Identification of one novel causative mutation in exon 4 of WFS1 gene in two Italian siblings with classical DIDMOAD syndrome phenotype

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ABSTRACT

The aim of the present paper is to describe a novel missense mutation (G107R) of WFS1 gene that was unexpectedly detected, in two siblings from Southern Italy, outside exon 8; a very unusual finding which has previously been reported only twice in Italian patients with Wolfram syndrome (WS). Although in Spanish pedigrees' WFS1 mutations are frequently located in exon 4, this finding is very infrequent in other pedigrees, particularly in Italian patients. Conclusions: a) our report of two siblings with one novel WFS1 mutation (G107R) expands the molecular spectrum of WS; b) this is the 3rd report of Italian patients harbouring one mutation outside exon 8 and the 2nd with one mutation in exon 4; c) on the basis of the present observations, and literature data we can infer that mutation locations outside exon 8 do not seem to be clearly associated with peculiar phenotype expressions of WFS1 gene.

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1. Introduction

Wolfram syndrome (WS), also known by the acronym DIDMOAD (diabetes insipidus DI, diabetes mellitus DM, optic atrophy OA and deafness D), is an autosomal-recessive disorder usually diagnosed in childhood when non-autoimmune, insulin-dependent DM is associated with OA. Additional characteristics include ureterohydronephrosis, neuropsychiatric and endocrinological impairment and cataract (d'Annunzio et al., 2008).

The gene involved (WFS1), which was identified in 1998 on chromosome 4p, spans 33.4 kb of genomic DNA and includes eight exons: the 1st is non-coding, 2–7 are coding and the 8th is 2.6 kb long (Inoue et al., 1998). WFS1 mRNA encodes an 890-amino acid polypeptide with nine putative transmembrane domains and a 100-kDa molecular mass (wolframin).

Until 2003, the WFS1 mutational spectrum included only 60 different causative defects (Colosimo et al., 2003), whereas a more recent systematic review of WS mutational studies, involving 219 patients, reported 172 WFS1 mutations, most of which were located in exon 8 (Yu et al., 2010), which corresponds to the transmembrane region and carboxyl tail of wolframin protein (Smith et al., 2004). The majority of these mutations are known to be inherited directly from the parents, whilst de novo mutations seem to be very rare in the WFS1 gene (Cryns et al., 2003). Sequencing studies of WS kindreds have shown that some mutations are more common in certain ethnic groups, which suggests that there is a founder effect in some populations (Gómez-Zaera et al., 2001).

The aim of the present report is to describe a novel missense mutation of WFS1 gene that was unexpectedly detected, in two siblings from Southern Italy, outside the exon 8 region, a very unusual finding which has previously been reported only twice in Italian patients with WS (Aloi et al., 2012; Colosimo et al., 2003).

2. Patients and methods

2.1. Case report

Two siblings of different sex and age were born and are still living in Telese Terme, a small town (7000 inhabitants and 2700 families), situated near Naples, in the district of Benevento (Campania region, Italy). Both parents and four grand-parents come from the same town, where consanguineous unions are not very unusual. Although patients' relatives do not acknowledge any consanguinity ties amongst themselves, nevertheless collateral relationships cannot be excluded.

In both siblings, the first clinical manifestation of WS was a non-autoimmune, insulin-dependent DM, that presented at the age of 7.1 and 8.4 years, respectively. Since then, they have been regularly followed in the Centre for Pediatric Diabetes of Naples University. The main data of patients' DM medical history are summarized in Table 1.
2.2. Methods

2.2.1. DNA extraction and PCR amplification

Genomic DNA was extracted from peripheral blood leukocytes by standard phenol chloroform procedure.

Fourteen primer pairs were used to amplify the entire coding region of the WFS1 gene, including the non-coding exon 1 and the exon–intron boundaries. Exon 8 was subdivided into seven regions (from 8a to 8g), as previously described (Colosimo et al., 2003). PCR amplifications were performed in a Gene Amp PCR System 9700 (Perkin Elmer, Foster City, CA) with an initial denaturation step at 94 °C for 15 min and then 30 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 45 s, followed by 7 min of final extension at 72 °C. WFS5 and WFS8c amplifications were performed at the annealing temperature of 62 °C.

Other signs and symptoms of WS progressively presented during the subsequent follow-up period and the entire spectrum of DIDMOAD manifestations became evident in both siblings by 16.0 and 14.6 years, respectively (Table 2). Apart from DIDMOAD manifestations, no further manifestations became evident in both siblings by 16.0 and 14.6 years, the subsequent follow-up period and the entire spectrum of DIDMOAD analysis in two Italian siblings with WS.

In both patients, DI management has required regular replacement therapy with antidiuretic hormone since the time of presentation.

In both patients, diagnosis of WS was initially assessed on the basis of the association of DM–OA and subsequently confirmed by genetic analysis, which was performed by studying all eight exons and the intron sequences of the entire WFS1 gene.

Clinical and/or biochemical abnormalities which might be compatible with WS diagnosis were not detected in either parents.

2.2.2. Sequencing analysis

DNA PCR samples underwent direct sequencing using a 3130 Genetic Analyzer (Applied Biosystem, Foster City, CA), according to PRISM Dye Terminator and Dye primer cycle sequencing chemistries. Sequences were compared with reference to human genomic and cDNA WSF1 sequences (GenBank accession nos. AC004689 and Y18064). To avoid potential artefacts due to AmpliTaq Gold™ DNA Polymerase (PE Applied Biosystem, Foster City, CA), each sequence alteration was confirmed by sequencing both DNA strands of three independent PCR products. The sequence variants were considered mutations when they: a) caused a nonconservative amino acid change; b) were absent in 300 ethnically-matched control chromosomes; and c) affected phylogenetically conserved residues. Other DNA variations that did not fulfil these criteria were considered polymorphisms.

3. Results

The results of the gene sequencing analysis are reported in Table 2. In both siblings, we identified a novel homozygous G-to-C nucleotide transversion resulting in a glycine-to-arginine substitution at codon 107 in exon 4 of the WFS1 gene (Fig. 1). The same mutation was also found in both parents in heterozygosis.

This G107R WFS1 mutation was not detected in 150 healthy and ethnically-matched subjects (300 chromosomes).

Sequence analysis also revealed a number of polymorphic variants in the coding sequence: I333I; F341F; A575A; K774K; S855S.

4. Discussion

In the present paper, we have reported an apparently nonconsanguineous family with both offspring affected by WS. In both

![Image](https://example.com/image.jpg)

**Fig. 1.** Sequences of WFS1 gene exon 4. The black arrow indicates the mutation G107R detected in homozygosis in both siblings, whilst the black square indicates the same mutation detected in heterozygosis in both parents.
siblings, a homozygous novel missense mutation (G107R) was found in exon 4 of the WFS1 gene. This mutation is not very different from that of Zalloua et al. (2008), who described a transition from Glycine to Glutamic Acid in position 107 of WFS1 (G107E) on the same exon 4.

According to literature data, WFS1 mutations are only rarely located outside exon 8: in exon 4 (Aloi et al., 2012; Cano et al., 2007; Hansen et al., 2005; Smith et al., 2004; Zalloua et al., 2008), or 5 (Colosimo et al., 2003; Gasparin et al., 2009), or 7 (Giuliano et al., 2005). To date, fourteen different variants have been described worldwide outside exon 8 (Rigoli et al., 2011). Although in Spanish WS pedigrees the most frequent WFS1 mutation was unexpectedly reported to be harboured in exon 4 (Gómez-Zaera et al., 2001), in Italian pedigrees the location of a WFS1 mutation in exon 4 has, to date, only been described in one patient (Aloi et al., 2012). In nearly all Italian reports WFS1 mutations are located in exon 8 (d’Annunzio et al., 2008; Lombardo et al., 2005; Rigoli et al., 2011; Tessa et al., 2001).

In the series by Aloi et al. (2012), the only patient harbouring one mutation in exon 4 showed a mild phenotype. By contrast, in the Australian series by Smith et al. (2004), the only patient harbouring one mutation in exon 4 exhibited a severe phenotype, with an early onset of cardinal clinical manifestations. Finally, in the present two cases the finding of a WFS1 mutation in exon 4 was not associated with a peculiar phenotype. At onset of the 3rd decade of life, one sibling in our case report exhibited no further clinical manifestations in addition to the classical quartet defining the DIDMOAD syndrome, whilst the other sibling exhibited only a psychiatric disorder. Even in other literature cases with different locations of mutations outside exon 8, no peculiar phenotypes were observed (Cano et al., 2007; Colosimo et al., 2003; Hansen et al., 2005) although a mild phenotype was described by Gasparin et al. (2009) in one patient harbouring one mutation in exon 5. However, on the basis of the present observations and literature data, we could argue that different locations of WFS1 mutations outside exon 8 are not able to clearly condition peculiar phenotype expressions in WS.

Another inference of the present report is that, although most mutations of the WFS1 gene occur in exon 8, molecular screening requires analysis of all exons, due to the possibility that mutations might also be located in other exons.

In the present case report, we have not performed functional analyses for novel mutation. We are aware that validation of the novel variants should await detailed functional analysis of mutations on a cellular and molecular level, nevertheless the criteria that we have adopted in the present case report, for definition of mutations, are currently adopted also by other Authors (Aloi et al., 2012).

To sum up: a) our report of two siblings with one novel WFS1 mutation (G107R) expands the molecular spectrum of WS; b) this is the 3rd report of Italian patients harbouring one mutation outside exon 8 and the 2nd with one mutation in exon 4; c) on the basis of the present observations, and the literature data we can infer that mutation locations outside exon 8 do not seem to be clearly associated with peculiar phenotype expressions of the WFS1 gene.

References