Wolfram syndrome 1 and Wolfram syndrome 2: clinical implications for pediatricians

Luciana Rigoli and Chiara Di Bella

Purpose of review
Wolfram syndrome 1 (WS1) is an autosomal recessive disorder characterized by diabetes insipidus, diabetes mellitus, optic atrophy, and deafness (DI DM OA D syndrome) associated with other variable clinical manifestations. The causative gene for WS1 (WFS1) encoding wolframin maps to chromosome 4p16.1. Wolframin has an important function in maintaining the homeostasis of the endoplasmic reticulum (ER) in pancreatic β-cells. Recently, another causative gene, CISD2, has been identified in patients with a type of Wolfram syndrome (WS2) resulting in early optic atrophy, diabetes mellitus, deafness, decreased lifespan, but not diabetes insipidus. The CISD2-encoded protein, ERIS (endoplasmic reticulum intermembrane small protein) also localizes to ER, but does not interact directly with wolframin. ERIS maps to chromosome 4q22.

Recent findings
Numerous studies have shown an interesting similarity between WFS1 and CISD2 genes. Experimental studies demonstrated that the Cisd2 knockout (Cisd2−/−) mouse shows a premature aging and typical symptoms of Wolfram syndrome. These researches provide interesting insight into the relation of neurodegenerative diseases, mitochondrial disorders, and autophagy and are useful for the pathophysiological understanding of both Wolfram syndrome and mitochondrial-mediated premature aging.

Summary
The knowledge of WS1 and WS2 pathogenesis, and of the interactions between WFS1 and CISD2 genes is useful for accurate diagnostic classification and for diagnosis of presymptomatic individuals.

Keywords
CISD2 gene, WFS1 gene, WS1, WS2

INTRODUCTION
Diabetes mellitus is a group of common metabolic disorders defined by hyperglycemia. One of the most important factors contributing to hyperglycemia is dysfunction and death of pancreatic β cells that characterize the progression of type 1 and type 2 diabetes mellitus as well as genetic forms of diabetes such as Wolfram syndrome [1–3].

Gene linkage and positional cloning analysis reveal that a subset of Wolfram syndrome patients belonging to the ‘WS1’ group (MIM 606201) carry a loss-of-function mutation in WFS1 gene (Fig. 1), which encodes a transmembrane protein, wolframin, localized in endoplasmic reticulum (ER) [4,5] (Fig. 2).

Recently, another causative gene, CISD2, has been identified from the analysis of different families of patients with Wolfram syndrome, and these patients have been classified as belonging to the ‘WS2’ group (MIM 604928) [6].

In WS1 and WS2, numerous experimental, clinical, and genetic studies have suggested a key role of ER stress in β cells dysfunction and death.

The ER has an essential function in the protein-folding process for secretory proteins, such as insulin, as well as cell-surface receptors and integral membrane proteins. Imbalance in ER homeostasis elicits stress in this organelle, leading to the accumulation of misfolded and unfolded protein in the organelle, a state called ‘ER stress’. Activation of the unfolded protein response (UPR), an adaptive response that counteracts the ER stress, serves to
Endocrinology and metabolism

KEY POINTS

- In this review, we outline the molecular mechanisms of ER-stress-mediated β-cell dysfunction and death during the progression of diabetes in WS1, underlying the role of wolframin.
- We report the recent findings concerning new WFS1 mutations, describing the novel clinical features of WS1.
- We describe the recent studies concerning CISD2, causative gene of WS2 and the associated phenotypes, analyzing the most recent research on the functional expression of CISD2.
- We analyze some studies in which there are described clinical cases of WF2 with phenotypes similar to mitochondrial diseases.
- We would like to urge the pediatricians and pediatric endocrinologists to be aware of WS1 and WS2 in order to permit early diagnosis and therapeutic intervention for these diseases.

Epidemiology

The syndrome is very rare with a prevalence of 1 in 770,000 in the UK [11], of 1 in 100,000 in the North America [15], and of 1 in 500,000 in children [16]. Carrier frequency is 1 out of 354 patients [11]. The highest prevalence of 1 in 68,000 is in the Lebanese population probably because of high rates of consanguinity [17]. As insulin-dependent diabetes mellitus is the first manifestation of WS1, some studies examined the groups of type 1 diabetes mellitus patients of different ethnic origin to assess how many of them could have been affected by WS1. The prevalence of this syndrome in diabetes mellitus patients was different between the studies, because it was estimated to be 4.8% in the Lebanese population as against 0.57% in UK [11,17].

Cause and Genetics

Initially, WS1 was considered to have mitochondrial involvement [18–20]. Subsequent studies showed that the syndrome is caused by loss-of-function mutations in WFS1 gene, encoding wolframin, a transmembrane protein localizing in ER. WFS1 is localized to 4p16.1 and consists of eight exons, of which only the first exon is a noncoding exon [4,5]. Wolframin is abundantly expressed in pancreas, brain, heart, and muscle, with lesser amounts being present in liver and kidneys. In the pancreas, the protein is expressed mainly in β cells and is absent in the exocrine glands [21]. It is thought to be involved in the regulation of ER stress and calcium homeostasis, and wolframin deficiency in mice leads to progressive loss of β cells and impaired glucose tolerance, which is presumably caused by increased stress and apoptosis in β cells [22].

Genetics

Genetic analyses in WS1 identified a wide spectrum of mutations, mainly localized in the largest exon 8 [23]. Recently, new mutations and new associated phenotypes were identified.

Seven patients were described for the first time from North India. Two out of seven were born of...
consanguineous marriage. In this series, new clinical manifestations have been found that include spastic myoclonus, short stature with pancreatic malabsorption, nephrogenic diabetes insipidus, cyanotic heart disease, and choledocholithiasis with cholangitis [24].

Chaussenot et al. [25] studied 59 WS1 patients, identifying 56 different WFS1 mutations, of which 10 were novel. Most of the mutations were missense (73.2%). Interestingly, in this cohort of WS1 patients, the onset of neurological symptoms was much earlier than previously reported, and the cognitive impairment was found in a high percentage (32%) of patients with neurologic signs. The most common neurologic complication was cerebellar ataxia. In patients with neurological manifestations, at least one mutation has been found in the hydrophilic carboxy-tail of the protein (44.4 vs. 26.9%). This study, therefore, showed a possible correlation between the location of WFS1 mutations and the development of neurological complications.

In a study of 9 patients and 22 first-degree relatives from Polish population, 9 different mutations were found of which 5 were novel. Six patients, including two sibling pairs, were homozygous for WFS1 mutations, suggesting a possible consanguinity between the parents of the patients. Patients were relatively young at the initial manifestation of the first signs of WS1, likely attributable to the severe nature of their mutations [26].

Yuca et al. [27] described a large inbred Turkish family with seven WS1 patients. Three women with a new homozygous WFS1 mutation (c.1532T>C; p.Leu511Pro) were affected by a very rapid progression to renal failure before age 12. The p.Leu511Pro wolframin mutant showed highly decreased expression compared to wild-type wolframin.

Allmadadi et al. [28] studied seven Iranian WS1 patients. The Glu717Lys (E717K) missense mutation was found in all patients, suggesting that it would be a potential founder mutation of WS1 in the Iranian population.

In an Italian study, nine WS1 young patients from nine unrelated families were evaluated [29]. Fourteen distinct variants were identified, almost all localized to the predicted transmembrane protein domains (64.3%). Two mutations, Ser888-TER (S888X) and Thr416Pro (T461P), were novel.

Yu et al. [30] showed a new 1962G>A WFS1 mutation in a WS1 Chinese patient. The mutation was identified in exon 8 along with two other previously described nonmutations: 2422A>G and 2565G>A.

Mezghani et al. [31] reported a Tunisian patient with clinical features of Wolfram syndrome, including diabetes mellitus, dilated cardiomyopathy, and neurological complications. His brother died of typical WS1. In the patient, multiple deletions of mitochondrial DNA (mtDNA) and a point mutation (m.3337G>A) in the mitochondrial gene encoding subunit ND1 were present in an almost homoplasmic state. Therefore, the patient’s cardiomyopathy was due to a complex I deficiency which is a major cause of mitochondrial disease.

Lieber et al. [32] studied a patient suspected to have a mitochondrial disease. He was affected by diabetes mellitus, diffuse brain atrophy, autonomic neuropathy, optic atrophy, and a severe amnestic syndrome. In addition to a previously reported Arg558Cys (p.R558C) WFS1 missense mutation [23*], multiple heteroplasmic mtDNA deletions...
Endocrinology and metabolism

and severe thiamine deficiency without nutritional causes were found. The finding of unexplained thiamine deficiency in a WS1 patient supports the hypothesis that there is a link between WFS1 and thiamine metabolism.

CLINICAL FEATURES OF WS1

WS1 is a progressive, neurodegenerative disorder. Diabetes mellitus and optic atrophy usually help make the diagnosis in the pediatric age group [11, 12].

Diabetes mellitus, a nearly constant finding, is the first manifestation to occur. Nonautoimmune and non-HLA-linked diabetes mellitus presents at an average age of 6 years (range 3 weeks to 16 years) and is characterized by rare microvascular complications that seem to develop more slowly than in the more common type 1 diabetes mellitus [14]. A decreased tendency of these patients to develop ketoacidosis has been reported [33]. Almost all patients require insulin replacement. When compared with age-matched and control-matched patients with type 1 diabetes mellitus, WS1 patients had lower daily insulin requirement and lower hemoglobin A1c (HbA1c) values, probably because of a persistence of some residual pancreatic β-cell activity [33].

Optic atrophy is required for the diagnosis of WS1 [11]. Because wolframin is expressed in the retinal ganglion cells, photoreceptors, and in glial cells in the proximal portion of the optic nerve, functional alterations of wolframin in these cell populations may explain the progressive optic atrophy [34].

Optic atrophy presents at an average age of 11 years (range 6 weeks to 19 years), with reduced visual acuity and loss of color vision. It is progressive, leading to vision of 6 of 60 or less in the better eye over an average of 8 years after initial diagnosis. Most patients go blind. Other less frequent ocular abnormalities reported are cataract (29.6–66.6%), pigmentary retinopathy (30%), diabetic retinopathy (7.6–34.6%), abnormal papillary light reflexes, nystagmus, pigmentary maculopathy, and glaucoma [35]. Often, optic atrophy is found during a screening for type 1 diabetes mellitus and may be an early clinical sign of WS1.

At an average age of 14 years (range 3 months to 40 years), 51–87% of patients present with partial central diabetes insipidus and respond well to intranasal or oral desmopressin. Neuroradiological and postmortem reports showed gliosis and atrophy of the hypothalamic paraventricular and supraoptic nuclei [36]. Other studies showed functional defects of these nuclei [37].

Slowly progressive high-frequency deafness is a feature seen in 62% of patients, developing at an average age of 16 years (range 5–39 years) [11], probably induced by dysfunctional neurons in the auditory-related structures of the brain [38].

Neurological abnormalities are present in 62% of the patients (mean age 30 years, range 5–44 years), probably because of loss of expression of WFS1 gene. The most common symptom is the ataxia of the trunk [11]. Other common neurological signs are loss of gag reflex, loss of olfaction, myoclonus, epilepsy, and nystagmus. Central apnea is a common cause of mortality.

Magnetic resonance imaging scans demonstrate generalized brain atrophy, especially in the cerebellum, medulla, and pons; absence of signal from the posterior pituitary; and reduced signal from optic nerve [36].

Frequently, WS1 patients (60%) are affected by episodes of severe depression, psychosis, or organic brain syndrome, as well as impulsive verbal and physical aggression. Presumed carriers of WFS1 mutations have been reported as being predisposed to psychiatric illness [13].

Primary hypogonadism (hypergonadotrophic or hypogonadotrophic) has been reported frequently in male patients [11, 17, 39]. In women, ovarian function is normal with only abnormalities in menstruation. Women usually retain their ability to become pregnant [17]. Short stature is commonly observed in WS1 patients, and growth hormone (GH) deficiency has been reported in these patients [17].

Medlej et al. [17] demonstrated pituitary hypofunction of probable hypothalamic origin in 15 of 20 WS1 patients. A deficient GH secretion was the most common abnormality (9 of 20 patients) followed by deficient corticotrophin secretion (4 of 20 patients). These data suggest that careful monitoring of WS1 patients for a possible severe growth retardation which could respond to GH administration is indicated. The requirement for steroid supplementation in these patients during periods of stress such as severe infection needs to be considered.

Hydroureter, urinary incontinence, and recurrent infections are common signs of neurogenic bladder. Fifty-five percentage of twenty-nine index patients had such signs with median age of onset of 22 years (range 10–44 years) [11]. Urodynamic examinations showed incomplete bladder emptying or complete bladder atony.

Gastrointestinal tract involvement is an unrecognized and often neglected facet of WS1. Bowel dysmotility (24%) [11], symptoms of gastroparesis (29%) [17], and bowel incontinence because of sphincter weakness [40] have been found.
Some studies reported a high number of WS1 patients affected by congenital heart diseases such as pulmonary stenosis, ventricular septal defect, and tetralogy of Fallot [17].

**DIAGNOSIS**

The proportion of patients affected by all the four clinical signs (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness) varies between 14 and 58% [11]. The combination of diabetes mellitus and optic atrophy has a positive predictive and a negative predictive value of 83 and 1%, respectively [11]. Therefore, a careful assessment of the patients’ siblings should be recommended because some features of the syndrome may be asymptomatic. Genetic studies also include prenatal diagnosis [41] and diagnosis in presymptomatic patients. Prenatal diagnosis for pregnancies at increased risk is only possible if the disease-causing mutations in the family are known.

**CISD2 GENE AND WS2**

WS2 (MIM 604928), diagnosed in three large, consanguineous Jordanian families and caused by mutations in CISD2 on chromosome 4q22, is characterized by juvenile-onset diabetes mellitus, optic atrophy, deafness, urinary tract dilatation, impaired renal function, hypogonadism, and severe gastrointestinal ulcer and bleeding, but not diabetes insipidus [6,42,43]. In one family, the facial features were abnormal [6]. An extensive phenotypic analysis showed additional symptoms in WS2 patients, such as significant bleeding tendency, as well as a defective platelet aggregation with collagen [42], which has not been previously described in WS1 families. The disorder is very rare and may be confined to a certain ethnic background.

Amr et al. [6] identified a single missense mutation in CISD2 gene (synonym ZCD2, Noxp70, Miner1, ERIS). A G-to-C transversion at nucleotide 109 predicted an amino acid change from glutamic acid to glutamine (E37Q) that disrupted the mRNA splicing by eliminating exon 2 and resulted in 15.3 kDa and contains a predicted transmembrane domain and a C-terminal CDGSH domain. It plays a role in calcium homeostasis [44], as well as wolframin. Lymphoblastoid cells from individuals with WS2 showed a significantly greater rise in intracellular calcium when stimulated with thapsigargin compared with controls, although no difference was observed in the resting concentrations of intracellular calcium [6]. Therefore, probably, both genes, WFS1 and CISD2, could reside in the same pathway, although CISD2 does not interact directly with wolframin. Probably, each gene has a specific mechanism that leads to nerve and pancreatic degeneration.

CISD2 is expressed in numerous tissues, including brain and pancreas [45**,46,47]. In adult mouse brain, CISD2 is expressed in a wide variety of structures, showing a substantial overlap of expression with Wfs1. These brain regions primarily control memory, emotions, and motor skills and probably the neurodegeneration in WS1 is correlated with the spatial expression of CISD2 [48].

Chen et al. [45**] suggested that CISD2 is involved in mammalian lifespan control. In mice, Cisd2 was primarily localized to the mitochondria and associated with the outer mitochondrial membrane. Cisd2-null mice showed early senescence and shortened lifespan compared to wild-type mice. The phenotype was characterized by prominent eyes, protruding ears, corneal opacities and degeneration, thinner bones and hair, and decreased muscle mass, all of which were consistent with premature aging. Tissue from mutant mice showed progressive mitochondrial breakdown and dysfunction accompanied by autophagic cell death, which precede nerve and muscle degeneration. It leads to a panel of phenotypic features suggestive of premature aging and associated with some features of Wolfram syndrome (early onset degeneration of the optic nerve and peripheral nerves, as well as impaired glucose tolerance) [46]. This experimental study clarifies the pathogenesis of WS2, establishing this disease as a mitochondrial-mediated disorder linked to an ER-related disorder. Interestingly, CISD2 maps to chromosome 4q22-q24, close to a region implicated in human longevity [47].

**CONCLUSION**

Wolfram syndrome is a rare genetic cause of diabetes mellitus. Early diagnosis is imperative in order to recognize treatable complications (and hence to reduce morbidity and mortality) and to prevent this disease in further progeny by genetic counseling. Routine ophthalmoscopic evaluation should be performed in all patients at the time of diagnosis of diabetes mellitus. The pediatricians and pediatric endocrinologists should suspect Wolfram syndrome in patients with nonautoimmune type 1 diabetes mellitus, history of early siblings’ death, family history of Wolfram syndrome or type 1 diabetes mellitus and deafness, and history of parental consanguinity. It must be stressed that most
Endocrinology and metabolism

complications of the Wolfram syndrome probably remain asymptomatic for a long time. The patients therefore should undergo routine screening.

A key role is played by the genetic mutation analysis for screening of Wolfram syndrome patients, perhaps enabling the development of novel therapeutic approaches based on the developing knowledge of gene and protein function. Genetic analysis is also available for prenatal diagnosis and for a diagnosis in asymptomatic individuals [41].

Acknowledgements

None.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

9. This study describes the role of ER stress in j cells dysfunction and death. This study is important because it summarizes the recent findings about the mechanisms of j cell death during diabetes.


Dear Author,

During the preparation of your manuscript for typesetting, some queries have arisen. These are listed below. Please check your typeset proof carefully and mark any corrections in the margin as neatly as possible or compile them as a separate list. This form should then be returned with your marked proof/list of corrections to the Production Editor.

### QUERIES: to be answered by AUTHOR/EDITOR

<table>
<thead>
<tr>
<th>QUERY NO.</th>
<th>QUERY DETAILS</th>
<th>RESPONSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;AQ1&gt;</td>
<td>As per style, the short title/running head can have a maximum of 65 characters including spaces and author names, and abbreviations/acronyms only as exceptions. Please check the suggested short title, “Wolfram syndrome 1 and Wolfram syndrome 2”.</td>
<td></td>
</tr>
<tr>
<td>&lt;AQ2&gt;</td>
<td>Please check the telephone and fax number for correctness.</td>
<td></td>
</tr>
<tr>
<td>&lt;AQ3&gt;</td>
<td>Ref. [14] was identical to Ref. [33]. Hence, Ref. [33], have been deleted from the bibliographic list and from the text as per house style, and subsequent references have been renumbered in the text and in the bibliographic list. Please check.</td>
<td></td>
</tr>
</tbody>
</table>