Application of Whole Exome sequencing in identifying sequence variants and copy number variants in phenotypic females with disorders of sexual development (DSD).

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BACKGROUND

Disorders of sexual development (DSD) are a heterogeneous group of congenital conditions associated with atypical development of internal and external genital structures.

- Combination of genetic tools such as Karyotype, microarray analyses, and next generation sequencing techniques are helpful in the detection of a wide range of possible pathogenic variants including aneuploidies, microdeletion/duplication syndromes, and sequence and copy number variation within coding and non-coding regions.
- Here we performed Whole Exome sequencing to identify the genetic cause of DSD in four patients.

46,XY DSD: Exome Sequencing Identifies Pathogenic Structural Variants

Patient 1: 3 mo old phenotypic female with multiple congenital anomalies, facial dysmorphism, epilepsy, cleft soft palate and poor feeding

- Karyotype performed elsewhere showed deletion of the short arm of chromosome X with 46,X,del(X)(q13)

Figure 1: Pt 1 initial Karyotype showing Sex chromosomes

- Exome sequencing revealed SRY positive, 42.7 Mb gain of Xp22.33p11.3 including the DAX1 gene and 10 Mb loss of Yq11.221q11.23, resulting in functional disomy of Xp which further helped explain the clinical phenotype.
- SRY positive individuals with two active copies of DAX1 gene is associated with 46,XY sex reversal and adrenal hypoplasia
- The final karyotype of the child was: 46,X,der(Y)t(X;Y)(p11.3;q11.22). The syndrome resulted from functional disomy Xp11.3-pter, with sex reversal related to the presence of two active copies of the DAX1 gene located in Xp21

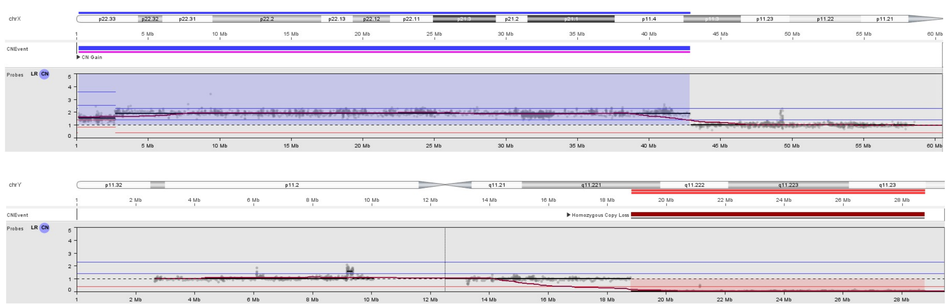

Figure 2: Pt 1 WES NxClinical result: gain of Xp22.33p11.3 including the DAX1 gene (Top) and loss of Yq11.221q11.23 (Bottom)

Figure 3: Pt 1 final Karyogram based on WES result: 46,X,der(Y)t(X;Y)(Ypter→Yq11.221::Xp22.33→Xp11,3)

Patient 2: 2 yr old phenotypic female with labial swelling, vaginal prolapse, bilateral corneal opacities, microphthalmia, bilateral gonadoblastoma and bilateral nephroblastoma

- Male dichorionic diamniotic twin was phenotypically normal
- Exome sequencing revealed XY sex chromosomes, SRY-positive, and 15 Mb deletion at chr 11p14.1p11.2 including the *PAX6* and *WT1* loci, consistent with WAGR syndrome
- WT1-related disorders include progressive glomerulopathy, Wilms tumor, and disorders of sex development

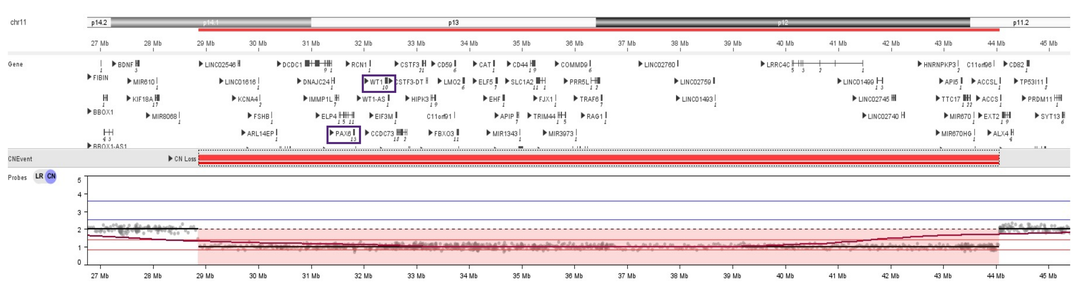

Figure 4: Pt 1 WES NxClinical result showing deletion of 11p14.1p11.2 region

46,XY DSD: Exome Sequencing Identifies Pathogenic Sequence Variants

Patient 3: Unexpected DSD diagnosis. 11 yr old phenotypic female with end stage renal disease (ESRD), glomerulonephritis; father with glomerulonephritis and chronic kidney disease, suspected Alport syndrome

- Trio exome sequencing identified XY chromosome constitution in proband
- Exome consistent with 46,XY *SRY*-pos
- *A De novo* pathogenic splice variant detected: *WT1* c.1432+5G>A associated with autosomal dominant Frasier syndrome.

Patient 4: 35 yr old phenotypic female with 46,XY karyotype, WES ordered to investigate cause of androgen insensitivity syndrome

- Exome consistent with 46,XY *SRY*-pos
- Hemizygous pathogenic missense variant detected: *AR* c.2324G>A (p.Arg775His) associated with X-linked recessive androgen insensitivity, X-linked recessive hypospadias, and X-linked recessive spinal and bulbar muscular atrophy of Kennedy

CONCLUSION

- Traditionally, cytogenetic analysis, fluorescent in situ hybridization (FISH), microarray technologies, and single gene sequencing are used to delineate the genetic cause of DSD.
- Here we used Whole Exome Sequencing (WES) data to detected pathogenic structural variants (deletion/duplication) and sequence variants in cases of XY DSD. WES also helped in identifying proper break points and identifying structural abnormality in case of DSD.
- These data demonstrate the advantage of a single, more cost effective and comprehensive whole exome sequencing approach in determining sequence variants and structural variants for highly heterogenous conditions such as DSD.

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