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CASE STUDY

The novel compound heterozygous mutations, V434del and W666X, in *WFS1* gene causing the Wolfram syndrome in a Chinese family

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Abstract Wolfram syndrome (WFS), also known as DIDMOAD, is an infrequent cause of diabetes mellitus. WFS is an autosomal recessive neurodegenerative disease characterized by various clinical manifestations such as diabetes mellitus, optic atrophy, diabetes insipidus, deafness, neurological symptoms, renal tract abnormalities, psychiatric disorders, and gonadal disorders. The majority of patients with WFS carry the loss of function mutations in the *WFS1* gene. The exons 2–8 of the *WFS1* gene from one Chinese WFS patient were amplified by the polymerase chain reaction (PCR), subcloning techniques and direct sequence determination was applied to the amplified fragments. The compound heterozygous mutation of a 3-bp (GAC) deletion (V434del) and another compound heterozygous mutation (G→N)(W666X) in exon 8 of *WFS1* gene was identified in the patient. Other seventeen members of her family were investigated. Four cases with heterozygotes had been found through screening for the mutation V434del and five cases for the mutation W666X in the whole family. This is the first report of WFS with the mutation V434del and W666X in the *WFS1* gene.

Keywords Wolfram syndrome · *WFS1* gene · Mutation · Diabetes mellitus · Diabetes insipidus

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Introduction

In 1938, Wolfram and Wagener [1] described a family in which siblings developed bilateral optic atrophy (OA) and diabetes mellitus (DM), followed by deafness, incontinence, and ataxia. The frequent association of diabetes insipidus (DI), DM, OA, and deafness (D) led to the acronym DIDMOAD, indicating four cardinal features [2]. DIDMOAD, commonly known as Wolfram syndrome (WFS), is a rare autosomal recessive disorder with demonstrable clinical and genetic heterogeneity [2–4]. The true incidence of WFS is not known, but is estimated as high as 1 in 770,000 in UK and 1 in 100,000 in South America [5, 6]. The majority of patients with WFS carry loss of function mutations in the *WFS1* gene [7–9]. Defects in mitochondrial [10–12] DNA were reported in some sporadic cases, but the mitochondrial genome was not found [13–16] to be systematically involved in the disease. Moreover, a recent publication suggested a further nuclear gene locus (chromosome 4q22-q24) associated with WFS

[17]. Some other studies carried out up to the present day also suggest that WFS is caused by alterations in genes located on chromosome 4 (on 4p16.1—gene *WFS1*, or on 4q22-q24—gene *WFS2*) [7, 17]; or, alternatively, in the mitochondrial DNA [5, 11, 12, 15, 18, 19]. The recently identified gene *WFS1* (from wolframin) encodes a transmembrane protein with 890 amino acids, which is continually synthesized, especially in the endocrine pancreas [17, 20]. In cases of WFS due to the mutation of the genes *WFS1* (OMIM-606201) and *WFS2* (OMIM-604928), the type of heredity is recessive and autosomal, with a risk of recurrence of 25%. On the other hand, deletions and mutations at the tip of the mitochondrial DNA (OMIM-598500) have also been described in patients with WFS [5]. In this way, WFS appears to be genetically heterogenetic. The onset of WFS is usually in juveniles, and most of the patients are referred to pediatricians or endocrinologists for treatment. Despite the striking neurologic and neuroradiologic features of the syndrome, the disorder has therefore made little impact on the neurologic and neuroradiologic literature. The diagnosis of WFS is essentially clinical and based on the obligatory presence of juvenile, insulin-dependent DM, and atrophy of the optic nerve. Although diabetes and OA represent the minimal diagnostic criteria, neurodegenerative or psychiatric features may evolve over time. Deafness is not always a prominent component. Involvement of the hypothalamus, brain stem (especially central sleep apnea), and cerebellum (ataxia) may develop in the third decade or later and is associated with the early mortality reported in this disease. Patients may also manifest central DI, sensorineural hearing loss, urinary tract atony and neurologic and/or psychiatric disorders [5]. There is an increase in susceptibility to psychiatric diseases and to DM in its adult form in carriers of WFS [5, 19]. In general, the disease evolves to the point of premature death at the age of about 35 years due to respiratory failure which is related to atrophy of the brainstem and the complications of urinary tract atony.

Here we report a family resident in China with WFS carrying a compound heterozygous mutation of 3-bp deletion (V434del GAC) in exon 8 and another compound heterozygous mutation (G→N) (W666X) also in exon 8 of the *WFS1* gene, and it is the first time to report a WFS family with these two mutations in *WFS1* gene all over the world.

Materials and methods

Subjects

The study protocols were approved by the Hospital Ethics Committee for Human Research, and informed consent was obtained from every subject participating in the study. The proband (Fig. 1; the index case number is subject III-2, representing generation III, subject number 2) was a 13-year-old Chinese girl. She complained of polyuria, polydipsia and polyphagia at the age of 5. Then she was diagnosed with Type 1 diabetes (non-autoimmune), for which she was receiving insulin with rather poor control of her blood sugar. A year later, she acquired DI, at the age of 7, bilateral OA with reduced color vision and visual acuity in the subsequent year; along with arrested growth in height and weight. The laboratory data of the proband revealed Type 1 diabetes (non-autoimmune) with high blood glucose and low C peptide (Table 1); however, her GAD antibody and IAA was negative. Regular urinalysis indicated that her urine specific gravity was lower than normal with no urine glucose and combine water deprivation with vasopressin confirmed her DI. Then, audiograms showed a bilateral low-tone hearing impairment in the patient. The hypogastric ultrasonic test showed ureterectasia and hydronephrosis. The photographic images of the patient's eyes showed bilateral papillary atrophy (Fig. 2). Cranium MR images showed signal-intensity abnormalities in optic radiation on both sides and intracranial optic nerve atrophy. Additionally, there was a diffuse area of high signal on PD and T2-weighted images (Fig. 3). Therefore these findings were useful to clinically diagnose her of WFS. The patient is the second child, and has one elder sister. The elder sister (III-1), 5 years older than the proband, never developed symptoms of WFS. Their parents are non-consanguineous and have no signs or symptoms. All the other third generation of the family are symptom-free and have no significant differences in height, weight, and intelligence, compared with age-matched healthy children.

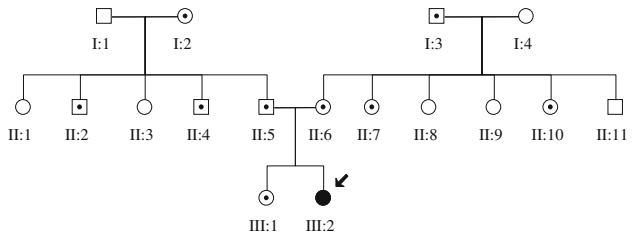

Fig. 1 Pedigree of a family with Wolfram syndrome. *Filled circle* represents the affected individual. *Open symbol* represents the unaffected individuals. *Arrow* represents the proband. *Square* represents the males and *circle* the females. Symbol with a *dot* in it represents asymptomatic carriers. (I-2, II-2 and II-4 carried the heterozygote of V434del and I-3, II-7, II-10 and III-n1 carried the heterozygote of W666X.) Generations are designated by roman numerals

Table 1 The laboratory data revealed high blood glucose and low C peptide

	0 min	120 min
Blood glucose (mmol/l)	11.2	16.2
C peptide (ng/ml)	0.512	0.572

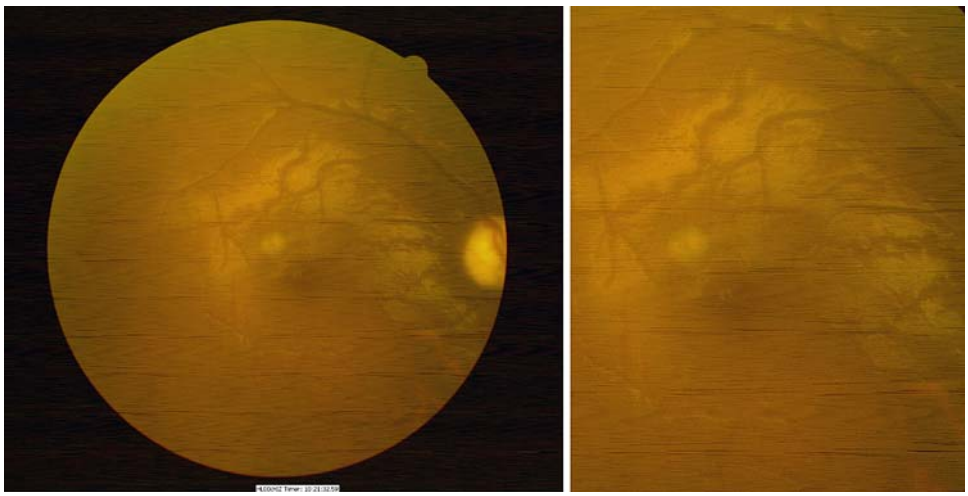

Fig. 2 Photographic image of the patient's eye showing bilateral papillary atrophy

Before their participation, all subjects gave informed written consent. The study was approved by the Institutional Review Board of Rui-Jin Hospital (Tables 2 and 3).

PCR and sequencing of *WFS1* gene

Genomic DNA from peripheral blood leukocytes of the proband and her family members were extracted using a commercially available kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The 2–8 exons of *WFS1* were amplified by PCR using 13 primer pairs (Table 4), which were designed with Primer Premier 5.0 software (PREMIER Biosoft International, Palo Alto, CA). PCR reactions were performed in a 25 μl volume containing standard PCR buffer 2 μl, 25 mM dNTP 0.5 μl, genomic DNA 200 ng, 0.5 μl of each specific primer, and 2.5 μ/μl Taq DNA polymerase 0.3 μl (Sangon, Shanghai, China). PCR amplifications were performed with denaturation step at 95°C for 10 min and then 36 cycles at 95°C for 30 s, 60°C for 30 s, 72°C for 45 s, followed by 7 min of final extension at 72°C. In case of exon 3 and exon 8, amplifications were performed for 30 cycles at an annealing temperature of 58°C. The purified PCR production of mutant exon was ligated into pGEM-T easy vector (Promega, Madison, WI) and then transformed to *Escherichia coli* strain DH-5α. Positive subclones were sequencing by ABI 3700 Genetic Analyzer (Applied Biosystems PerkinElmer, Foster City, CA).

Results

Clinical features of the WFS family

The pedigree of this family is shown in Fig. 1. The proband is a 13-year-old female patient. She was diagnosed with WFS because of presenting all the four symptoms, while her family members showed no signs of the syndrome. The physical examination (heart rate, blood pressure, respiration rate, temperature, overall health status) and some other WFS correlated examinations of all the other family members seemed to be normal.

Mutation analysis of the affected pedigree

As shown in Fig. 4, in the analyses of the *WFS1* gene for the family, the affected girl was identified with a novel heterozygotic mutation of 3-bp deletion (V434del GTC) in exon 8, and another heterozygous point mutation in exon 8 TGG to TAG (W666X), resulting in the introduction of a STOP codon. The mother was found to have the heterozygous for the mutation W666X, while the father was heterozygous for the mutation V434del. With her other family members, three (I-2, II-2, II-4) carried the heterozygote of V434del and the other four (I-3, II-7, II-10, III-1) carried the heterozygote of W666X.

Discussion

Wolfram syndrome is a rare autosomal recessive disorder with demonstrable clinical and genetic heterogeneity. It is classified as a progressive neurodegenerative disease. This means the disorder primarily affects the body's nervous system and that the nervous system gradually declines in its ability to work correctly. DM, together with the presence of OA, is the hallmark traits of WFS, and a person with these two traits is presumptively considered to have WFS until proven otherwise. Then, DI is the third most common aspect of WFS. Neurological and psychiatric abnormalities are also well-known components of WFS [3, 5, 21–23] and their frequency and severity were positively correlated with the patient's age. It has also been reported that patients

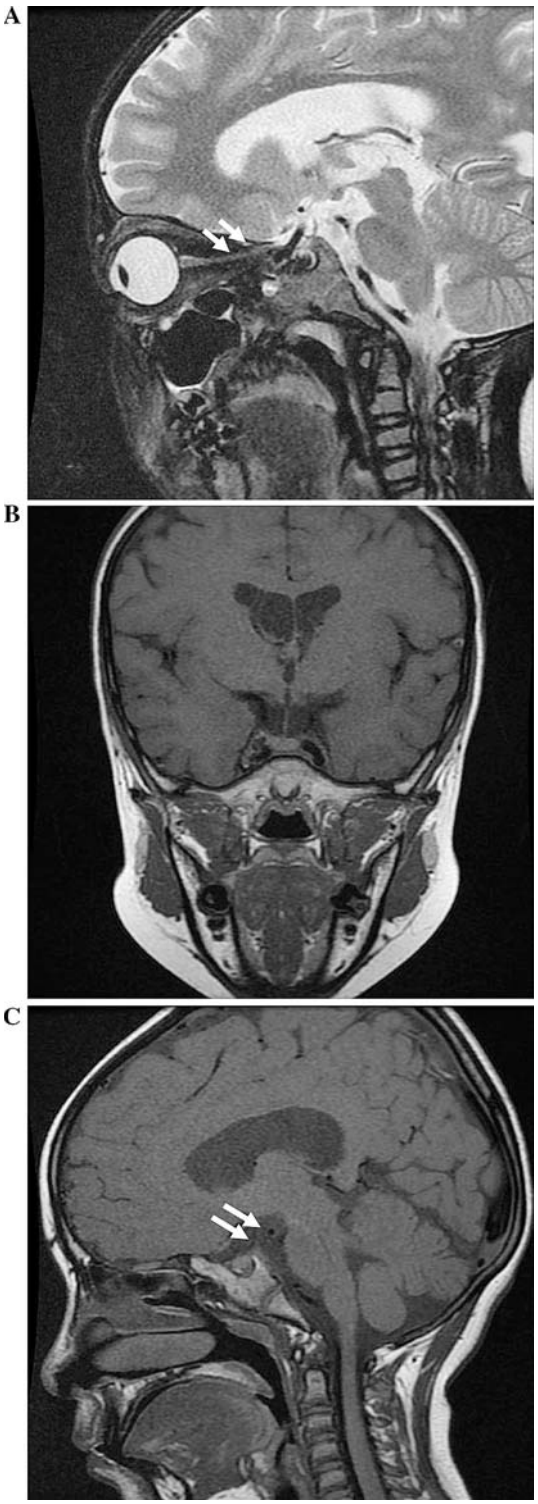

Fig. 3 Cranium MR images showed signal-intensity abnormalities in optic radiation on both sides and intracranial optic nerve atrophy (**a**, *arrowheads*) and absence of T2-hyperintensity normally recognized in the posterior pituitary lobe (**b**, **c**, *arrowheads*)

with WFS could be found to have short stature and GH deficiency [24], but till now no pituitary exploration was performed in any other study.

Table 2 Growth hormone arginine stimulation test showed that GH can be stimulated

	0 min	30 min	60 min	90 min
GH (ng/ml)	2.14	32.1	8.96	2.92

Table 3 GnRH stimulation test showed that LH and FSH can also be stimulated

	−15 min	0 min	25 min	45 min	90 min	120 min
LH (mIU/ml)	1.79	1.51	7.7	14.63	21.74	23.54
FSH (mIU/ml)	24.12	22.15	31.02	42.91	66.7	71.52

Table 4 Primers used in PCR/sequencing reaction of *WFS1* coding region

Exon	Primers	Primers sequences (5′–3′)	Product size (bp)
2	2F	AAGCGGTGCTGGCCCATG	464
	2R	CCGTTCCCACCCAGCTATC	
3	3F	GACCGGAAGGCAAACAGT	376
	3R	GATCTCAGGCACCGACACT	
4	4F	AGTGGCCGGAGGCTCAGT	436
	4R	CCAACAGCATCACCAGCGT	
5	5F	AGTCAGATGTCCATGCATCC	510
	5R	CTCTACAGGAAGGTTCTGGT	
6	6F	GAGCACGCTACGTGGTGCT	406
	6R	GGAGGCACGGGTGAGATAG	
7	7F	CCTGAACCCACTCAGCTC	409
	7R	CCAGCGGCACGGCTGTAA	
8-1	8-1F	TTCCCACGTACCATCTTTCC	334
	8-1R	CACATCCAGGTTGGGCTC	
8-2	8-2F	AGAACTTCCGCACCCTCAC	330
	8-2R	TCAGGTAGGGCCAATTCAAG	
8-3	8-3F	CCTGAGCCTGAGCACCCAT	470
	8-3R	CGGTGACTGCGATCTTGG	
8-4	8-4F	GTGAGCTCTCCGTGGTCATC	346
	8-4R	CCCTCTGAGCGGTACACATAG	
8-5	8-5F	ATCCTGGTGTGGCTCACG	300
	8-5R	GTAGAGGCAGCGCATCCAG	
8-6	8-6F	GCGTGACTGACATCGACAAC	356
	8-6R	GCTGAACTCGATGAGGCTG	
8-7	8-7F	CAGCAGCGAGTTCAAGAGC	300
	8-7R	CCTCATGGCAACATGCAC	

In 1998, the nuclear gene for WFS, *WFS1*, was discovered and mapped to chromosome 4p16.1 by positional cloning [7, 8]. The *WFS1* gene is composed of eight exons spanning 33.4 kb of genomic DNA. The 3.6-kb mRNA encodes an 890-amino acid protein named wolframin [8]

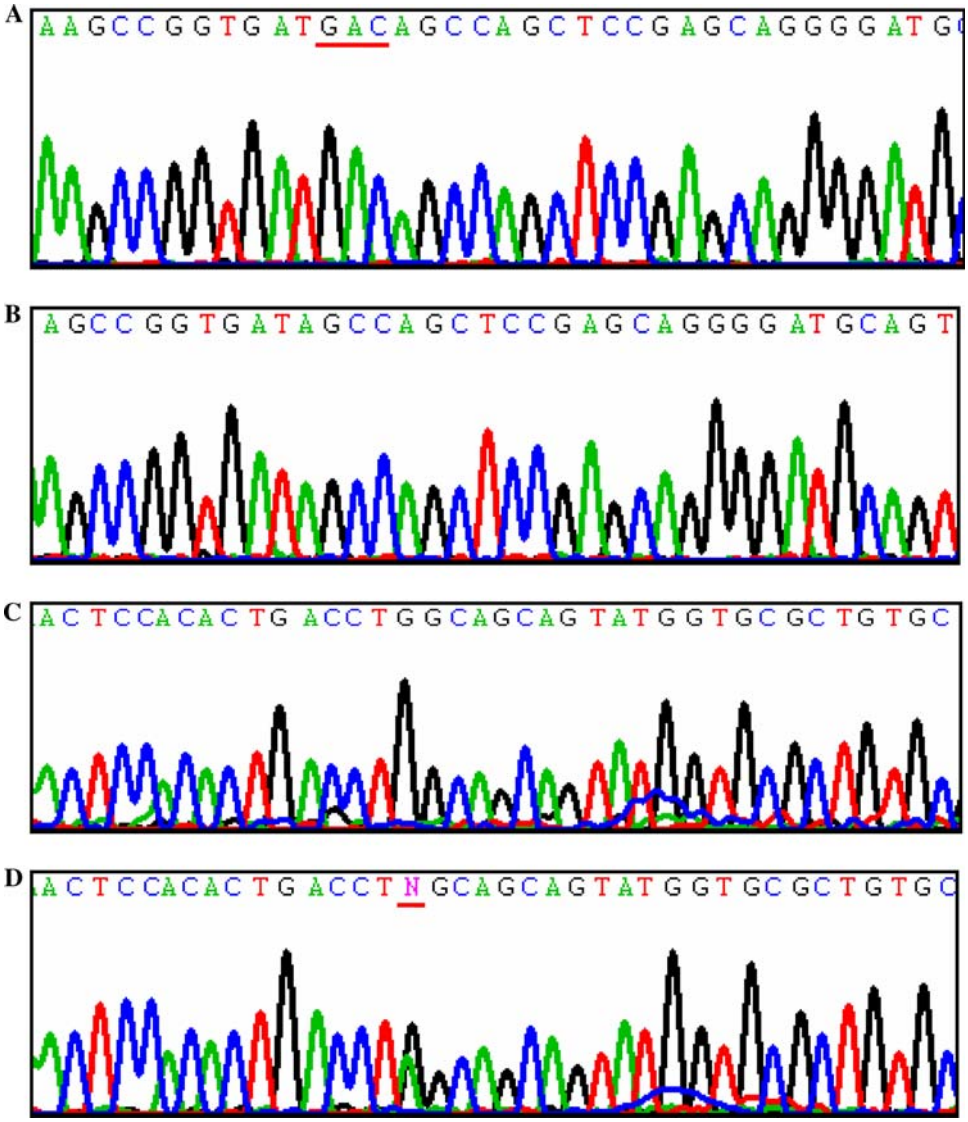

Fig. 4 Identification of mutation by direct sequencing of the *WFS1*. The exon 8 of *WFS1* gene, amplified by PCR, was directly sequenced in this family. Comparing with the wide-type sequences (**a**, **c**), the heterozygous mutation (c.1470_1472 del GAC) presented with singlet signals on chromatogram after subcloning (**b**) and another heterozygous point mutation (G→N)(W666X) presented with doublet signals on chromatogram (**d**)

with nine predicted transmembrane domains belonging to a novel gene family. Biochemical studies in cultured cells indicate *WFS1* to be an integral, endoglycosidase H-sensitive membrane glycoprotein that primarily localizes in the endoplasmic reticulum [25]. Recent evidence suggests that *WFS1* is either a novel endoplasmic reticulum calcium channel or a regulator of channel activity [17]. And the wolframin was suggested to play a role in the survival of certain populations of cells, particularly neuronal and endocrine cells [7, 25]. As the sequence of the human *WFS1* gene, which is for WFS, had been known (NC_000004.10), the molecular diagnostic method appears to be extremely beneficial and should become routine for this hereditary disease due to its simple and noninvasive to scan for known and unknown mutations. Homozygous or compound heterozygous mutations in *WFS1* may lead to the autosomal recessive inherited WFS, presenting a variety of phenotypes, including DI, DM, AO, and deafness, referred to as DIDMOAD (OMIM:#222300) [8]. Genetic studies of WFS have been performed by several groups of researchers, and the results have provided evidence of genetic heterogeneity [17, 26, 27]. Linkage of WFS to markers on chromosome 4p has been reported during the last decade [28]. More recently, a candidate gene (*WFS1*) was mapped to the 4p16.1 and isolated [29]. In addition, mutations in this gene have been identified in patients affected with Wolfram disease [7, 9, 30, 31]. To date, over 60 different mutations causing WFS have been identified in the *WFS1* gene, including missense and nonsense mutations, large and small deletions, and most of the causative changes occurred in exon 8.

Treatment consists of managing the complications of WFS as they occur, and preventing the secondary complications when possible. No individual person will follow the above-described patterns exactly, and not everyone develops each of the manifestations before their death. For

instance, in some people the auditory nerve is spared, in others the digestive system nerves. Other parts of the nervous system are universally affected, such as the optic nerve and the mechanism that regulates sugar metabolism. Therefore, although the specific path that each person takes will be different, the overall progression of the disease to premature death is not disputed. There have been no reported cases of people diagnosed with WFS that have not progressed to life-threatening complications and premature death, and there currently is no treatment for the underlying mechanism of neurodegeneration in WFS. Once the diagnosis of WFS is established through genetic studies and ruling out other causes, care management focuses on screening for and treating the other predictable disorders of WFS.

The patient in our study presented with juvenile-onset Type 1 diabetes (non-autoimmune) of nonautoimmune nature followed by OA in the first decade [20] and subsequent central DI. Sensory nerve deafness developed during her second decade, whereas urinary tract and neurological abnormalities appeared later in life. This is concordant with previously reported series particularly the one by Barrett et al. [5] with only one difference concerning the onset of DI, which happened earlier in this patient. Urinary tract dilation and abnormal urodynamics were also found in this patient. This finding is concordant with the literature in which urologic abnormalities are reported as frequent complications of WFS [5, 32, 33]. These complications have been mainly attributed to the high output urine state resulting from DI. Besides, defective GH secretion is also an alteration documented in this patient which leads to her short stature. The diagnosis of WFS in the proband was based on history, typical clinical symptoms, laboratory tests, imaging examinations and the direct sequencing analysis of the *WFS1* gene. In this family, we have identified two novel mutations responsible for WFS in the *WFS1* gene (i.e., V434del, W666X). By screening exon 8 for the two mutations among the other 17 family members, four asymptomatic members, including her father, were detected to carry the mutation (V434del) on one of their alleles, and another five, including her mother and a sister, were detected to carry the mutation (W666X) on one of their alleles, which confirmed to follow Mendelian inheritance.

Treatment of WFS usually consists of managing the complications as they occur, and preventing the secondary complications when possible. This patient was treated with insulin injection and desmopressin acetate tablets, and also advised to take urethral catheterization when necessary.

In summary, we describe the clinical and genetic features in a three-generation family with WFS. It is caused by a compound heterozygous mutation of 3-bp deletion V434del GAC and another compound heterozygous mutation TGG to TAG (W666X) in exon 8 of the *WFS1* gene, these two mutations are first identified in WFS subjects in our study. Although previous studies had got some achievements in WFS, our knowledge about this disease is still limited. WFS is a multisystem, progressive disorder with many manifestations which are being discovered because of developments in new investigative technology, as well as improvement in life expectancy. The disorder should be kept in mind particularly in our part of the world, where consanguinity is prevalent. Therefore, more researches should focus on understanding the molecular basis of the disease and a more complete picture with respect to the global distribution of *WFS1* gene mutations is required. And we hope that this information will open new horizons to future genetic and molecular investigations that may lead to a better elucidation of the pathophysiology of WFS and hopefully to improvement in means of prevention and treatment of this devastating disease.

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