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Case Presentation

Prenatal Diagnosis of Denys-Drash Syndrome

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2. Key words

Triad; Nephropathy; Microarray; Ambiguous; Genitalia; Failure; Micro deletion; CMA, WT1

1. Abstract

Denys-Drash syndrome consists of the triad of progressive nephropathy characterised by diffuse mesangial sclerosis (DMS), genital abnormalities, and Wilms tumour. Nephropathy may range from early onset proteinuria to nephrotic syndrome to end stage renal failure. Genital malformations affects both external and internal genitalia. It may range from penoscrotal hypospadias, bilateral cryptorchidism to an enlarged clitoris with fused labia and a urogenital sinus to atrophic uterus to streak ovaries or dysgenetic testes. The risk of developing Wilms' tumor may be as high as 50%.

A primigravida with screen positive for trisomy 21 was evaluated for further work up. Antenatal ultrasound showed oligohydramnios with failure of growth. Both foetal kidneys were visualized with normal echogenicity; with suspicion of genital ambiguity. Antenatal case with suspected ambiguous genitalia, oligohydramnios and failure of growth; amniocentesis was done and chromosomal microarray analysis (CMA) was done. It identified a micro deletion of 20 kilo base pairs (Kbp) in WT1 gene located at 11p13 including both exonic and intronic regions. Advantage of chromosomal micro array is higher resolution which helps in identifying additional clinical significant abnormality.

3. Prenatal Diagnosis of Denys-Drash Syndrome

In 1967, Denys et al. described the triad of ambiguous genitalia, nephrotic syndrome and Wilms' tumor in an XX/ XY mosaic [1]. Drash et al. [2] described this triad in two patients and suggested that it may be a syndrome [2].

4. Case Presentation

31 years old primigravida with quadruple test report screen positive for trisomy 21 as 1:181 was being referred to the foetal medicine department for further work up. Antenatal ultrasound showed oligohydramnios (amniotic fluid index – 8.0 cm) with failure of growth **(Figure 1)**.

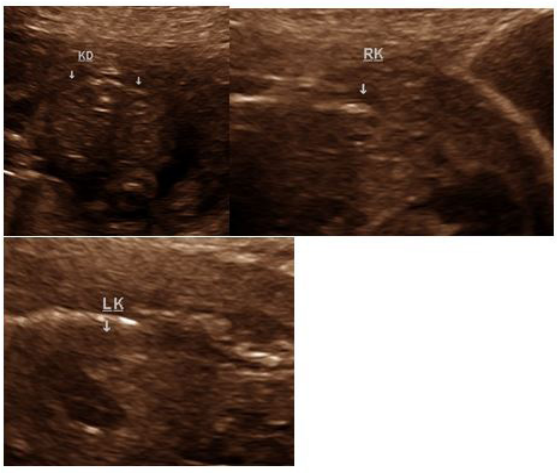

Figure 1: Antenatal transabdominal ultrasound showing bilateral renal tissues with respective renal pelvis.

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Both foetal kidneys and bladder were visualized **(Figure 2**) with normal echogenicity. On antenatal imaging we failed to identify specific foetal genital structures which suggestive of genital ambiguity.

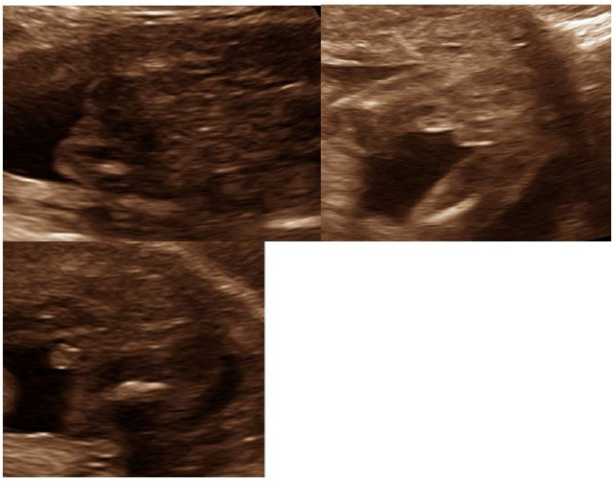

Figure 2: Antenatal ultrasound with failure to identify any specific foetal genital structures is suggestive of genital ambiguity.

With this background with suspected ambiguous genitalia, oligohydramnios and failure of growth; the patient and the consultand were counselled regarding the need for further foetal evaluation. An informed consent was taken, amniocentesis was done and amniotic fluid was sent for chromosomal microarray analysis (CMA) **(Figure 3)**.

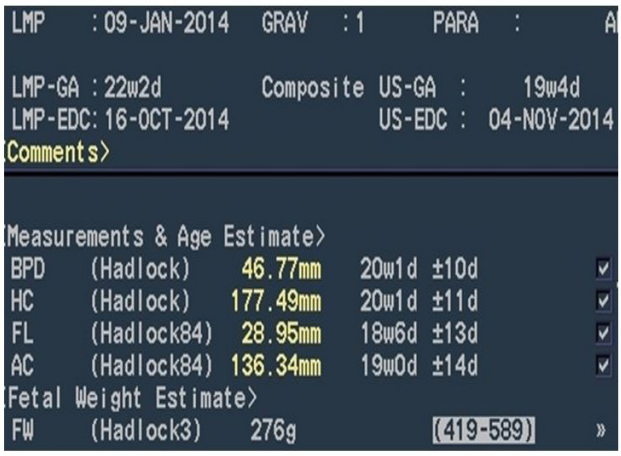

Figure 3: Biomerty of the said fetus showing failure to grow.

5. Methodology

CMA was performed using affymetrix cytoScan 750K microarray. This microarray consisted of 750,000 oligonucleotide probes across the genome, including unique non polymorphic probes, and SNP (single nucleotide polymorphism) probes. These SNP probes help in the identification of long contiguous stretches of homozygosity (LCSH) that may suggest uniparental disomy (UPD), or regions of the genome 'identical by descent'. Patient hybridization parameters were then compared to data derived from phenotypically normal individuals **(Table 1)**.

Table 1: Description of abnormality detected on microarray.

Serial No	Exon of WT1 gene	Abnormality detected
1	Exon 7	Complete Exon is deleted
2	Exon 8	Complete Exon is deleted
3	Intron 6	Partially Deleted
4	Intron 7	Complete Intron is deleted
5	Intron 8	Partially Deleted

250ng of genomic DNA was isolated from lymphocytes, digested with Nsp1 and then ligated by Nsp1 adapter. Titanium Taq amplified PCR (polymerase chain reaction) products were purified using AMP pure beads. Purified DNA was fragmented to the product size of 25bp to 125 bp, biotin labelled, hybridized on Cyto Scan 750K array and then scanned for cell file generation. The sample was processed for microarray analysis after 5 QC checks. Microarray analysis identified a microdeletion of 20 kilo base pairs (Kbp) in WT1 gene located at 11p13. The reported region included both exonic and intronic regions.

6. Interpretation

arr [hg19] 11p13(32,416,454-32,436,111)x1; Break Points: 32,416,454-32,436,111 array; human genome version 19; chromosome location 11p13; base pair position 32,416,454-32,436,111; deletion of copy number 1; Size of Microdeletion: 20Kbp

7. Discussion

Mutation in a single copy of the Wilms tumour suppressor gene 1 (WT1) is sufficient to produce nephropathy and disorders of sexual development. Constitutional point mutation in the zinc finger domain of WT1 in one allele causes diffuse mesangial sclerosis (DMS) and abnormal sex differentiation by a dominant negative effect i.e. abnormal product of a single copy of mutant WT1 gene interferes with the function of the unaffected copy of the WT1 gene and changes its normal regulatory function which is sufficient to produce nephropathy and disorders of sexual development.

However, Wilms tumor results from mutations in both copies of the WT1 gene. In contrast, Wilms tumor is a result of two independent events (two-hit hypothesis) that sequentially lead to loss of function of both copies of the WT1 gene. The first mutation in a single copy of the WT1 gene (first hit) leads to persistence of an undifferentiated tissue in the developing kidney, called mesenchyme. Subsequently; another mutation (second hit) in the second copy causes uncontrolled cell growth in the kidney resulting in Wilms tumor.

Nakadate H [3] in their paper had discussed in great detail regarding the different genetic aetiology for WT1. Of the 7 patients with malformation-associated WTs, all had WT1 abnormalities. 4 had hemizygous WT1 deletion and 1 had homozygous WT1

deletion; 3 of the 5 had a frame shift or missense mutation in exon 7, 9 or 10 in the remaining allele.

WT1 is located on chromosome 11p13, it encodes zinc finger domains and its product plays a key role in the regulation of gene transcription [4]. Expression of WT1 is observed in the glomerular epithelium of the kidneys and the genital ridge during the embryonic period, thus WT1 is thought to have a functional role in renal and gonadal organogenesis [5]. The WT1 tumour suppressor gene encodes a transcriptional factor containing four zinc fingers. [6,7] WT1 gene has two alternative splicing regions, one consisting of 17 amino acids which are encoded by the whole of exon 5 and the other comprising three amino acids (lysine, threonine, and serine (KTS)) situated between the third and fourth zinc fingers encoded by the 3' end of exon 9. Four isoforms of the gene thus occur depending on the presence or absence of these regions [8].

These isoforms are present in a fixed proportion in tissues where they are expressed. WT1 is expressed from the condensing mesenchyme to mature podocytes in fetal kidneys, genital ridges and fetal gonads. Therefore, this gene is thought to play an important role in the development of the kidneys and gonads [9,10].

This intron 9 mutation leads to impairment of exon9 alternative splicing with a consequent decrease in the +KTS isoform, thereby resulting in a quantitative +KTS/−KTS isoform imbalance. Depending on exonic or intronic mutations, clinical features vary amongst the patients.

Exonic mutations clinically had either Denys-Drash syndrome or IDMS. WT1 mutations were missense mutations within the second or the third zinc finger encoded by exon 8 or 9, respectively. Intron 9 splicing donor site mutations, the clinical features are consistent with Frasier syndrome.

These isoforms have different DNA binding properties [11]; the –KTS isoform has greater binding affinity for growth related genes [12]. Two of these isoforms have been shown to vary in subnuclear localisation. The +KTS isoform appears to be involved in post-transcriptional RNA processing in association with splicing factors, while the −KTS isoform is situated in the transcriptional factor domain [13,14].

Denys-Drash syndrome (DDS) is characterised by WT1 mutations, early onset renal failure, abnormal sex differentiation and a predisposition to Wilms tumour [1,2]. WT1mutations have missense changes in exon 8 or 9 affecting zinc finger 2 or 3. Germline mutations in WT1 have been reported in the majority of DDS patients [15,16] Missense point mutations in exon 7 are very rare [15].

Usually, WT1 missense mutations are detected in exons 8 or 9 and affect zinc fingers 2 or 3, which show a high level of homology to the three zinc fingers of EGR1 and are believed to be important for their binding capacity to WT1 DNA targets [17].

Functional impairment of this gene is considered to give rise to urogenital abnormalities and Wilms tumours. Denys-Drash syndrome and Frasier syndrome, both of which are characterised by nephropathy with genital abnormalities

Denys-Drash syndrome consists of the triad of progressive nephropathy characterised by diffuse mesangial sclerosis (DMS), genital abnormalities, and Wilms tumour [18]. The incomplete form also exists. All patients with DDS have point mutations in the zinc finger domain encoded by exons 7 to 10 of theWT1gene [19]. Most of them are missense and in exon 8 and 9 that encode the second and third zinc fingers.

Moorthy et al. [20] proposed an allelic disorder known as Frasier syndrome; characterised by a slowly progressing nephropathy, male pseudohermaphroditism and no Wilms tumour [20]. Focal segmental glomerular sclerosis (FSGS) is often observed in cases of Frasier syndrome meanwhile diffuse mesangial sclerosis (DMS) is noted in Denys-Drash syndrome. Frasier syndrome arises from heterozygous mutation at the intron 9 splicing donor site of the WT1 gene [21,22].

The amount of the +KTS isoform is less as compared to −KTS isoform owing to the intron 9 mutation in Frasier syndrome, in whom masculinisation is impaired, suggestive of +KTS isoform playing a role in masculinisation. The incidence of Wilms tumour is considered to be markedly lower in patients with intron 9 mutations than in those with exonic mutations [23,24].

8. Postnatal

The genital malformations vary considerably, ranging from penoscrotal hypospadias with bilateral cryptorchidism to an enlarged clitoris with fused labia and a urogenital sinus, in patients with a 46, XY karyotype. The internal genitalia may also be affected; the abnormality varying from a small atrophic vagina and uterus to the presence of streak ovaries or dysgenetic testes [25].

The nephropathy is characterized by onset of proteinuria between birth to two years of age [26]. With more than 50% of children presenting with proteinuria before one year of age. Proteinuria may evolve into nephrotic syndrome and to end stage renal failure (ESRF) [27]. Varying degrees of focal and diffuse mesangial sclerosis are the most consistent histopathological findings in the kidneys. A 50% risk of developing Wilms' tumor has been reported in this syndrome [26].

9. Conclusion

Primary advantage of micro array is higher resolution which yields more genetic information; array has been reported to identify additional clinical significant abnormality in approximately 6% of cases which might be missed on conventional karyotype. Thus based on the results of NICHD multicentric trial 2012; prenatal chromosomal micro array analysis is most beneficial when foetal structural abnormalities are detected on antenatal ultrasound [28].

In conclusion, there is a genotype phenotype concordance. Clinical profile, progression of nephropathy, degree of genital abnormalities and incidence of Wilms tumours vary with the type of underlying genetic mutation; i.e. exonic or intronic mutations. Thus, detection of types of WT1 mutations is useful for prognostic estimation of the clinical course in children with progressive nephropathy.

It is important to consider the diagnosis of Denys-Drash syndrome in any patient with unexplained nephropathy, particularly young girls and children with ambiguous genitalia or those presenting with an early Wilms' tumor.

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