Alpha Fetoprotein and Congenital Nephrotic Syndrome

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Abstract

Nephrotic syndrome is characterized by heavy albuminuria consequent hypoalbuminemia and oedema. All types of congenital nephrotic syndrome have renal protein loss invariably beginning in utero and leading to markedly elevated maternal serum (MSAFP) and amniotic fluid alpha-fetoprotein (APP) levels.

Persistent renal parenchymal echogenicity with persistent raised MSAFP suggests underlying renal pathology; kidney damage and possibly renal failure. High-throughput gene sequencing revealed a heterozygous missense mutation in exon 5 of the TBX18 gene, resulting in amino acid substitution of Serine for Tyrosine at codon 309.

Heterozygous truncating mutation in TBX18 (OMIM*604613) gene leads to congenital anomalies of the kidney and urinary tract-2 (CAKUT2) (OMIM#143400), accounting for 49.1% for chronic kidney disease manifesting before 25 years of age.

High-throughput gene sequencing has rendered delineation of causative genetic mutations feasible, enabling molecular analysis of early CKD-causing genes.

Keywords: Congenital; Nephrotic Syndrome; Alpha-Fetoprotein; Echogenic Renal Parenchyma; TBX18; Sequencing

Introduction

Chronic kidney disease (CKD) in children is defined by the presence of kidney damage or by a glomerular filtration rate that has remained below 60 ml/min/1.73 m^2 for more than 3 months [1]. Progression of CKD to end-stage renal disease (ESRD) requires dialysis or transplantation for survival [2].

Nephrotic syndrome is a clinical diagnosis characterized by heavy albuminuria consequent hypoalbuminemia and oedema [3]. It is classified as congenital if it presents at birth or during the first months of life [4].

Finnish type of congenital nephrotic syndrome (CNF) is an autosomal recessive disease presenting with severe proteinuria and secondary hypoalbuminemia. The kidneys are enlarged and have diffuse punctuate echogenicities reflecting microcysts due to dilatation of

the proximal convoluted tubules, fusion of basement membrane foot processes, and proliferation of mesangial cells [5]. CNF is the frequent in Finland with an incidence rate of 1.2/10,000 live births [6] but has been reported in other ethnic groups also [7].

Majority (90%) of the protein loss in CNF is albumin leading to secondary hypogammaglobulinemia, placentomegaly, in-utero non-immune hydrops and increased risk for infection. Neonatal signs of CNF include proteinuria, hypoalbuminemia, oedema and failure to thrive with respiratory difficulties.

The prognosis for CNF is generally poor, with half of conservatively managed neonates dying before the age 6 months and nearly all by 4 years. It usually doesn't respond to steroids or immunosuppression; the only treatment is renal transplantation. However, 20% have recurrence of nephrotic syndrome, which may respond to rituximab [8].

Finnish CNF and diffuse mesangial sclerosis are the two major congenital nephrosis, with renal protein loss invariably beginning in utero and leading to markedly elevated maternal serum and amniotic fluid alpha-fetoprotein (APP) levels.

Diagnosis

Prenatal diagnosis of Finnish CNF has been reported as early as Seppala., *et al*. (1976) by elevated levels of alpha-fetoprotein (AFP) in amniotic fluid (AF) [9]. Morris., *et al*. (1995) has described AF AFP levels can be exploited for prenatal diagnosis of Finnish CNF [10].

Elevated AFP in maternal serum is nonspecific and may varied aetiologies. Foetal reasons may open neural tube defect (NTD), foetal non NTD reasons include cleft lip/palate, gastroschisis, omphalocele, congenital diaphragmatic hernia, cutis aplasia (maternal exposure to propylthiouracil), congenital nephrosis, multiple gestations, incorrect estimation of gestational age. Placental chorioangioma and uteroplacental insufficiency with maternal hepatic and ovarian lesions might also lead to increased levels of maternal AFP.

However, markedly elevated AFAFP (greater than 10 MoM) has been reported in congenital nephrotic syndrome, maternal hepatoma, and abdominal pregnancy, but rarely with neural tube defects, abdominal wall defects, foetal demise.

In all types of congenital nephrotic syndrome, the maternal serum alpha fetoprotein level is markedly elevated. The severe proteinuria characteristic in nephrotic syndrome results in an increased excretion of alpha fetoprotein into the amniotic fluid, from which it enters the maternal circulation [11]. The two main causes of congenital nephrotic syndrome is the Finnish type and diffuse mesangial sclerosis which are both inherited in autosomal recessive fashion [12].

In conclusion, a markedly elevated maternal serum and amniotic fluid AFP should alert the sonographer to the possible diagnosis of congenital nephrosis of the Finnish type. The sonogram of the foetal kidneys may show normal or echogenic parenchyma and normal or enlarged renal size.

Case Report

A primigravida was seen at the department of foetal medicine at Artemis hospital with a quadruple report of increased maternal serum AFP (MSAFP), 7.53 Mom. Ultrasound examination of the foetus was invariably normal with slight impression for bilateral echogenic kidneys which were regarded to be a normal variant at 14 weeks (Figure 1).
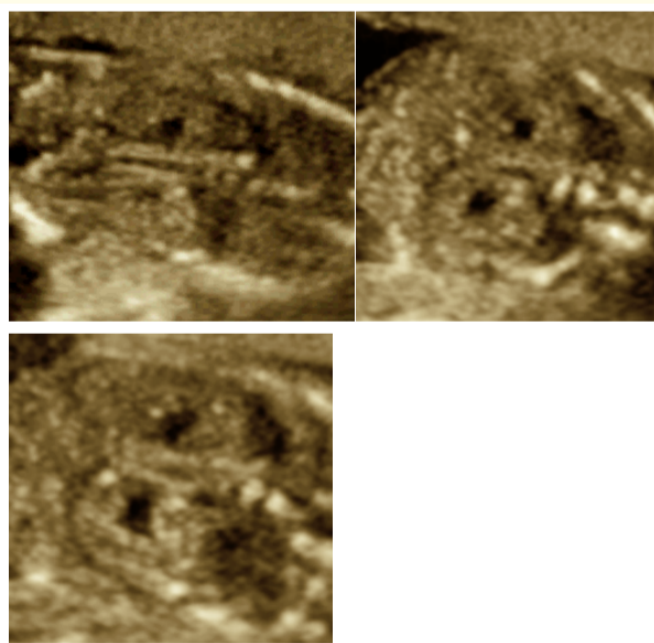

Figure 1: *Antenatal ultrasound images showing bilateral echogenic kidneys at 14 weeks, regarded as normal variant in differential.*

She was counselled regarding the normal variation in the foetal kidneys and was asked to repeat the quadruple marker and to come back with the reports. Her MSAFP in second quadruple marker was even raised then earlier 8.3 Mom with essentially normal antenatal ultrasound apart from echogenic kidneys (Figure 2).
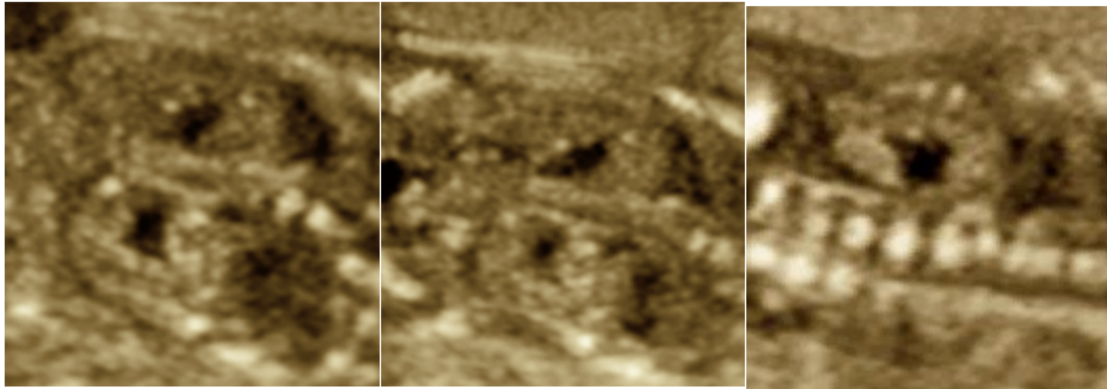

Figure 2: Antenatal ultrasound images showing persistent bilateral echogenic kidneys at 16 weeks with persistent raised MSAFP suspecting Finnish CNF.

She and her partner were counselled regarding the finite possibility for congenital nephrotic syndromes particularly Finnish CNF, for which she agreed for invasive amniocentesis procedure and for amniotic fluid to be subjected to molecular analysis. This took around 4 weeks' time for the results; however the echogenicity of the kidneys persisted at 20 weeks (Figure 3) contrary to the normal variant which we supposed it to be at our first examination at 14 weeks. In the serial ultrasound examination of the foetus the amniotic fluid volume remained within normal limits.
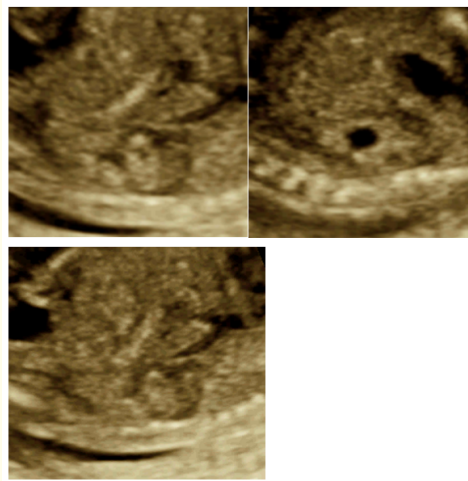

Figure 3: Antenatal ultrasound images persistent showing bilateral echogenic kidneys at 20 weeks with suspected CAKUT2.

With due informed consent, Amniocentesis was done; foetal DNA extracted was subjected to targeted gene sequencing on Illumina sequencing platform and Sentieon (v201808.07) was used for identification of variants.

Molecular testing revealed it to be a heterozygous missense mutation in exon 5 of the TBX18 gene, resulting in amino acid substitution of Serine for Tyrosine at codon 309. It has been classified as mutation of uncertain significance (Table 1).

Reports		TBX18 gene	Mutation	
7 affected members of a 4-generation (14)	Congenital anomalies of the kidney and urinary tract-2 (CAKUT2), ureteropelvic junction obstruction (UPJO)	Heterozygous 1-bp deletion (c.1010delG),	Exon 7, frameshift and premature termination (Gly337ValfsTer19)	Mutation resulted in a loss of function with a dominant-negative effect.
4 individuals, 2 families (14)	CAKUT2 manifesting as renal duplex, renal asymmetry, hydronephrosis, and small kidneys	Heterozygous c.1570C-T transition (c.1570C-T)	Exon 8, substitution his524-to-tyr (H524Y)	Mutation resulted in a loss of function.
Single patient (14)	CAKUT2 manifesting as small echogenic kidneys, vesicoureteral reflux, and renal dysplasia	Heterozygous c.487A-G transition (c.487A-G)	Exon 2, lys163-to-glu (K163E) substitution	
Present case	Congenital anomalies of kidney and urinary tract-2 presenting a persistent bilateral echogenic kidneys with raised MSAFP	Heterozygous c.926A>C (p.Tyr309Ser)	Exon 5, missense variation Serine for Tyrosine at codon 309 (p.Tyr309Ser)	Missense mutation

Table 1: Describing the previous mutational analysis of TBX18 gene and the present case.

The clinical phenotype raised the suspicion of Finnish CNF; however the molecular genetic testing pointed to autosomal dominant heterozygous missense mutation in exon 5 of the TBX18 gene. As this p.Tyr309Ser variant has not been reported in the literature (1000 genomes, gnomAD) it has been classified to be variant of uncertain significance.

Persistent echogenicity of the renal parenchyma with persistent raised MSAFP points towards the possibility of underlying renal pathology; kidney damage and possibly renal failure due to this heterozygous mutation. As the literature is deficient of the supportive data for the said mutation to be causative; the said variant needs to be correlated carefully with the serial foetal renal evaluation and the classification might change to likely pathogenic or pathogenic depending upon postnatal course.

	POG - 15 weeks	POG - 15 weeks 5 days
AFP	261 ng/ml - 7.53 MoM	300 IU/ml - 8.73 MoM
uE3	0.27 ng/ml - 0.53 MoM	3.54 nmol/L - 1.74 MoM
HCG	31518 mIU/ml - 0.82 MoM	32.27 IU/ml - 0.80 Mom
Inh-A	275.6 pg/ml - 1.50 MoM	282.20 - 1.06 MoM

Table 2: Depicting persistent raised MSAFP levels (different labs).
MSAFP - 7.53 MoM; 8.73 MoM.

Discussion

T-Box transcription factor 18; (TBX18), OMIM 604613, TBX18 gene is a member of the Tbx1 subfamily of T-box transcription factor genes expressed at multiple sites and likely to participate in paraxial mesoderm formation and somatogenesis in human embryo. TBX18 maps at 6q14.3. Mutations in human TBX5 and TBX3 causes Holt-Oram syndrome and ulnar-mammary syndrome respectively.

Heterozygous truncating mutation in TBX18 (OMIM*604613) gene leads to congenital anomalies of the kidney and urinary tract-2 (CAKUT2) (OMIM#143400). This disorder encompasses a spectrum of developmental disorders of the urinary tract that can range from mild vesicoureteral reflux to severe renal agenesis. Other phenotypes include renal duplication, small kidneys, ureteropelvic junction obstruction, hydronephrosis, and renal dysplasia. These abnormalities can result in kidney damage, and possibly renal failure [13].

Literature confirmed that truncating mutation have a dominant-negative dose-dependent effect. Vivante., *et al*. (2015) [14] described that the clinical phenotype resulted from impaired ureter smooth muscle cell development during nephrogenesis. It is transmitted in autosomal dominant manner.

The primary aetiologies of chronic kidney disease (CKD) in children is different from those of adults onset CKD. The most common reasons for CKD manifesting before 25 years of age are: i) congenital anomalies of the kidneys and urinary tract (CAKUT) (49.1%), ii) steroid-resistant nephrotic syndrome (SRNS) (10.4%), iii) chronic glomerulonephritis (8.1%), iv) renal cystic ciliopathies (5.3%), encompassing >70% of CKD together.

More than 200 monogenic causative genes have been identified for the 70% most common aetiologies of early onset CKD. These genetic mutations are singularly sufficient for disease causation without any additional biological or environmental insult which is known as "full penetrance" of the mutation [15-17].

Currently there are approximately 36 genes known to be mutated in CAKUT [14,17], 39 genes in SRNS [18], 10 genes in chronic glomerulonephritis, and over 95 genes in renal cystic ciliopathies [19,20].

The aetiologies for early onset CKD were unknown earlier, however high-throughput gene sequencing has made identification of single-gene (monogenic) causes of CKD possible. Thus, the revelation of the primary aetiologies, delineation of the related pathomechanisms and improved understanding of disease has been made possible.

It has to noted that in about one-third (30%) of cases, genetic variants published as likely disease causative and described in genetic databases are not confirmed as deleterious [21]. Consequently, any attribution of pathogenicity to a given genetic variant has to subjected to a strict criteria with multiple levels of evidence to be considered (amino acid sequence conservation, segregation analysis, tissue specific gene expression, functional studies and animal models) before labelling as causative [22,23].

Conclusion

High-throughput gene sequencing has rendered delineation of causative genetic mutations feasible, enabling molecular analysis of early CKD-causing genes. Gene panel analysis with next generation sequencing is an emerging tool which has a potential to change clinical research and practice.

Identifying a monogenic cause for patients with early CKD has already several immediate clinical implications which include:

1. Providing patient and families with definitive genetic aetiology;

2. Seeing the clinical phenotype through the specific genetic context and planning personalized medicine;

3. Precise genetic counselling;

4. Identification of at risk, previously unrecognized family members;

5. Avoiding unnecessary diagnostic procedures, tests and treatments;

6. Early detection and treatment of extra renal manifestations;

7. Surveillance and early monitoring of potential future complications and

8. Guiding advanced medical management on a gene specific basis.

Ethical Statement

- The manuscript has not been submitted any other for simultaneous consideration.

- The manuscript has not been published previously (partly or in full).

- A single study is not split up into several parts.

- No data have been fabricated or manipulated (including images) to support our conclusions.

- No data, text, or theories by others are presented as if they were the author's own ("plagiarism").

- Co-Authors have adequately contributed to the work.

- This is a retrospective study - "For this type of study formal consent is not required".

Conflict of Interest

The authors declare that they have no conflict of interest.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

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