CASE REPORT

Familial focal segmental glomerulosclerosis associated with a WT1 gene missense mutation: A case report

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Abstract

Focal segmental glomerulosclerosis (FSGS) can cause proteinuria and loss of kidney function, leading to end-stage renal disease (ESRD). Podocyte injury is the central pathophysiological mechanism of hereditary FSGS. Numerous mutations in genes encoding or affecting the transcriptional regulation of podocyte cell compartments have been detected in patients with genetic FSGS. Herein, we report a rare case of familial FSGS with an autosomal dominant WT1 mutation. A 63-year-old man developed proteinuria; his reading showed over 1g protein/day. A pathological diagnosis of FSGS was made after renal biopsy. His elder brother and a 36-year-old son also had ESRD. Heterozygous variant of WT1 (NM_024426.4) c.1373G>A (p.Arg458Gln) missense was detected in the patient and his son, by whole-exome sequencing. Although genetic screening is not a part of routine practice, it should be performed in such cases to aid appropriate treatment options selecting, revealing extrarenal symptoms, and family planning.

Keywords: Focal segmental glomerulosclerosis, WT1 gene, Missense mutation.

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Introduction

Focal segmental glomerulosclerosis (FSGS) is characterised by focal scarring of the glomerular capillary tuft and podocyte injury. The prevalence of biopsy-proven FSGS varies according to geographical areas, race, age, accessibility to health care services, and indication for renal biopsy. The causes of FSGS are categorised into primary, secondary, genetic, and undetermined.[1]

Podocyte injury is the central pathophysiological mechanism in hereditary FSGS.[2] The WT1 transcription factor gene (WT1) plays a critical role in genitourinary development, and its mutations are involved in several diseases that involve the kidneys and genitals.[3] Recent studies have revealed that WT1 deletion is associated with podocytopathy.[4,5] Herein, we report a case of familial FSGS with an autosomal dominant WT1 mutation and provide a review of the literature.

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Case Report

A 63-year-old man was referred to the nephrology department of CHA Bundang Hospital in Republic of Korea, on February 22, 2018, due to a 10-year history of proteinuria. Additionally, he had a 20-year history of hypertension and a two-month history of gout. Physical examination revealed a maximum blood pressure of 160/100 mmHg and a body mass index of 24.8 kg/m^2. A urinary dipstick test showed urine protein 2+ and the spot urine protein/creatinine ratio was 1.91 g/g (normal below 0.2 g/g). Serum blood urea nitrogen, creatinine, and albumin levels were 15.7 mg/dL (normal 5-20 mg/dL), 1.0 mg/dL (normal 0.7-1.2 mg/dL), and 3.6 g/dL (normal 3.5-5.2 g/dL), respectively. An abdominopelvic computed tomography scan showed that both kidneys were normal in size (left, 12.5 cm; right, 12.3 cm). He was asymptomatic, with no extrarenal manifestations.

The kidney biopsy samples revealed the presence of up to 17 glomeruli, of which 8 (47%) showed total sclerosis, and 3 (18%) showed segmental sclerosis. The glomerular basement membrane had a normal thickness with relatively smooth contours and without electron-dense deposits. The podocyte foot processes were widely effaced. The tubules had marked focal atrophy with mononuclear cell infiltration and fibrosis in the interstitium. Hyaline arteriolosclerosis and fibrointimal thickening of small arteries were also observed. Immunofluorescence microscopy showed no immunocomplex or autoantibody deposition. Overall, a pathological diagnosis of moderately advanced FSGS was made (Figure 1 A and B).

The patient's deceased older brother had ESRD and a 36-year-old son too has end-stage renal failure (ESRD). His son was diagnosed with FSGS at the age of 18 and is currently on chronic haemodialysis (Figure 1 C). Based on family history, genetic analysis on the patient and his family

members was performed. This study was approved by the IRB of CHA Bundang Medical Centre and the patient and his son gave informed written consent. Whole-exome sequencing was performed using SureSelect Human All Exon V6 (Agilent, Santa Clara, CA, USA) and sequencing was performed using the NovaSeq platform (Illumina, San Diego, USA).[6] The mean depth of coverage was 100× (>10×=99.2%). The pathogenicity of each variant was evaluated according to the recommendations of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology guidelines.[7] The heterozygous variant of WT1 (NM_024426.4) c.1373G>A (p.Arg458Gln) missense, was detected in the patient and his son. This was previously reported to be associated with FSGS and nephrotic syndrome. Moreover, the variant is absent in the gnomAD v2.1.1 dataset and functional studies provide strong evidence that it has a damaging effect on the gene or gene product with in silico tools.[2] Therefore, this variant was classified as pathogenic according to the ACMG guidelines.[7]

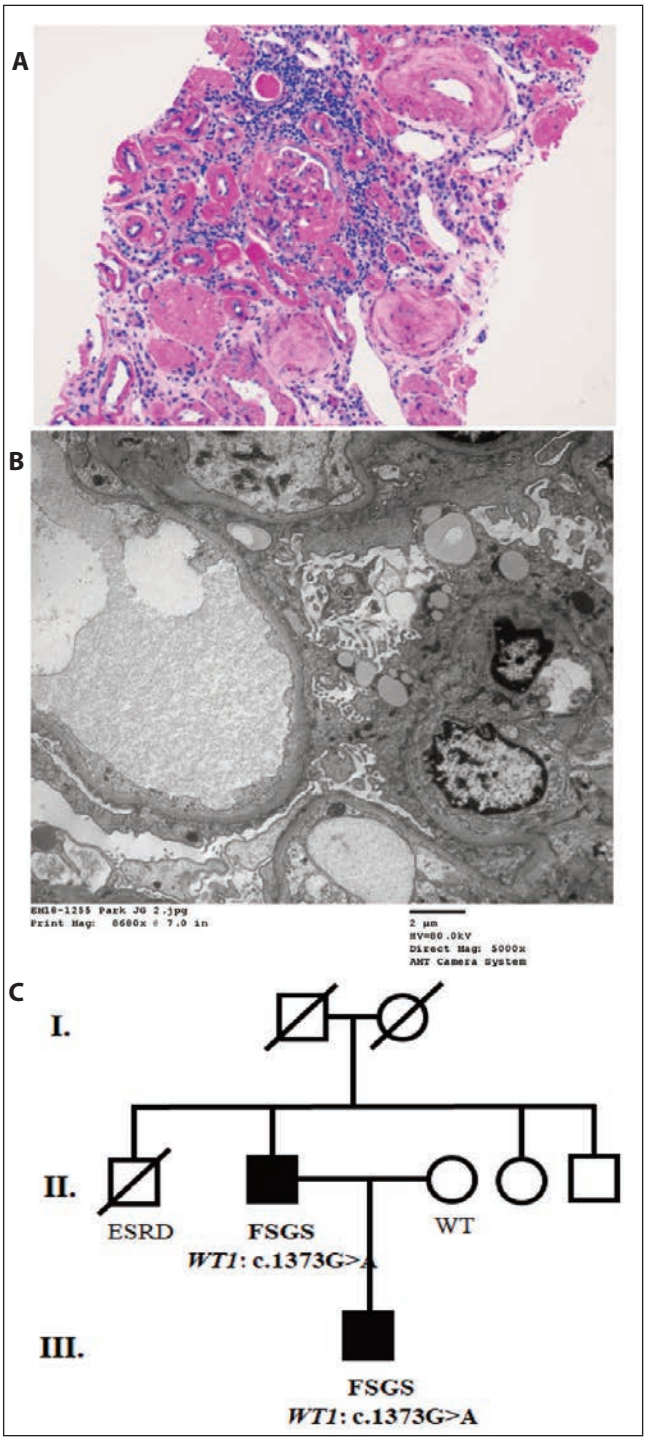

Figure: (A) Light microscopy shows focal atrophy and loss of tubules with interstitial infiltration of lymphocytes, hyaline arteriolosclerosis, and intimal fibrosis of the artery (periodic acid-Schiff stain, x200). (B) Electron microscopy of the renal biopsy sample shows diffuse foot process effacement of podocytes without electron-dense deposits (x5,000). (C) Three-generation family pedigree.

Angiotensin receptor blockers and calcium channel blockers (Fimasartan 60mg or Amlodipine 5mg) were prescribed to the patient. During follow-up over one year, his serum creatinine was stable at 1.1–1.3 mg/dL (normal 0.7-1.2 mg/dL), and the urine protein level decreased to 0.362g/g (normal below 0.2g/g).

Discussion

We describe a case of familial FSGS caused by a missense mutation in WT1. Kidney biopsy revealed FSGS with diffuse foot process loss, although the amount of proteinuria and normal serum albumin levels were not consistent with the pathological findings. Genetic testing was performed because of the patient's family history of ESRD; the WT1 mutation, which appeared to be the cause of podocyte abnormalities, was observed in both the patient and his son. Many inherited forms of FSGS have been identified, and most are caused by mutations in podocytes or glomerular basement membrane proteins. WT1 mutations usually present as the autosomal dominant form of hereditary kidney disease, and its penetrance is nearly 90% with various ages of onset. A WT1 mutation is one of the most common causes of familial FSGS and genetic steroid-resistant nephrotic syndrome (SRNS), and has been reported to cause 10–20% of nonsyndromic genetic FSGS.[2] The possible genetic causes of FSGS are summarised in Table after reviewing OMIM database (https://www.omim.org/).

WT1 is a 50-kb gene with 10 exons on chromosome 11, band *p*13. WT1 encodes a DNA-binding protein that functions as a transcription factor. Exons 1–6 encode proline-rich regions for the transcriptional regulation of

Table: List of genes that cause focal segmental glomerulosclerosis.

	Gene	Cytogenetic Location	Inheritance	Further symptom
**	ACTN4	19q13.2	AD	Renal failure 5th decade of life
**	ANLN	7p14.2	AD	Onset age is varied greatly (9-69 years)
**	APOL1	22q12.3		
	C6	5p13.1	AR	C6 deficiency
**	CD2AP	6p12.3		Early-onset nephrotic syndrome associated
	COQ6	14q24.3	AR	Primary coenzyme Q10 deficiency-6
*	COQ8B	19q13.2	AR	Renal failure usually occurred in the fifth decade of life.
**	CRB2	9q33.3	AR	Ventriculomegaly with cystic kidney disease
*	DAAM2	6p21.2	AR	The disorder is slowly progressive, and most patients eventually develop end-stage renal disease.
	DNM2	19p13.2	AD	Charcot-Marie-Tooth disease
	DSTYK	1q32.1	AD	Genitoureteral anomaly
	FARSB	2q36.1	AR	Interstitial lung disease and brain calcification
	G6PC	17q21.31	AR	Glycogen storage disease Ia
	GON7	14q32.12	AR	Mental retardation
	HPRT1	Xq26.2-q26.3	XLR	Lesch-Nyhan syndrome
**	INF2	14q32.33	[a]	Charcot-Marie-Tooth disease
	ITGA3	17q21.33	AR	Epidermolysis bullosa, junctional 7 interstitial lung disease
*	KIRREL1	1q23.1	AR	Onset of proteinuria in the first or second decade of life.
	LAGE3	Xq28	XLR	Mental retardation
	LMX1B	9q33.3	AD	Developmental defects of dorsal limb structures, the kidney, and the eye, manifested by nail dysplasia, patellar abnormalities, elbow dysplasia, iliac horns, nephropathy, and glaucoma,
	MPV17	2p23.3	AR	Mitochondrial DNA depletion syndrome 6 (hepatocerebral type)
**	MYO1E	15q22.2	AR	Childhood onset
	NARS2	11q14.1	AR	Delayed neurodevelopment, refractory seizures, hypotonia, and hearing impairment
	NFKB2	10q24.32	AD	Immunodeficiency
	NPHS1	19q13.12	AR	Finnish congenital nephrosis
*	NUP107	12q15	AR	Mental retardation
*	NUP133	1q42.13	AR	Mental retardation
*	NUP160	11p11.2	AR	
*	NUP205	7q33	AR	
*	NUP85	17q25.1	AR	
*	NUP93	16q13	AR	
	OSGEP	14q11.2	AR	Mental retardation
**	PAX2	10q24.31	AD	Papillorenal syndrome, hearing loss, central nervous system anomalies, soft skin, ligamentous laxity, and/or genital anomalies
*	PDCN/NPHS2	1q25.2	AR	
*	PLCE1	10q23.33	AR	
	PLIN1	15q26.1	AD	Loss of subcutaneous adipose tissue primarily affecting the lower limbs, insulin-resistant diabetes mellitus, hypertriglyceridaemia and hypertension
*	PTPRO	12p12.3	AR	
	SCARB2	4q21.1	AR	Progressive myoclonic epilepsy
*	SGPL1	10q22.1	AR	Primary adrenal insufficiency; fetal hydrops and foetal demise; ichthyosis, acanthosis, adrenal insufficiency, immunodeficiency, and neurologic defects
	SLC37A4	11q23.3	AR	Glycogen storage disaese
	SMARCAL1	2q35	AR	Combination of a spondyloepiphyseal dysplasia, slowly progressive immune defect, immune-complex nephritis
*	TBC1D8B	Xq22.3	XL	
	TP53RK	20q13.12	AR	Mental retardation
	TPRKB	2p13.1	AR	Mental retardation
	TRIM8	10q24.32	AD	Lobal developmental delay
**	TRPC6	11q22.1	AD	
	VPS33A	12q24.31	AR	Mucopolysaccharidosis-plus syndrome
	WDR4	21q22.3	AR	Mental retardation
	WDR73	15q25.2	AR	Mental retardation
*	WT1	11p13	AD, SMu	Genitoureteral anomaly
	ZMPSTE24	1p34.2	AR	Facial dysmorphism

Nephrotic syndrome (NS) and FSGS are genetically heterogeneous disorders that represent a spectrum of hereditary renal diseases. The variation of * genes could be more represented as nephrotic syndrome and ** could be more represented as FSGS. OMIM was also added to NS-listing genes: LAMB2, DGKE, ARHGDIA, EMP2 (602334), MAGI2, KANK2, AVIL, and NOS1AP. [a] AD inheritance pattern in Charcot-Marie-Tooth disease, but an unknown pattern in FSGS. AR, autosomal recessive; AD, autosomal dominant; XLR, X-linked recessive; XL, X-linked; SMu, spontaneous mutation.

target genes and homodimerisation of gene products, while exons 7–10 encode a DNA-binding domain consisting of four zinc fingers. In the kidneys, WT1 is required for the development and maintenance of podocytes homeostasis; indeed, several podocyte genes, including NPHS1 (nephrin), NPHS2 (podocin), SYNPO (synaptopodin), and Podxl (Podocalyxin), have been identified as WT1 targets. These genes are important components of the slit diaphragm and cytoskeleton of podocytes and are required for cell viability.[5]

WT1 DNA binding has been suggested to perform both activator and repressor functions, although the exact mechanism remains unknown.[5] Interestingly, podocyte-specific genes have multiple WT1 binding sites, which can epigenetically increase the podocyte-specific gene expression.[5] In addition, WT1 forms a complex along with enhancer proteins to regulate the transcription of target genes.[5] In Adriamycin-induced podocyte injury models, it has been proposed that multiple WT1 DNA binding recruits a series of transcription factors and enhancers to induce a repair response at an early stage.[foetal] WT1:c.1371G> A(pArg458Gln), the missense mutation noted in our case, is presumed to cause modification of the zinc finger portion of the WT1 protein, which affects target DNA binding affinity.[8] In the United States, Hall et al first reported the missense mutation (R458Q) in WT1 isoform D in two kindred Northern Europeans with nonsyndromic FSGS.[2] They observed that WT1R458Q overexpression significantly downregulated nephrin and synaptopodin expression, leading to impaired podocyte homeostasis.

Mutations in WT1 cause various pathologies of the genitourinary system.[9] The Frasier and Denys-Drash syndromes, associated with WT1 mutation, show abnormal gonadal development and progressive glomerulopathy.[9] Wilm's tumour and kidney-urinary dysplasia were observed in 40% and 11% of patients with

WT1 mutations, respectively.[10] Therefore, additional tests may be needed to rule out extrarenal disease in FSGS patients with suspected WT1 mutation, which may provide appropriate treatment for the patient's extrarenal disease. Interestingly, the age of onset and the severity of the disease differed between the father and son despite the same mutated gene. It is likely to be caused by an autosomal dominant genetic disease that is well expressed as a heterogeneous phenotype.

Genetic FSGS is known to respond poorly to steroids and other immunosuppressants.[11] Therefore, if hereditary FSGS is suspected, immunosuppressive treatment is generally stopped and the treatment is focussed on conservative therapies such as Renin-Angiotensin-Aldosterone blockade, optimal blood pressure control, and a low-salt diet. In the present case, the patient maintained kidney function and achieved reduced proteinuria with a Renin-Angiotensin-Aldosterone blocker and antihypertensive treatment. However, in randomised controlled trials, apolipoprotein L1 risk variants have not been reported to affect the response to immunosuppressants in patients with FSGS.[11] Further studies on the effects of immunosuppressants in genetic FSGS therapy are needed.

Conclusion

Genetic analysis is not contemplated as routine care for adults with FSGS; however, it may help in selecting treatment options, identifying extrarenal symptoms, and family planning. FSGS patients with positive family history and nonsyndromic glomerulonephritis should be evaluated for genetic causes. Despite not being able to identify all genetic causes, next-generation sequencing (NGS) or target gene panels are useful tools for detecting genetic diseases, including hereditary FSGS.

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YJK, SR: Writing the original draft.
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