystic hygroma (which resolved by the second trimester)
- an echogenic bowel
- anasarca (generalized edema)

Postnatally identified features include:

- microcephaly, mild dysmorphic facial features, brachydactyly
- redundant skin
- hypotonia, joint laxity, hip dysplasia
- bilateral sensory neural hearing loss
- congenital hypothyroidism
- diaphragmatic hernia, congenital heart defect, unilateral cystic renal dysplasia, bilateral inguinal testes

Prior NEGATIVE genetic testing included:

- Amniocentesis karyotype and microarray (UNC)
- PrenatalSEQ trio genome (UNC)
- Heritable Disorders of Connective Tissue panel with del/dup (GeneDx)
- Clinical WES and Mito genome (GeneDx)
- Clinical trio genome (Undiagnosed Disease Network at Duke)

Expression Analysis

A new blood sample was obtained from the trio and RNA extracted. Gene expression was performed in five technical replicates using *GAPDH* as an endogenous control. Expression was analyzed using the comparative CT (ΔΔCT) method and plotted relative to the father's *WDR44* expression, demonstrating that the proband has reduced *WDR44* expression (Figure 2).

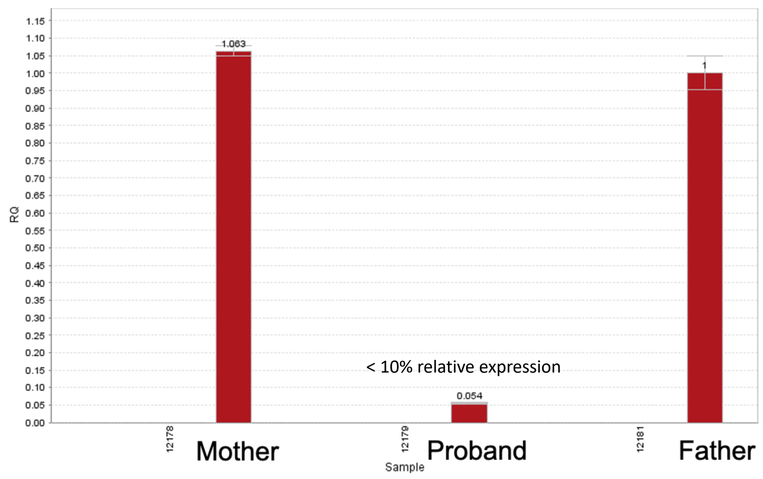

Figure 2: *WDR44* relative gene expression (RQ) plot for the trio (error bars indicate the standard error)

Conclusions

Reanalysis of negative genome data for structural variants can identify additional genomic variation missed by standard sequence variant analysis pipelines. Structural variants of non-coding regions can affect gene transcription and, when phenotypic information is not utilized to prioritize or filter variants based on known gene-disease associations, can point to candidate genes such as *WDR44*. However, identification of additional individuals with *WDR44* variants will be necessary to further elucidate the role of loss-of-function *WDR44* variants in disease. In summary, this study highlights the benefits of genomic reanalysis and the opportunities provided by research studies and the UNC GENYSIS core facility to inform and improve clinical care and clinical genetic testing.

References

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