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Identification of a novel *WFS1* homozygous nonsense mutation in Jordanian children with Wolfram syndrome

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ABSTRACT

Wolfram syndrome (WS) is a rare autosomal recessive neurodegenerative disorder characterized by the presentation of early onset type I diabetes mellitus and optic atrophy with later onset diabetes insipidus and deafness. *WFS1* gene was identified on chromosome 4p16.1 as the gene responsible for WS disease given that most of the WS patients were found to carry mutations in this gene. This study was carried out to investigate the molecular spectrum of *WFS1* gene in Jordanian families. Molecular and clinical characterization was performed on five WS patients from two unrelated Jordanian families. Our data indicated that WS patients of the first family harbored two deletion mutations (V415del and F247fs) located in exon 8 and exon 7 respectively, with a compound heterozygous pattern of inheritance; while in the second family, we identified a novel nonsense mutation (W185X) located in exon 5 in the N-terminal cytoplasmic domain with a homozygous pattern of inheritance. This mutation can be considered as loss of function mutation since the resulting truncated protein lost both the transmembrane domain and the C-terminal domain. Additionally, the W185X mutation lies within the CaM binding domain in wolframin protein which is thought to have a role in the regulation of wolframin function in response to calcium levels.

1. Introduction

Wolfram syndrome (WS) was first described in 1938 by Wolfram and Wagener as a rare progressive neurodegenerative disorder with an autosomal recessive pattern of inheritance (Wolfram and Wagener, 1938). WS is also known by the acronym DIDMOAD given that it is characterized by the presentation of multisystem symptoms: diabetes insipidus (DI), type I diabetes mellitus (DM), optic atrophy (OA), and deafness (D) (Blanco-Aguirre et al., 2015). Other clinical symptoms were found to be associated with WS including ataxia, peripheral neuropathy, psychiatric problems, and renal tract abnormalities (Haghighi et al., 2013). WS is diagnosed early in life with a juvenile onset of DM and OA (Hardy et al., 1999); patients die young at a median age of 30 years mostly due to respiratory failure caused by brainstem atrophy (Pizzolanti et al., 2014). WS prevalence is estimated to be 1 in 770,000 with a carrier frequency of 1 in 354 (Nakamura et al., 2006). Inoune and colleagues identified the gene responsible for Wolfram syndrome as *WFS1* (Inoue et al., 1998). *WFS1* gene is located within the short arm of chromosome 4 (4p16.1), spanning about 33.4 kb of DNA and composed of eight exons, where exon 1 is noncoding, exon 2 is the start point of translation, and exon 8 is the largest exon encompassing 2.6 kb of DNA (Rigoli et al., 2010). *WFS1* gene codes for the wolframin protein; an 890 amino acid with an estimated molecular mass of 100 kDa (Hardy et al., 1999). Wolframin is an endoglycosidase H-sensitive integral membrane glycoprotein with nine hydrophobic transmembrane domains and large hydrophilic regions at the N- and C-termini (Fonseca et al., 2005).

Wolframin is expressed in in the β-cells of pancreas, heart, brain, muscle tissues, liver, spleen and kidney (Hofmann et al., 2003). Wolframin is an endoplasmic reticulum (ER)-localized protein and studies indicate a role in protein synthesis, protein folding and modification, membrane trafficking, and ER stress signaling (Pizzolanti et al., 2014). Additionally, studies have shown that wolframin protein has a calmodulin (CaM) binding site at the N-terminal cytoplasmic domain between the residues Glu90 and Trp186 (Yurimoto et al., 2009), suggesting a role for wolframin in maintaining the intracellular homeostasis of calcium ions by controlling their levels in the ER (Qian et al., 2015). Most WS patients have a mutation(s) in the WFS1 gene and >230 mutations in the gene have been identified so far (Qian et al., 2015).

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Accordingly, these mutations were found to be mostly distributed in exon 8 and they include missense, nonsense, splicing mutations, frameshift insertions, and frameshift deletions, with homozygous and compound heterozygous genotypes (Chaussenot et al., 2011). Most of the mutations found in WS patients result in the expression of a loss of function wolframin; however, the specific role of the mutated wolframin in the expression of WS symptoms and the exact genotype-phenotype relationship in WS patients are not clear yet (Blanco-Aguirre et al., 2015).

Interestingly, it was reported that WS patients show some level of phenotypic heterogeneity where certain patients did not develop diabetes insipidus but they demonstrated certain features as upper gastrointestinal ulceration with bleeding tendency (Gasparin et al., 2009). A Jordanian group studied the possibility of locus heterogeneity and the presence of mutations in other genes that might be associated with expressing different symptoms in WS patients. Linkage analysis in Jordanian WS patients who developed different symptoms resulted in identifying another locus associated with the disease at chromosome 4q22-25 (Shanti et al., 2000). Amr et al. identified a zinc-finger gene, *ZCD2/WFS2*, in this region and were able to show that three consanguineous Jordanian families with WS harbor mutations in this gene resulting in a different clinical manifestation of WS such as patients show no signs of diabetes insipidus or hearing loss however, patients show upper gastrointestinal ulceration with bleeding tendency (Amr et al., 2007).

In this study, we investigated the molecular status of *WFS1* gene in two unrelated Jordanian families, including five WS patients, to identify mutations associated with the disease which will be pivotal in genetic screening for parental counseling prior to conception and aid in early diagnosis of the disease.

2. Methods

2.1. Patients

Five patients (4 females and 1 male) from two unrelated families from northern Jordan were recruited in this study. The minimal criteria to diagnose WS were early onset diabetes mellitus and optic atrophy. Clinical records of the selected patients were retrieved from the files of Jordanian Royal medical services (JRMS). All study subjects underwent comprehensive clinical examination including ophthalmological, endocrinological, audiographic, urological, and neurological evaluations. Blood was collected from all patients as well as their parents, siblings, 39 close family members, and 100 control volunteers (unrelated) in EDTA tubes under aseptic conditions and stored at 4 °C. Written consent was obtained from subjects participating in this study and the research was approved by the human research ethics committee of the Jordan University of science and technology. The main clinical features of the patients are listed in Table 1.

2.2. DNA extraction

Genomic DNA was extracted from whole blood using GeneJET Genomic DNA Purification Kit (Thermo Scientific) according to the manufacture's recommendations. Nanodrop (ND-1000) was used to check the concentration (>50 ng/μL) and purity (against RNA and protein) of the isolated DNA.

2.3. Mutational analysis

Mutational screening was done on the coding region of *WFS1* gene of exons 2–8 by PCR and direct sequencing. Primers used to amplify these exons were previously described (Colosimo et al., 2003). PCR reactions were performed in a final volume of 30 μL containing 100 ng (2–4 μL) genomic DNA, 15 μL master mix (GoTaq® Green Master Mix, Promega, USA), 1–2 μL of 5 pmol of forward and reverse primers, and nuclease free water was added to a final volume of 30 μL. PCR conditions were performed as follows: an initial denaturation at 95 °C for 7 min, followed by 30 cycles of denaturation step at 95 °C for 1 min, annealing step for 30 s, and extension step at 72 °C for 30 s, then a final extension step at 72 °C for 7 min. PCR products were visualized by gel electrophoresis with ethidium bromide dye. The purified PCR products were sequenced using the big dye terminator cycle sequencing reaction kit and analyzed on an ABI prism 3100 Genetic Analyzer (Applied Biosystem, USA). Mutational analysis was carried out using mutation surveyor software (SoftGenetics, PA, USA). Validity of variations in the results was done by sequencing both DNA strands of two independent PCR products.

3. Results

3.1. Patients and clinical data

Five patients from two unrelated families were studied. Pedigrees of the families are shown in Fig. 1 and the main clinical features of the patients are shown in Table 1. The first family (Fig. 1a) consists of three WS patients, as three of seven siblings fulfilled the minimal diagnostic criteria. Diabetes mellitus was diagnosed in the first decade of life, patients received insulin with poor compliance to treatment. Ophthalmological examination revealed optic atrophy at a mean age of 12.4 ± 2.05 (range 10–15) and a recent eye examination showed visual acuity <6/60 in both eyes. All patients were subjected to audiography with abnormal results in all cases. Analyses of urine and serum osmolarity confirmed the diagnosis of diabetes insipidus. Urinary tract abnormalities were observed in all patients. Furthermore, all patients showed neurological abnormalities, while two of them (patient IV-18 and patient IV-22) were diagnosed with epilepsy. The second family (Fig. 1b) had four siblings with two of them diagnosed as WS patients. They were known as diabetic case since the age of three. On fundoscopic examination, bilateral optic atrophy without diabetic retinopathy was confirmed at the age of five. Urinary tract abnormalities were observed in the two patients and ultrasonography examination diagnosed patient II-1 with hydronephrosis and gall bladder stones. Audiometric studies showed bilateral high frequency sensorineural hearing loss by the age of five. According to neurological evaluation, the patients had poor concentration and moderate intellectual disability.

3.2. Mutation analysis data

Two unrelated families with five WS patients were examined in this study. Direct sequencing of exons 2–8 of *WSF1* gene revealed several genetic variations including deletion mutations, nonsense mutations, and several polymorphisms in both families (Tables 2 & 3). Two deletion mutations were detected in family 1, V415del and F247fs (Table 2).

Table 1
Clinical feature of the WFS patients.

Family	Patient	Gender	Age	DM (age at diagnosis)	OA (age at diagnosis)	DI (age at diagnosis)	Deafness (age at diagnosis)	Urinary tract abnormalities	Neurological abnormalities	Other complication
F1	IV-18	Female	20	3	15	4	15	+	+	Epilepsy
F1	IV-19	Female	17	5	12	5	12	+	+	−
F1	IV-22	Female	11	4	10	4	9	+	+	Epilepsy
F2	II-1	Female	19	3	5	4	5	+	+	Hydromephrosis Remove gall bladder stone
F2	II-2	Male	15	3	5	4	5	+	+	−

DM, diabetes mellitus; DI, diabetes insipidus; OA, optic atrophy. All Ages in years. (+) denotes complication present. (−) denotes complication absent.