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BASIC SCIENCE ARTICLE

Wolfram syndrome 1 in the Italian population: genotype–phenotype correlations

Luciana Rigoli[1], Concetta Aloi[2], Alessandro Salina[2], Chiara Di Bella[1], Giuseppina Salzano[1], Rosario Caruso[1], Emanuela Mazzon[3], Mohamad Maghnie[4], Giuseppa Patti[4], Giuseppe D'Annunzio[4] and Fortunato Lombardo[1]

OBJECTIVES: We studied 45 patients with Wolfram syndrome 1 (WS1) to describe their clinical history and to search for possible genotype–phenotype correlations.
METHODS: Clinical criteria contributing to WS1 diagnosis were analyzed. The patients were classified into three genotypic classes according to type of detected mutations.
RESULTS: WS1 prevalence in Italy is 0.74/1,000,000. All four manifestations of DIDMOAD were found in 46.7% of patients. Differently combined WS1 clinical features were detected in 53.3% of patients. We found 35 *WFS1* different mutations and a novel missense mutation, c.1523A>G. WS1 patients were homozygotes or compound heterozygotes for *WFS1* mutations except for 2 heterozygote patients (4.5%). Each genotypic group exhibited a different age onset of DM, D, and DI but not of OA. Genotypic Group 2 patients manifested a lower number of clinical manifestations compared to Groups 1 and 3. Moreover, genotypic Group 1 patients tended to have a shorter survival time than the other groups. No differences were found regarding type of clinical pictures.
CONCLUSIONS: Our study suggested that molecular *WFS1* typing is a useful tool for early assessment of clinical history, follow-up, and prognosis of WS1.

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INTRODUCTION

Wolfram syndrome 1 (WS1; OMIM 222300) is a rare, autosomal-recessive, neurodegenerative, and progressive disease.[1] WS1 prevalence in the general population has been appraised from 1/770,000 individuals[2] to 1/54,478, in different ethnic groups.[3] The minimum ascertainment criteria for WS1 clinical diagnosis are the occurrence of diabetes mellitus (DM) (usually during the first decade of life) and bilateral optic atrophy (OA) before the age of 15 years, which are usually associated with diabetes insipidus (DI), deafness (D), renal tract abnormalities, or neuropsychiatric disorders.[4] Therefore, WS1 is also known by the acronym DIDMOAD due to its main features (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and Deafness).[2,5] Urinary tract dysfunctions (UD) seem to be more frequent than expected, prompting some to suggest that the acronym DIDMOADUD is better suited than the more commonly used DIDMOAD.[6] Cognitive problems and mood disorders have also been reported.[4] However, WS1 clinical diagnosis is established in a patient exhibiting the two major criteria (DM+OA), or with one major criterion along with two minor criteria, or in a patient with two of any of the DIDMOAD anomalies.[2,6,7]

DM is due to insulinopenia secondary to degeneration of β-cells in the absence of autoimmunity, occurs as the first manifestation, has a non-autoimmune origin, and is insulin requiring. Previous autopsy studies showed loss of β-cells or atrophy of the islets in the pancreas from WS patients, while the exocrine portion of the gland was normal or with focal areas of fibrosis. Therefore, WS-associated DM is not caused by a functional defect in β-cells but by actual β-cell depletion.[4]

Good metabolic control of diabetes with a lower frequency of microangiopathic complications and less glycemic variability is frequently observed.[8]

At present, many patients affected by WS1 remain undiagnosed due to misdiagnosis of type 1 diabetes, with inadequate treatment and evaluation.[9]

OA is usually diagnosed in the first decade of life. The pathogenesis of OA could result from the effects of *WFS1* mutations on the survival of retinal ganglion cells, leading to anterograde atrophy of retinal axons and shrinkage of the optic nerve.[10] Analysis of unfolded protein response signaling revealed an activation of endoplasmic reticulum (ER) stress in mutant mouse, suggesting functional impairment in optic pathways.[10]

Sensorineural deafness (D) is usually diagnosed at a median age of 16 years in 60% of cases,[5] even if hearing loss can be diagnosed earlier than previously, i.e., median age 7.3 years.[5] Audiometric features include a severe auditory threshold shift, more evident for the medium/high frequencies. D could be a consequence not only of a dysfunction of cochlear neurons and VIII nerve fibers but also of the central nervous pathways in brainstem and inferior colliculus.[5]

A wide spectrum of abnormalities affecting the central nervous system has been described; anosmia, ataxia, seizures, nystagmus, gaze palsies, dysarthria, dysphagia, psychiatric disturbances, cognitive impairment, neurogenic bladder, central apnea, and neurogenic upper airway collapse myoclonus are the most frequently reported.[4] Endocrine dysfunctions include primary and secondary hypogonadism, more frequent in male gender, while in females only menstrual abnormalities are frequently encountered.[5] Anterior pituitary hypofunction seems to have a

[1]Department of Human Pathology, University of Messina, Messina, Italy; [2]LABSIEM (Laboratory for the Study of Inborn Errors of Metabolism), Istituto Giannina Gaslini, Genoa, Italy; [3]IRCCS Centro Neurolesi "Bonino-Pulejo", Messina, Italy and [4]Department of Pediatrics, Istituto Giannina Gaslini, Genova, Italy
Correspondence: Giuseppe D'Annunzio (GiuseppeDAnnunzio@gaslini.org)

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hypothalamic origin and includes growth hormone deficiency and impaired corticotrophin secretion. Growth velocity and pubertal development need to be carefully followed. Moreover, steroid supplementation during periods of stress or during infectious diseases needs to be considered.[5] Gait, balance impairment, and orthostatic dysregulation can be detected also at a young age.[11]

Mortality is ~65% before 30–40 years and the average age of death is 30 (range 25–49) years,[12] thus demonstrating a more severe natural history compared with type 1 DM.[8,9] WS1 patients usually die from central respiratory failure as a result of brainstem atrophy or asphyxia by food aspiration due to swallowing incoordination, as reported in young patients with various neurological diseases.[4,6] Other causes of precocious death include end-stage renal disease secondary to infections and suicide.[6,12]

WS1 is caused by mutations in *WFS1*, a gene located on 4p16.1 composed of eight exons, of which only the first exon is a noncoding exon.[13,14] *WFS1* encodes wolframin, an 890-amino acid glycoprotein which participates in the regulation of endoplasmic reticulum (ER) stress responses.[14] Wolframin is a hydrophobic and tetrameric protein with nine transmembrane segments and large hydrophilic regions at both termini. It is a resident of the ER, with an N_{cyt}/C_{lum} orientation in the ER membrane.[14]

Despite *WFS1* being ubiquitously expressed, differences between tissues with high (i.e., pancreatic β-cells and brain) and low (i.e., whole blood or kidney) expression are quite significant.[13]

WFS1 mutations can be demonstrated in 75–90% of patients meeting clinical criteria for WS.[15] In animal and cell models of WS, *WFS1* mutations lead to elevated ER stress levels, pancreatic β-cell dysfunction, and initiation of ER stress-associated cell death.[16]

WS1-causing *WFS1* mutations include missense, frameshifting, nonsense, and splice mutations and are predominantly located in exon 8.[7] Patients with WS1 are homozygous or compound heterozygotes, while heterozygotes have an increased risk of psychiatric hospitalization and an increased risk of DM and hearing loss.[7] It has been observed that severe WS1 phenotypes are associated with extensive intragenic deletions,[7] and that compound heterozygote for two missense mutations leads to a relatively mild phenotype.[7] However, a clear genotype–phenotype correlation has not been established.

Several years ago, a different phenotype was described in three large, consanguineous Jordanian families, including 16 Wolfram syndrome patients who had specific features in addition to those previously described in WS1. In all the affected members, there was absence of DI and psychiatric disorders. Several patients had profound upper gastrointestinal ulceration and bleeding, as well as defective platelet aggregation with collagen.[17,18] This different phenotype has been called WS2 and is caused by mutations of *CISD2* (CDGSH iron-sulfur domain-containing protein 2) gene on chromosome 4q24.[18,19] *CISD2* gene encodes for a highly conserved zinc-finger protein of the endoplasmic reticulum intermembrane small (ERIS), playing a pivotal role in calcium homeostasis (Ca2H).[19]

The first Italian case report of WS2 syndrome was reported in a girl with DM, optic neuropathy, intestinal ulcers, sensorineural hearing loss, and defective platelet aggregation to ADP. Genetic testing showed a novel homozygous intragenic deletion of *CISD2*.[20]

In our study, we performed clinical and molecular analyses of 44 WS1 Italian patients to evaluate the prevalence of WS1 in different geographic areas of Italy and its natural history. We also studied one WS1 patient from Morocco. We looked for a possible correlation between clinical features of our patients and *WFS1* mutations, and we estimated the progression rate of the syndrome according to different genotypes.

PATIENTS AND METHODS

Study design and methods

Prevalence rates of WS1 in Italy was calculated by evaluation of total Italian population (60,589,445).

Our study population consisted of 44 ethnically Italian patients and 1 Arab male (Morocco). They were recruited from the Pediatric Clinic of IRCCS G. Gaslini Institute of Genoa (Italy) and from the Pediatrics Department of Messina University Hospital (Italy). These are the only two Italian laboratories in which genetic diagnosis of WS1 is performed.

Once the first-degree relatives gave their consent, they were subjected to a detailed clinical assessment, including relevant biochemistry, to rule out any features of WS1. The patients were enrolled from 1998 to 2017.

In our patients, basic clinical criteria contributing to WS1 diagnosis were the coexistence of insulin-treated, juvenile-onset DM and OA occurring before 15 years of age.[6] All subjects were subjected to a complete family, medical, and neurological history.

In the patients, the following data were evaluated: age, glycated hemoglobin (HbA1c) concentrations and diabetic ketoacidosis (DKA) prevalence at DM diagnosis, honeymoon duration, and daily insulin requirements at DM onset. DKA was defined by blood glucose levels >250 mg/dl, pH < 7.3 and serum bicarbonate levels <15mEq/l. To define honeymoon duration, we used the criteria that are generally used for definition of partial remission in T1DM: HbA1c concentrations <7% and daily insulin requirement <0.5 IU/kg/day. HbA1c was measured by an immunoassay method with a monoclonal antibody directed against a sequence of the HbA1c.

Autoantibodies against pancreatic β-cells (anti-glutamic acid decarboxylase (GADA), anti-thyrosin phosphatase-like protein (IA-2A), anti-insulin (IAA)) were evaluated by radio-immunological assay. The analyses of HLA-DQA1 and -DQB1 genetic polymorphisms were performed by polymerase chain reaction (PCR)/sequence-specific primer technique.

All patients were subjected to a physical examination; complete neurological exam; visual acuity, refraction, assessment of nystagmus, color vision testing, pupillary testing and dilated fundus exams; audiology and vestibular exams; urologic evaluation; and neuroimaging.

DI was diagnosed according to the following clinical findings: polyuria, polydipsia, an osmolality of <300 mOsm per kilogram of water, or a specific gravity of <1010 in a 24-h urine sample without glycosuria and ketonuria.

Other clinical features were assessed by either a physician or a specialist.

Subjects and/or parents provided written informed consent.

Molecular analyses

DNA from probands, parents, and controls were extracted from whole blood using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany). Exons and flanking regions of *WFS1* were amplified by PCR using previously described primers.[21] Amplicons were purified with exonuclease I and shrimp alkaline phosphatase (ExoSap-IT, USB Corporation, Staufen, Germany) and then sequenced for both sense and antisense strands using an automated fluorescent sequencing method (Big Dye Terminator Kit v1.1, Applied Biosystems). The products were separated on an ABI PRISM sequencing apparatus 3730 (Applied Biosystems). All variations were validated 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 fulfill these criteria were considered polymorphisms.

Bioinformatics. All the identified *WFS1* variants were checked for novelty utilizing HGMD (http://www.hgmd.cf.ac.uk/ac/index.php), Exac (http://exac.broadinstitute.org/), EVS (http://evs.gs.washington.edu/EVS/), and dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/), and Varsome (https://varsome.com/).

Table 1. Statistics of age onset and frequency of the clinical features of our WS1 patients

Clinical features	Min. (years)	Median (years)	Mean (years)	Mode	Max. (years)	P95 (years)	SD (years)	Number of patients	% of patients ($n = 45$)
Diabetes mellitus	1	6	6.5	3	15	13.4	3.4	45	100%
Optic atrophy	5	11.5	12.3	11	29	22.2	4.6	45	100%
Hearing defects	8	13	13.1	10	45	22.9	8.0	26	57.7%
Diabetes insipidus	3	14	14.4	14	41	30	8.0	27	60%
Neuro-psychiatric symptoms	17	20.5	20.5	23	23	23	2.3	6+14	44.4%
Urological defects	5	14	13.2	n.e.	18	17.8	5.1	5+6	24.4%
Endocrinological defects	7	8	8	n.e.	9	8.9	1	2+1	6.6%
Congenital heart defect	9	10.5	10.5	n.e.	12	11.85	2.1	2	4.4%
Bilateral cataract	22	25.5	25.5	n.e.	29	28.6.	4.9	2	4.4%

n.e. not evaluable, *WS1* Wolfram syndrome 1

To better define the potential pathogenic role of mutations on *WFS1* functionality, several computational analyses were performed, such as Mutation Taster, Sift, and Polyhen2, which classified the variants as "*disease causing*", "*damaging*", and "*probably damaging*", respectively.

Genotype classification

The high genetic heterogeneity of *WFS1* complicates genotype–phenotype correlations in WS1 patients, and thus we subdivided the mutations into three groups according to the predicted functional consequences.[7] In Group 1, we included patients with nonsense and frameshift mutations and/or multiple amino acid insertion/deletions in both alleles. These mutations lead to complete depletion of wolframin. Group 2 consisted of patients with missense mutations and/or single amino acid insertions in both alleles. The functional consequences of these mutations are still unknown, but most of them could cause a milder degradation of the *WFS1* protein than the mutations in Group 1. Group 3 included patients with compound heterozygous mutations that were not found in Groups 1 and 2.

Furthermore, the patients were divided into two groups based on their geographical origin (coming from North and Central Italy, and from South Italy and Italian Islands) to verify a possible different geographical distribution of genotype groups.

Statistical analysis

Data analysis was performed using the STATA software (version 9.0; Stata Corp. LT, College Station, TX). A p value $< .05$ was considered as statistically significant. Categorical variables were analyzed by Chi-square test of contingency tables to evaluate a correlation between each genotypic group and age at onset of the clinical manifestations (DM, OA, D, DI). Ages of onset of each clinical feature were analyzed as a categorical variable by using the median value. Moreover, we analyzed the correlation between the genotypic groups and the type and number of clinical manifestations.

Follow-up was calculated from the date of first diagnosis to time of death (or last follow-up). Survival curves were illustrated using the Kaplan–Meier method, and the differences were compared using the log-rank test.

RESULTS

Clinical results

Our study group consisted of 20 males and 25 females. At the beginning of the study, they were aged between 12 and 51 years (median age 24 years). Forty-four WS1 patients were born, and were living, in different districts of Italy. One patient was from Morocco.

We found a WS1 prevalence of 0.74/1,000,000 in Italy. In our WS1 patients, DM was a hallmark manifestation and it usually appeared earliest among the other clinical manifestations of WS1. As shown in Table 1, recruited patients manifested the following clinical features: DM in 45 (100%), OA in 45 (100%), D in 26 (57.7%), DI in 27 patients (60%), neuro-psychiatric symptoms in 20 (44.4%), renal tract abnormalities in 11 (24.4%), endocrinological manifestations in 3 (6.6%), congenital heart defects in 2 patients (4.4%), and bilateral cataract in 2 patients (4.4%).

In the group of 20 patients with neuro-psychiatric symptoms, 7 patients were affected by neurological symptoms such as ataxia (4 patients), epilepsy (2 patients), and polyneuropathy (1 patient). Morphological abnormalities of the pituitary gland were found in 2 patients. Moreover, one patient was affected by a mild cognitive impairment. Finally, we found that 10/20 (50%) patients were affected by psychiatric disorders.

Endocrinological features were Hashimoto's thyroiditis in two patients and abnormalities in menstrual cycle in one patient.

Onset age of clinical features followed the subsequent pattern: DM during the first decade (39/45 patients, 86.7%), OA during the second decade (27/45 patients, 60%), and D during the second decade (12/26 patients, 46.1%) and the third decade (13/26 patients, 50%). DI presented more frequently during the second decade (16/27 patients, 59.2%) (Fig. 1). Unfortunately, we did not have the onset age of neuro-psychiatric symptoms, urological defects, and endocrinological manifestations (Fig. 1).

The pattern of onset of WS1 clinical features was not followed by all patients. In 3 patients (6.6%), OA was diagnosed prior to DM. In 1 patient (2.2%), DM and DI were diagnosed simultaneously at the age of 5.0 years. There was one patient in whom D and DM were diagnosed prior to OA. In six patients, D was diagnosed prior to OA.

Six subjects (13.3%) died, of whom 5 due to respiratory failure and 2 due to chronic renal failure. The patients died at a median age of 29.5 ± 21.6 years.

All four clinical characteristics of DIDMOAD were found in 21 patients (46.7%), while in the remaining 24 patients (53.3%) the clinical features of WS1 were variously associated.

We found 2 clinical features of WS1 in 7 patients (15.6%); 3 clinical manifestations in 10 (22.2%); 4 clinical features in 10 (22.2%), 5 clinical manifestations in 10 patients (22.2%); and 6 clinical manifestations in 7 patients (15.6%). One patient (2.2%) manifested a severe WS1 with 7 clinical characteristics (DIDMOAD, renal tract abnormalities, and neuro-psychiatric symptoms). Our WS1 patients presented a median of four clinical features.

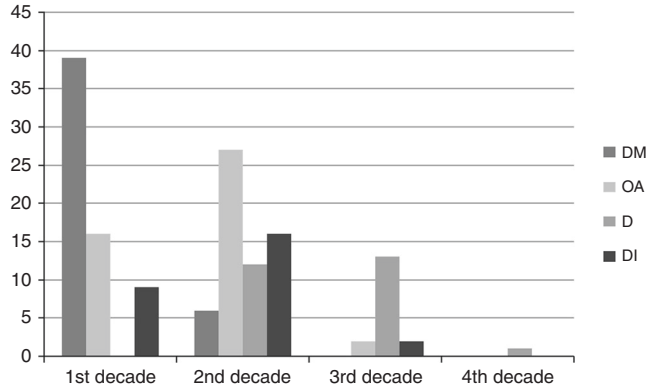

Fig. 1 Proportion of patients for each clinical feature at onset age

WFS1 mutations

In our study, we found a total of 35 different mutations in *WFS1*, which were all already reported in literature and linked to WS1 (Table 2)[21–36] except for a novel missense substitution, c.1523A>G (Tyr508Cys) located in exon 8. The mutational spectrum included missense, nonsense, frameshift, splicing, deletion, and duplication mutations. Two patients were heterozygotes (4.5%).

Most mutations were located in exon 8 (82.8%). Three mutations were situated in exon 4 (8.6%) and 3 in exon 5 (8.6%).

The most frequent mutation was a c.1362_1377del16 deletion (37.1%) localized in exon 8. The deletion c.1230_1233delCT in exon 8 was detected in 14.2% of the mutations. IVS6+16G>A and c.1381A>C mutations were found in 8.6% of the cases. Other mutations or deletions were present in <6% of patients. The missense change Tyr508Cys identified in a 42-year-old female (patient 12) was reported in Varsome data base (https://varsome.com/) as a very rare variant of "*uncertain significance*" with a population frequency of 1/110,514 (0.000009049) analyzed alleles. In patient 12, DM started at age 11 years, and OA appeared at 23 years. Moreover, she had hyporeflexia at four limbs and depressive syndrome.

Finally, in our study group, we found 2 heterozygote patients (no. 7 and no. 34).

Patient 7 was a 19-year-old female who had been suffering from D since the age of 1 year. Moreover, she was affected by DM and OA at the age of 14 years. This patient had also a mild cognitive impairment. In this WS1 case, we found anAla684Val missense mutation.

Patient 34 was a 21-year-old male who had been suffering from DM since the age of 7.4 years, from DI at the age of 8 years, and from OA at the age of 11 years. In this patient, we detected a Tyr528Stop nonsense mutation.

The Ala684Val and Tyr528Stop *WFS1* mutations were found in exon 8.

Genotypic groups and correlations between genotype–phenotype

To study whether different mutation types play a role in the WS1 phenotype, we classified the patients into genotypic classes as indicated in the "Patients and methods" section. We found a statistically significant difference between the distribution of genotypic groups in North and Central Italy and in Southern Italy and Italian Islands ($p = 0.05$). Genotypic Group 1 was particularly frequent in Sicily (Fig. 2).

We found 20 patients in Group 1 (47.7%), 6 in Group 2 (14.2%), and 16 in Group 3 (38.1%).

Comparison of the onset ages of each clinical feature (DM, OA, D, and DI) revealed significant differences for DM ($p = 0.05$), D ($p = 0.01$), and DI ($p = 0.02$) when we grouped the patients into three genotypic classes. In particular, the patients of Group 1 manifested DM, D, and DI earlier than those of Groups 2 and 3. No significant difference for OA was found between the three genotypic groups (Table 3).

The number of clinical features ranged from 2 to 7 in our patients. A statistically significant correlation was found between their median value (≤4 versus ≥5) and genotypic groups ($p < 0.05$). Indeed, Group 2 patients were significantly characterized by a lower number of clinical manifestations (≤4) compared to Groups 1 and 3. No significant differences were found between the three genotypic groups and the type of WS1 clinical features of our patients.

Figure 3 shows Kaplan–Meier survival plots in the three genotypic groups of WS1 patients. Five patients in Group 1 and 1 patient in Group 3 died of disease, whereas all Group 2 patients survived. The survival plots for three patient groups overlapped for approximately 15 years, and thereafter Group 1 plot diverged exhibiting a trend ($p = 0.06$) toward shorter survival time.

DISCUSSION

Wolfram syndrome is an uncommon hereditary recessive disorder characterized by multiple clinical manifestations with a variable presentation. To date, there are few studies on genotype–phenotype correlations.[7,31] To our knowledge, we have characterized the clinical and genetic data of a large number of Italian patients with WS1 for the first time. In our study, the prevalence of WS1 in the Italian population was 0.74/1,000,000, whereas it was 1:770,000 in the UK,[2] and 1:805,000 in North India.[37] The prevalence of WS1 here reported was related to the global Italian population without distinguishing the different geographical areas. In fact, in Sicily, WS1 prevalence was 3.7/1,000,000, which is higher compared to that of the entire Italian population. We excluded the possibility that it may be a result of selection bias. Instead, we suggest that the high prevalence of WS1 in Sicily could be due to ancestral consanguinity or due to different ancestral origins of the Sicilians.

Excluding DM and OA present in all our patients, clinical features such as D and DI were found in ~60% of patients. Neurological symptoms such as ataxia, epilepsy, and polyneuropathy were more frequent (44.4%) than nephrological complications (22.2%). Fifty percent of our WS1 patients with neurological manifestations were also affected by psychiatric disorders such as depression or obsessive–compulsive symptoms. In an analysis of data literature regarding 412 patients, De Heredia et al.[7] reported the following frequencies of WS1 clinical features: 98.21% for DM; 82.14 for OA; 48.21 for D; 37.76% for DI; 19.39% for urological manifestations; and 17.9% for neurological manifestations. In our study, the percentages of WS1 patients with D (57.7% versus 48.21%) and DI (60% versus 37.76%) were higher respect to those reported by De Heredia et al.[7] The high variability of clinical picture that characterizes WS1 includes other clinical manifestations. In our series, we also found patients affected by endocrinological symptoms (3 patients), congenital heart defects (2 patients), and also bilateral cataract (2 patients), which is an uncommon manifestation of WS1.[10] In our study, the picture of the natural history of Italian WS1 patients was similar to those previously described.[1,2,7] However, the proportion of our patients showing the four clinical features of DIDMOAD was 46.7%, slightly lower than the data reported by Barrett et al. in a population from the UK (53%),[2] and by Matsunaga et al. (49%),[38] but considerably lower than the data reported by Medlej et al. from Lebanon (58%).[39] A high percentage of consanguineous marriages over many generations characterized the 31 Lebanese studied WS1 patients, and two putative different *WFS1* mutations were only in 23.7% of the 17 Lebanese examined families. Different methods of recruitment of patients or different genetic factors could also explain the discrepancies between patients from Italy and those from Lebanon.

Table 2. Mutations and genotype groups of our WS1 patients

Family	Mutation group	Exon	Nucleotide changes	Amino acid change	Type of mutation	Zygosity	Reference	HGMD-Public accession number
1	3	8	c.1628T>G c.2104G>A	p.Leu543Arg p.Gly702Ser	Missense Missense	Compound heterozygote	Colosimo et al.[21] Gasparin et al.[22]	CM031400 CM112218
2	3	8	c.1060_1062delTTC c.2663C>A	p.Phe354fs p.Ser888*	Frameshift Nonsense	Compound heterozygote	Hardy et al.[23] Aloi et al.[24]	CD993077 CM120065
3	3	8	c.1620delGTG	p.Val540fs	Frameshift	Compound heterozygote	Colosimo et al.[21]	CD031551
4	3	8	c.1885C>T	p.Arg629Trp	Missense	Compound heterozygote	Kadayifci et al.[25]	CM014754
4, 10, 44	3, 3, 3	8	c.1230_1233delCTCT	p.Val412fs	Frameshift	Compound heterozygote	Gasparin et al.[22]	CD090458
20, 31	1, 1					Homozygote		
5	2	8	c.1582T>G	p.Tyr528Asp	Missense	Homozygote	Zalloua et al.[26]	CM087003
6	1	8	c.2106_2113del8	p.Val644fs	Frameshift	Homozygote	Zalloua et al.[26]	CD086068
7	—	8	c.2051C>T	p.Ala684Val	Missense	Heterozygote	Tessa et al.[27]	CM015844
45	3					Compound heterozygote		
8, 13	1	8	c.1456C>T	p.Gln486*	Nonsense	Homozygote	Colosimo et al.[21]	CM031396
9	3	4	c. 409_424dup16	p.Val142fs	Frameshift	Compound heterozygote	Gomez-Zaera et al.[28]	CI012867
10	3	8	c.1546delTTC	p.Phe516del	Deletion	Compound heterozygote	Colosimo et al.[21]	CD031550
11	3	8	c.2165_2188dup24 c.2504_2505insC	p.Met722_Trp730_dup p.Arg835fs	Duplication Frameshift	Compound heterozygote	Strom et al.[14] Colosimo et al.[21]	CN984518 CI031593
12	**2**	**8**	**c.1523A>G**	**p.Tyr508Cys**	**Missense**	**Homozygote**	**Novel (data unpublished)**	
14	3	8	c.1514G>A	p.Cys505Tyr	Missense	Compound heterozygote	Colosimo et al.[21]	CM031397
14	3	8	c.1620_1622delGTG	p.Trp540del	Deletion	Compound heterozygote	Colosimo et al.[21]	CD031551
38	3							
15	2	5	c.605A>G	p.Glu202Gly	Missense	Homozygote	Piccinno et al.[29]	nd
16	3	5 8	c.505G>A c.1332C>G	p.Glu169Lys p.Tyr444*	Missense Nonsense	Compound heterozygote	Hardy et al.[22] Pizzolanti et al.[30]	CM992979 CM1413605
17, 18, 22, 23, 24, 25, 26, 27, 28, 29, 36, 37	1	8	c.1362_1377del16	p.Tyr454fs	Frameshift	Homozygote	Colosimo et al.[21]	nd
44	3					Compound heterozygote		
19	2	8	c.1346C>T	p.Thr449Ile	Missense	Homozygote	Smith et al.[31]	CM043877
21, 30	3, 3	8	1328G>T	p.Ser443Ile	Missense	Compound heterozygote	Tessa et al.[27]	CM015195
21, 30, 45	3, 3, 3	IVS6	IVS6+16G>A	—	Splicing	Compound heterozygote	Tessa et al.[27]	CS013118
32, 33	2	4	c319G>C	p.Gly107Arg	Missense	Homozygote	Rigoli et al.[32]	CM136855
34	—	8	c.1584C>G	p.Tyr528*	Nonsense	Heterozygote	Colosimo et al.[21]	CM031398
35	3	8	c.1675G>A	p.Ala559Thr	Missense	Compound heterozygote	Furlong et al.[33]	CM993371
38	3	8	c.2020G>A	p.Gly674Arg	Missense	Compound heterozygote	Khanim et al.[34]	CM011519
3								
39	1	8	c.2099G>A	p.Trp700*	Nonsense	Homozygote	Giuliano et al.[35]	CM050357
40	1	8	c.873C>A	p.Tyr291*	Nonsense	Homozygote	Domenech et al.[36]	CM041101
9, 35	3, 3	8	c.1381A>C	p. Thr461Pro	Missense	Compound heterozygote	Aloi et al.[24]	CM120066
41	2					Homozygote		
42	3	5 8	c.532_537del6 c.1673G>A	p.Lys178_Ala179del p.Arg558His	Deletion Missense	Compound heterozygote	Colosimo et al.[21]	CD031549 CM031402
43	1	4	c.387G>A	p.Trp129*	Nonsense	Homozygote	Smith et al.[31]	CM043876

The genotype groups are indicated
Group 1: patients with nonsense and frameshift mutations and/or multiple amino acid insertion/deletions in both alleles
Group 2: patients with missense mutations and/or single amino acid insertions in both alleles
Group 3: patients with compound heterozygous mutations that have been found in Groups 1 and 2
Patients 7 and 34 have only one heterozygous mutation
New identified WFS1 mutation is presented in bold
nd: no data in "The Human Gene Mutation Database (HGMD)"
WS1 Wolfram syndrome 1

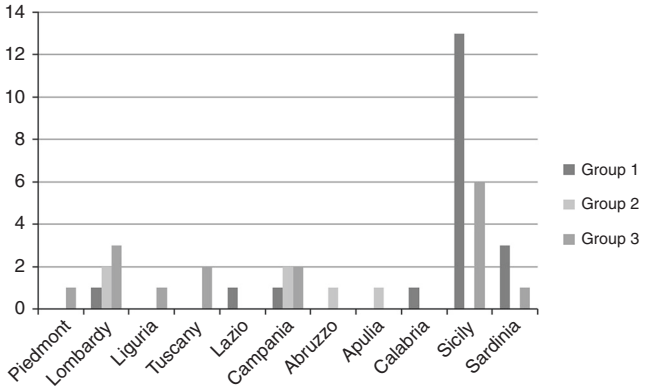

Fig. 2 Genotypic distribution of Wolfram syndrome 1 patients among Italian geographic areas

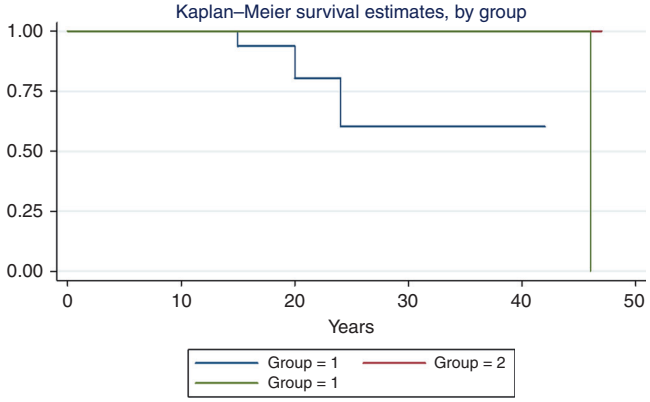

Fig. 3 Kaplan–Meier patient survival in the three study groups

Table 3. Statistics of the correlation between the genotypic groups and clinical features at onset age

Genotype	Min. (years)	Median (years)	Max. (years)	Number of patients	% patients	*p*
Group 1	2	4.5	10	20	47.7	
DM						
Group 2	3	7.5	11	6	14.2	
Group 3	4	11	16	16	38.1	0.05
Group 1	5	11.5	18	20	47.7	
OA						
Group 2	8	11.5	29	6	14.2	
Group 3	7	12	25	16	38.1	n.s.
Group 1	5	10	18	15	57.8	
D						
Group 2	10	15	16	3	1.5	
Group 3	3	14	45	8	30.7	0.01
Group 1	3	11.5	18	16	59.2	
DI						
Group 2	12	14	41	3	11.1	
Group 3	5	12.5	30	8	29.7	0.02

D deafness, *DI* diabetes insipidus, *DM* diabetes mellitus, *OA* optic atrophy, *n.s.* not significant

Median age onset of DM and OA of our patients was 6 years and 12.3 years, respectively, according to other studies.[6] In our study, the main differences are in the median age at onset of neuropsychiatric and nephrological features. Age at onset of neuropsychiatric manifestations was 20.5 years, which is higher than that of the French population (15 years of age),[35] and lower than that of patients from the UK.[2] Median age at onset for urological defects was 14 years, similar to the French population (12 years), but different from that reported by De Heredia et al.[7] We believe that genetic factors, probably not yet fully known, could explain the complex clinical picture of WS1. Recent studies have attempted to clarify the clinical aspects of this serious neurodegenerative disease searching for possible genotype–phenotype correlations.[7]

We also looked for genotype–phenotype correlations in Italian patients. Thirty-five different *WFS1* mutations were found mainly in exon 8 (82.8%). The c.1362_1377del16 deletion in exon 8 appeared to be very frequent in Italy (37.1%), especially in Sicily, while the other *WFS1* mutations had a frequency <14.2%. In Italian WS1 patients, recessive *WFS1* mutations were in homozygosis in a high percentage of cases (59.1%). In rare cases, single mutated alleles were seen (4.5%). The classification of mutations in three different groups according to the type of mutation suggests interesting results. We found a different distribution of genotypic groups in Italian geographic areas. In fact, Group 1 was significantly more frequent in Southern Italy/Italian Islands compared to North/Central Italy ($p = 0.05$).

Differences of median age onset of DM, D, and DI were found in the three genotypic groups. In particular, an earlier onset age of these clinical features was shown in Group 1 patients. Group 1 included nonsense and frameshift mutations and/or multiple amino acid insertion/deletions in both alleles that lead to a complete depletion of wolframin. Accordingly, Group 1 patients exhibited a trend toward shorter survival time. Moreover, we found a correlation between the genotype groups and the number of clinical pictures. Genotypic Group 2 showed a lower number of clinical manifestations of WS1. This could be due to a milder degradation of wolframin caused by missense mutations and/or single amino acid insertions in both alleles that identify Group 2. No correlations were detected between the type of manifestations and genotypic groups.

The prevalence, the clinical picture, and the genetic aspects of WS1 in Italy is not known and is limited to reporting of few cases.[3,21,24,27,29,30,32] Our study could help broaden the series of WS1 patients from different geographical areas. We believe that an expansion of the number of clinical and genetic studies could lead to the definition of genetic and clinical criteria that allow early diagnosis of the disease. This is very important as WS1 is a complex genetic neurodegenerative disease and many pathogenetic mechanisms remain elusive. Recently, it has been hypothesized that WS1 is a mitochondriopathy due to alterations of "mitochondria-associated ER membranes" (MAMs).[40] In particular, Del Prat et al. highlighted an interaction between *WFS1* and CISD2, a protein expressed in MAMs suggesting a role of mitochondrial abnormalities in the pathogenesis of neurologic features that characterize WS1.[40]

Recent advances in clinical and genetic research improve the knowledge of the syndrome. A multidisciplinary team managing patients with the aim of arriving at a prompt diagnosis and treatment of all clinical pictures of WS1 is mandatory.

AUTHOR CONTRIBUTIONS

L.R., G.D. and F.L. gave substantial contributions to conception and design of the study. C.A., A.S., C.D.B., G.S., R.C., E.M. and G.P. gave their contribution to acquisition of data or analysis and interpretation of data; L.R., R.C., G.D., F.L. and G.P. drafted the article and revised it critically for important intellectual content; L.R., G.D. and F.L. gave the final approval of the version to be published.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

Ethics: The research was conducted according to the Declaration of Helsinki and approved by IRCCS Giannina Gaslini Institute and University of Messina Ethical Committees.

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