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Autoimmune Disease in a DFNA6/14/38 Family carrying a Novel Missense Mutation in *WFS1*

Michael S. Hildebrand¹, Jessica L. Sorensen¹, Maren Jensen², William J. Kimberling², and Richard J.H. Smith^{1,3}

¹Department of Otolaryngology - Head and Neck Surgery, University of Iowa, Iowa City, IA, USA

²Department of Genetics, Boys Town National Research Hospital, Omaha, NE, USA

³Interdepartmental PhD Program in Genetics, Department of Otolaryngology, University of Iowa, Iowa City, Iowa City, IA, USA

Abstract

Most familial cases of autosomal dominant low frequency sensorineural hearing loss (LFSNHL) are attributable to mutations in the Wolfram syndrome 1 (*WFS1*) gene at the DFNA6/14/38 locus. *WFS1* mutations at this locus were first described in 2001 in six families segregating LFSNHL that was non-progressive below 2000 Hz; the causative mutations all clustered in the C-terminal domain of the wolframin protein. Mutations in *WFS1* also cause Wolfram syndrome (WS), an autosomal recessive neurodegenerative disorder defined by diabetes mellitus, optic atrophy and often deafness, while numerous single nucleotide polymorphisms (SNPs) in *WFS1* have been associated with increased risk for diabetes mellitus, psychiatric illnesses and Parkinson's disease.

This study was conducted in an American family segregating autosomal dominant LFSNHL. Two hearing impaired family members also had autoimmune diseases - Graves disease (GD) and Crohn's disease (CD). Based on the low frequency audioprofile, mutation screening of *WFS1* was completed and a novel missense mutation (c.2576G→A) that results in an arginine-to-glutamine substitution (p.R859Q) was identified in the C-terminal domain of the wolframin protein where most LFSNHL-causing mutations cluster. The family member with GD also carried polymorphisms in *WFS1* that have been associated with other autoimmune diseases.

Keywords

Crohn's disease; DFNA6/14/38; Graves disease; Missense mutation; *WFS1*

INTRODUCTION

The Wolfram syndrome 1 gene (*WFS1*) was originally implicated in human disease as the cause of Wolfram syndrome (WS), an autosomal recessive, progressive neurodegenerative disorder defined by diabetes mellitus, optic atrophy and frequently sensorineural deafness and psychiatric disorders [Inoue et al., 1998; Strom et al., 1998; Swift and Swift 2000]. The morphological characteristics of this syndrome are atrophy and cell death within the endocrine system and neural networks [Hofmann et al., 2003]. Later, *WFS1* was implicated in autosomal dominant, non-syndromic hearing loss at the DFNA6/14 locus in six families segregating delayed-onset, slowly progressive LFSNHL [Bespalova et al., 2001]. These families all carried

missense mutations in a region of exon 8 that encodes the C-terminal domain of the wolframin protein [Bespalova et al., 2001]. The hearing loss in some of these families was associated with tinnitus [Bespalova et al., 2001].

Of the original LFSNHL-causing *WFS1* mutations, one — V779M — was identified in 1/336 control individuals [Bespalova et al., 2001]. This frequency is comparable to that of heterozygous carriers of WS, which is estimated to be 0.3-1% [Ohata et al., 1998]. Since an increased risk of sensorineural hearing loss had been reported for these carriers [Ohata et al., 1998], it was predicted that *WFS1* is a common cause of LFSNHL. This assumption has been verified by the discovery of *WFS1* mutations in the majority of families segregating LFSNHL in different ethnic groups [Cryns et al., 2002; Fukuoka et al., 2007; Hansen et al., 2005]. To date, at least 26 different missense mutations causing non-syndromic LFSNHL and 110 mutations responsible for WS have been identified in *WFS1* [Fukuoka et al., 2007; Khanim et al., 2001]. The former are non-inactivating missense mutations predominantly located within the C-terminal domain [Cryns et al., 2003a], while the latter are inactivating mutations that do not cluster in any particular region of the protein [Cryns et al., 2003a]. This genotype-phenotype association suggests that WS is the result of loss of function while non-syndromic LFSNHL is due to a dominant-negative effect.

The wolframin protein is known to contain nine putative transmembrane domains and its function and role in causing diabetes mellitus in WS has been partially elucidated. There is evidence that wolframin functions as a calcium channel in the endoplasmic reticulum (ER) [Osman et al., 2003] and is crucial for maintaining homeostasis of the ER in pancreatic β -cells [Fonseca et al., 2005]. This role was confirmed by generation and analysis of *WFS1*-null mice that were shown to have progressive β -cell loss and impaired stimulus-secretion coupling in insulin secretion [Ishihara et al., 2004]. However, the function of wolframin in the inner ear and the mechanisms by which missense mutations cause non-syndromic LFSNHL have not been extensively explored [Takeda et al., 2001]. The expression of *WFS1* has been localized in the mouse cochlea at different developmental stages and is widely distributed in different cochlear cell types, including inner and outer hair cells, a variety of supporting cells, and cells of the lateral wall, spiral ganglion and vestibule [Cryns et al., 2003b]. *WFS1* localizes to the ER and similarity is observed between *WFS1* expression and the presence of the canalicular reticulum (CR), a specialized form of ER believed involved in transcellular ion transport [Cryns et al., 2003b]. Thus wolframin may be involved in regulation of inner ear ion homeostasis as maintained by the CR [Cryns et al., 2003b].

The present study was conducted to determine the genetic basis of the novel phenotypic syndromic presentation of LFSNHL that included GD and CD in an American family. The LFSNHL was fully penetrant while GD (n=1) and CD (n=1) were present in only a subset of hearing-impaired individuals. The *WFS1* gene was screened based on the low frequency audioprofile and reported tinnitus, and a novel missense mutation was detected that affects the C-terminal domain of the wolframin protein. Polymorphisms in the *WFS1* gene were also evaluated that potentially confer susceptibility to autoimmune diseases.

MATERIALS AND METHODS

Family Report

All procedures were approved by the Institutional Review Board at the University of Iowa and Boys Town National Research Hospital. A five-generation Wisconsin family that presented with low frequency sensorineural hearing loss (LFSNHL), autoimmune diseases and Parkinson's disease was studied (Fig. 1). Individuals I:1 and I:2 were both reported to have hearing loss, however one of them is likely to represent a phenocopy. The lack of phenotypic information and DNA from these individuals precluded further characterization of their disease

status. The self-reported age of onset of hearing loss ranged from 2 to 45 years and was non-progressive, with a general trend of mild-to-moderate low and mid frequency (< 2000 Hz) hearing loss (Fig.2). In younger affected persons, the audioprofile was up-sloping preserving hearing in the high frequencies; with advancing age, high frequency loss developed and the audioprofile became flattened.

Individual IV:4 is typical of the affected family members and her clinical presentation is described here. Otoscopy revealed clear ear canals and tympanometry indicated normal middle ear pressure and compliance. Acoustic reflex thresholds were normal and acoustic reflex decay was absent, consistent with a cochlear hearing loss in both ears. Pure tone tests revealed a bilateral mild-to-moderate hearing loss in the low and mid frequencies with normal hearing sensitivity above 2000 Hz. Speech reception thresholds were in agreement with pure tone averages, indicating good test reliability. Auditory word recognition scores were excellent with 96% for the right and 92% for the left ear. Sudden onset atypical tinnitus was reported in the right ear at age 29. The tinnitus was described as having a 'rattling quality' and being like a 'constant roar that sounds like the ocean'. The tinnitus was accompanied by an apparent deterioration in hearing sensitivity.

To assist with sound amplification individual IV:4 was fitted with a Qualitone behind-the-ear hearing aid and reported improved speech and word recognition in the quiet. In the presence of competing background noise scores were reduced to 72%. Although she also had dizzy spells, vestibular function testing was not done. Two other affected family members (II:2 and II:4) also reported balance problems, although the balance problems of II:4 may be related to his Parkinson's disease. No family member has received a cochlear implant as the hearing loss is not severe enough to warrant such intervention.

In addition to the described otologic features, two affected family members have autoimmune diseases. III:1 was diagnosed with GD and IV:2 with CD, meaning autoimmune diseases are present in two generations of this family. One hearing impaired male (II:4) has Parkinson's disease. No person in this family has been diagnosed with WS.

Genetic Analyses

The coding exons (2-8) of the *WFS1* gene were amplified using gene-specific primers and a standard PCR protocol (Table SI, supplementary material). Amplicons from exons 2-7 were pooled post-PCR and heteroduplexes were formed by denaturing at 95°C for 5 min in a thermal cycler and cooling at a rate of 1°C/min to room temperature. DHPLC analysis of each amplicon was performed at three different temperatures. The analysis was conducted using Navigator™ Software (Transgenomic™, Omaha, NE) to estimate optimal temperature, run time and acetonitrile gradient. The best predicted temperature was then bracketed by $\pm 2^\circ\text{C}$ to optimize sensitivity. Sequencing of any variants and exon 8 amplicons was completed using a BigDye™ v3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA) and sequencing products were read using an ABI 3730s Sequencer (Perkin Elmer, MA, USA). Sequencing chromatograms were compared to published cDNA sequence using Sequencher v4.5 (Gene Code Corp, MI, USA).

RESULTS

Genetic Screening Revealed a Novel *WFS1* Mutation

Based on the characteristic low frequency (< 2000 Hz) audioprofile of all affected family members and reported tinnitus in at least one affected family member (IV:4), *WFS1* was considered an excellent candidate for causing the SNHL in this family. Direct sequencing of *WFS1* showed that all available affected family members (II:2, III:1, III:3, IV:2, IV:4, V:2)

except II:4 carried a novel missense G-to-A nucleotide transposition (c.2576G→A) in exon 8 that results in substitution of an arginine residue for a glutamine residue at position 859 (R859Q) (Fig.3A). Consistent with the segregation of this variant with the hearing loss, unaffected family member IV:6 and unrelated spouse IV:5 did not carry this variant. Both affected individuals with diagnosed autoimmune diseases (III:1 and IV:2) also carried this variant, although the hearing impaired person (II:4) with Parkinson's disease did not. A screen of 177 CEPH control individuals (354 chromosomes) was negative for the c.2576G>A (p.R859Q) mutation.

Further confirmation of this disease-causing mutation was demonstrated by constructing a multisequence alignment of the wolframin (WFS1) protein (Fig.3B). This analysis revealed that R859 is conserved between human and mouse WFS1 protein and therefore is likely to be important for the structure and/or function of the protein. In addition, a missense mutation affecting the same residue (p.R859P) has previously been reported to cause autosomal dominant LFSNHL in a family from Utah (Fig.3C) [Gurtler et al., 2005]. In this case the mutation substituted R859 for a proline residue that was thought to disrupt helical chains in the C-terminal domain of the protein [Gurtler et al., 2005].

Polymorphisms of *WFS1* in the American Family

Of the numerous polymorphisms identified in *WFS1*, a significant number have been associated with diabetes mellitus, psychiatric disorders and Parkinson's disease [Awata et al., 2000; Cryns et al., 2003a; Domenech et al., 2002; Inoue et al., 1998; Minton et al., 2002; Sequeira et al., 2003; Shadrina et al., 2006; Strom et al., 1998; Swift and Swift 2000; Swift and Swift 2005; Swift et al., 1991; Swift et al., 1998; Torres et al., 2001]. We analyzed all available members of this family for polymorphisms in exon 8 of *WFS1* and identified six (I333V, V395V, N500N, H611R, K811K, S855S) (Table II). I333V, V395V, N500N and H611R were shared in heterozygous or homozygous state by all affected individuals and were also present in at least one normal hearing family member. Four of these polymorphisms, V395V, H611R, K811K and S855S, have been associated with diabetes mellitus [Awata et al., 2000; Domenech et al., 2002; Minton et al., 2002; Sequeira et al., 2003], and homozygosity for the H611R variant (611R/611R) has been associated with psychiatric disorders [Sequeira et al., 2003]. All four of these variants have also been reported in control individuals [Domenech et al., 2002; Sequeira et al., 2003]. The two family members with LFSNHL and autoimmune disease (III:1 and IV:2) carry at least one copy of the c.1185T→C (p.V395V) and c.1832A→G (p.H611R) variants (Table II). The c.1645C→T (L549L) variant was not found in any person in this family, including II:4 who has Parkinson's disease.

DISCUSSION

The clinical spectrum in WS has fueled interest in the large number of polymorphisms that have been identified in *WFS1*. To date, over 70 synonymous and non-synonymous variants in the coding region have been reported, most of which are likely to be benign polymorphisms as they have been detected in control populations (reviewed in [Cryns et al., 2003a]). However, it is difficult to definitively exclude pathogenicity since synonymous variants may alter splice enhancer sites and non-synonymous variants (K193Q, L432V, L499F, G576S, A559T, A684V, R708C, R818C, D866N, V871M) may impact protein function [Cryns et al., 2003a]. Indeed, *WFS1* polymorphisms have been associated with diabetes mellitus, psychiatric illnesses and Parkinson's disease [Awata et al., 2000; Cryns et al., 2003a; Inoue et al., 1998; Minton et al., 2002; Sequeira et al., 2003; Shadrina et al., 2006; Strom et al., 1998; Swift and Swift 2000; Swift and Swift 2005; Swift et al., 1991; Swift et al., 1998; Torres et al., 2001]. Awata and colleagues demonstrated that *WFS1* allele variants R456H, H611R and I720V are significantly correlated with type 1 diabetes in a case-control association study in a Japanese

population [Awata et al., 2000]. A separate study in a Spanish population identified four *WFS1* variants (V395V, H611R, K811K, S855S) associated with diabetes mellitus and deafness [Domenech et al., 2002]. These data combined with that from other studies (reviewed in [Cryns et al., 2003a]) suggest that *WFS1* may be an important susceptibility gene for diabetes mellitus.

A strong correlation between *WFS1* variants and a variety of psychiatric illnesses has also been demonstrated [Swift and Swift 2000; Swift and Swift 2005; Swift et al., 1991; Swift et al., 1998; Swift et al., 1990]. A study of obligate carriers of specific *WFS1* variants from 36 families showed that they suffered a higher proportion of psychiatric illness as compared to their spouses [Swift et al., 1991], and a subsequent study has reported that heterozygous carriers of *WFS1* disease-causing mutations are 26-fold more likely to require psychiatric hospitalization than non-carriers [Swift et al., 1998]. A number of *WFS1* polymorphisms have since been associated with psychiatric illness, including a significantly higher frequency of individuals homozygous for the H611R variant amongst suicide completers [Sequeira et al., 2003]. Some affected members of the American family described here carry the H611R variant. However, none of these family members have reported psychiatric illness.

Recently, a *WFS1* polymorphism has been associated with Parkinson's disease (PD) in the Russian population [Shadrina et al., 2006]. Investigation of a cohort of PD patients and controls revealed that individuals carrying the c.1645C→T (L459L) SNP in *WFS1* were at higher risk of developing PD [Shadrina et al., 2006]. Wolfram syndrome is a neurodegenerative disorder associated with optic atrophy and sensorineural deafness, and it is therefore likely to involve selective degeneration of different neuron populations, suggesting that alterations in *WFS1* could also be associated with degeneration of dopaminergic neurons in the pathogenesis of PD [Hofmann et al., 2003]. However, family member (II:4) who was diagnosed with Parkinson's disease was not found to carry the c.1645C→T variant.

The major clinical manifestation in GD is overproduction of thyroid hormone (hyperthyroidism) that leads to goiter and in many cases, extrathyroid pathologies including myxedema and orbitopathy [Davies 2000; Fung et al., 2003]. Genetic susceptibility to autoimmunity triggered by unknown environmental factors is considered the etiology for this disease [Philippou and McGregor 1998; Tomer and Davies 1993]. Antibody and cell-mediated autoimmunity are involved in the pathogenesis of GD. Activated thyroid-specific T-cells infiltrate the thyroid gland where they activate B-cells to produce thyroid stimulating hormone receptor (TSHr) antibodies [Kim and Hatton 2008]. This activation cascade induces hyperproliferation of thyrocytes and oversecretion of thyroid hormone.

There is significant evidence for a major genetic component in the etiology of GD. (1) A positive family history is the single most important risk factor, implying hereditary transmission and/or a shared environment [Brix et al., 1998a; Brix et al., 2001; Brix et al., 1998b; Philippou and McGregor 1998]. (2) Pooled data from a series of twin studies in the Danish population indicates that 79% of the liability for the development of GD is attributable to genetic factors [Brix et al., 2001]; the remaining 21% is due to specific environmental factors not shared by the twins. Non-twin siblings of individuals with GD are also at increased risk of developing the disease, with the relative risk 5-10% for females and 0.9-4% for males, compared to approximately 1% for the control population [Brix et al., 1998b].

The presence of hyperthyroidism in one hearing-impaired family member (III:1) and CD in another (IV:2) is interesting in light of the reported links between *WFS1* mutations and type 1 diabetes, which is likely to have an autoimmune etiology [Awata et al., 2000; Cryns et al., 2003b]. Individual IV:2 is the daughter of III:1 and it is noteworthy that her affected sisters (IV:1 and IV:4) and affected nephew (V:2) are not reportedly symptomatic for GD or CD. Thus

autoimmune diseases are present in two generations of this family. Supporting for a possible role for wolframin in thyroid and gastrointestinal function is the presence of one *WFS1* expressed sequence tag (EST) in an adult human thyroid gland cDNA library (GenBank ID: BF830038) and at least 11 ESTs in human intestinal tissue, including from the colonic mucosa of patients with CD (GenBank ID: AI991248.1).

The novel *WFS1* mutation that causes the LFSNHL in this family affects the same residue of wolframin that has been implicated in LFSNHL in a Utah family (p.R859P) [Gurtler et al., 2005]. It is worth speculating what changes to protein structure may be conferred by substituting the positively charged R859 residue for either the nonpolar sidechain of a proline residue (R859P, the Utah family) or an uncharged, polar glutamine sidechain (R859Q, this family). Substitution with proline is predicted to alter the secondary structure of the wolframin protein by introducing a bending point in the amino acid chain and disrupting helical chains [Gurtler et al., 2005]. In both cases the loss of positively charged arginine may interfere with the ability of the C-terminal domain to interact with other proteins in the ER lumen. A recent yeast two-hybrid screen using a human brain cDNA library has shown that the C-terminal (amino acids 652-890) and transmembrane domains (amino acids 322-652) of wolframin interact with the Na⁺/K⁺ ATPase β 1 subunit [Zatyka et al., 2008]. Disruption of this interaction was speculated to lead to reduction in α 1 and β 1 sodium pump subunit expression in pancreatic β -cells and the diabetes mellitus pathology in WS [Zatyka et al., 2008]. Since ion transport including potassium ion recycling is crucial for auditory function, disruption of the *WFS1*-Na⁺/K⁺ ATPase β 1 interaction in the inner ear may also contribute to the hearing loss seen in WS and LFSNHL families with *WFS1* mutations. It is possible that the R859 residue substituted in this family is involved in this interaction since it is located in the C-terminal domain.

Individual II:4 is a phenocopy since his audioprofile is distinct from that of other members of the family. His hearing loss is asymmetric and his low frequency hearing is preserved. Furthermore, his hearing loss was not considered bothersome until age 74 years, when he had his first audiogram.

In summary, we have identified a novel mutation, p.R859Q, in *WFS1* as the cause of dominantly inherited LFSNHL in an American family. We have also noted the possible association of this mutation and other *WFS1* polymorphisms with the GD and CD, two autoimmune diseases that affect two hearing-impaired members of this family. The p.R859Q mutation is predicted to interfere with the structure and/or binding activity of the C-terminal domain of the wolframin protein. This report provides further evidence for the diverse roles of wolframin in human biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

GD, Graves disease; PD, Parkinson's disease; CD, Crohn's disease; SNHL, sensorineural hearing loss; LFSNHL, low frequency sensorineural hearing loss; WFS1, wolframin syndrome gene 1; WFS1, wolframin protein; WS, Wolfram syndrome; SNP, single nucleotide polymorphism; CR, canalicular reticulum.

REFERENCES

- Awata T, Inoue K, Kurihara S, Ohkubo T, Inoue I, Abe T, Takino H, Kanazawa Y, Katayama S. Missense variations of the gene responsible for Wolfram syndrome (WFS1/wolframin) in Japanese: possible contribution of the Arg456His mutation to type 1 diabetes as a nonautoimmune genetic basis. *Biochem Biophys Res Commun* 2000;268(2):612–6. [PubMed: 10679252]
- Bespalova IN, Van Camp G, Bom SJ, Brown DJ, Cryns K, DeWan AT, Erson AE, Flothmann K, Kunst HP, Kurnool P, Sivakumaran TA, Cremers CW, Leal SM, Burmeister M, Lesperance MM. Mutations in the Wolfram syndrome 1 gene (WFS1) are a common cause of low frequency sensorineural hearing loss. *Hum Mol Genet* 2001;10(22):2501–8. [PubMed: 11709537]
- Brix TH, Christensen K, Holm NV, Harvald B, Hegedus L. A population-based study of Graves' disease in Danish twins. *Clin Endocrinol (Oxf)* 1998a;48(4):397–400. [PubMed: 9640404]
- Brix TH, Kyvik KO, Christensen K, Hegedus L. Evidence for a major role of heredity in Graves' disease: a population-based study of two Danish twin cohorts. *J Clin Endocrinol Metab* 2001;86(2):930–4. [PubMed: 11158069]
- Brix TH, Kyvik KO, Hegedus L. What is the evidence of genetic factors in the etiology of Graves' disease? A brief review. *Thyroid* 1998b;8(7):627–34. [PubMed: 9709918]
- CrynsKPFisterMPenningsRJBomSJFlothmannKCaethovenGKremerHSchattelmanIKolnKATothTKupkaSBlinNNurnbergPThieleHvan de HeyningPHReardonWStephensDCremersCWSmithRJVan CampGMutations in the WFS1 gene that cause low-frequency sensorineural hearing loss are small non-inactivating mutations. *Hum Genet*2002110538994 Epub 2002 Apr 9. [PubMed: 12073007]
- Cryns K, Sivakumaran TA, Van den Ouweland JM, Pennings RJ, Cremers CW, Flothmann K, Young TL, Smith RJ, Lesperance MM, Van Camp G. Mutational spectrum of the WFS1 gene in Wolfram syndrome, nonsyndromic hearing impairment, diabetes mellitus, and psychiatric disease. *Hum Mutat* 2003a;22(4):275–87. [PubMed: 12955714]
- CrynsKThysSVan LaerLokaYPfisterMVan NassauwLSmithRJTimmermansJPVan CampGThe WFS1 gene, responsible for low frequency sensorineural hearing loss and Wolfram syndrome, is expressed in a variety of inner ear cells. *Histochem Cell Biol*2003b119324756 Epub 2003 Feb 19. [PubMed: 12649740]
- Davies, TF. Lippincott Williams & Wilkins; Philadelphia: 2000. Graves disease: pathogenesis; p. 518 p
- Domenech E, Gomez-Zaera M, Nunes V. WFS1 mutations in Spanish patients with diabetes mellitus and deafness. *Eur J Hum Genet* 2002;10(7):421–6. [PubMed: 12107816]
- FonsecaSGFukumaMLipsonKLNguyenLXAllenJROkaYUranoFWFS1 is a novel component of the unfolded protein response and maintains homeostasis of the endoplasmic reticulum in pancreatic beta-cells. *J Biol Chem*2005280473960915 Epub 2005 Sep 29. [PubMed: 16195229]
- Fukuoka H, Kanda Y, Ohta S, Usami S. Mutations in the WFS1 gene are a frequent cause of autosomal dominant nonsyndromic low-frequency hearing loss in Japanese. *J Hum Genet* 2007;52(6):510–5. [PubMed: 17492394]. Epub 2007 May 11.
- Fung S, Malhotra R, Selva D. Thyroid orbitopathy. *Aust Fam Physician* 2003;32(8):615–20. [PubMed: 12973869]
- GurtlerNKimYMhatreASchlegelCMathisADanielsRSheltonCLalwaniAKTwo families with nonsyndromic low-frequency hearing loss harbor novel mutations in Wolfram syndrome gene 1. *J Mol Med*200583755360 Epub 2005 May 24. [PubMed: 15912360]
- Hansen L, Eiberg H, Barrett T, Bek T, Kjaersgaard P, Tranebjaerg L, Rosenberg T. Mutation analysis of the WFS1 gene in seven Danish Wolfram syndrome families; four new mutations identified. *Eur J Hum Genet* 2005;13(12):1275–84. [PubMed: 16151413]

- Hofmann S, Philbrook C, Gerbitz KD, Bauer MF. Wolfram syndrome: structural and functional analyses of mutant and wild-type wolframin, the WFS1 gene product. *Hum Mol Genet* 2003;12(16):2003–12. [PubMed: 12913071]
- Inoue H, Tanizawa Y, Wasson J, Behn P, Kalidas K, Bernal-Mizrachi E, Mueckler M, Marshall H, Donis-Keller H, Crock P, Rogers D, Mikuni M, Kumashiro H, Higashi K, Sobue G, Oka Y, Permutt MA. A gene encoding a transmembrane protein is mutated in patients with diabetes mellitus and optic atrophy (Wolfram syndrome). *Nat Genet* 1998;20(2):143–8. [PubMed: 9771706]
- Ishihara HTakeda STamura ATakahashi RYamaguchi STakei DYamada TInoue HSoga HKtagiri HTanizawa Yoka YDisruption of the WFS1 gene in mice causes progressive beta-cell loss and impaired stimulus-secretion coupling in insulin secretion. *Hum Mol Genet* 2004;13(11):1597–1604. Epub 2004 Mar 31. [PubMed: 15056606]
- Khanim F, Kirk J, Latif F, Barrett TG. WFS1/wolframin mutations, Wolfram syndrome, and associated diseases. *Hum Mutat* 2001;17(5):357–67. [PubMed: 11317350]
- Kim N, Hatton MP. The role of genetics in graves' disease and thyroid orbitopathy. *Semin Ophthalmol* 2008;23(1):67–72. [PubMed: 18214794]
- Minton JA, Hattersley AT, Owen K, McCarthy MI, Walker M, Latif F, Barrett T, Frayling TM. Association studies of genetic variation in the WFS1 gene and type 2 diabetes in U.K. populations. *Diabetes* 2002;51(4):1287–90. [PubMed: 11916957]
- Ohata T, Koizumi A, Kayo T, Shoji Y, Watanabe A, Monoh K, Higashi K, Ito S, Ogawa O, Wada Y, Takada G. Evidence of an increased risk of hearing loss in heterozygous carriers in a Wolfram syndrome family. *Hum Genet* 1998;103(4):470–4. [PubMed: 9856492]
- Osman AA Saito MM Makepeace C Permutt MASchlesinger PMueckler MWolframin expression induces novel ion channel activity in endoplasmic reticulum membranes and increases intracellular calcium. *J Biol Chem* 2003;278(25):27556–27562. Epub 2003 Oct 3. [PubMed: 14527944]
- Philippou G, McGregor AM. The aetiology of Graves' disease: what is the genetic contribution? *Clin Endocrinol (Oxf)* 1998;48(4):393–5. [PubMed: 9640403]
- Sequeira A, Kim C, Seguin M, Lesage A, Chawky N, Desautels A, Tousignant M, Vanier C, Lipp O, Benkelfat C, Rouleau G, Turecki G. Wolfram syndrome and suicide: Evidence for a role of WFS1 in suicidal and impulsive behavior. *Am J Med Genet B Neuropsychiatr Genet* 2003;119(1):108–13. [PubMed: 12707947]
- Shadrina MNikopensius TSlominsky PIlarioshkin SBagyeva GMarkova EIvanova-Smolenskaia IKurgALimborska SMetspalu AAssociation study of sporadic Parkinson's disease genetic risk factors in patients from Russia by APEX technology. *Neurosci Lett* 2006;405(3):212–216. Epub 2006 Jul 28. [PubMed: 16876316]
- Strom TM, Hortnagel K, Hofmann S, Gekeler F, Scharfe C, Rabl W, Gerbitz KD, Meitinger T. Diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) caused by mutations in a novel gene (wolframin) coding for a predicted transmembrane protein. *Hum Mol Genet* 1998;7(13):2021–8. [PubMed: 9817917]
- Swift M, Swift RG. Psychiatric disorders and mutations at the Wolfram syndrome locus. *Biol Psychiatry* 2000;47(9):787–93. [PubMed: 10812037]
- Swift M, Swift RG. Wolframin mutations and hospitalization for psychiatric illness. *Mol Psychiatry* 2005;10(8):799–803. [PubMed: 15852062]
- Swift RG, Perkins DO, Chase CL, Sadler DB, Swift M. Psychiatric disorders in 36 families with Wolfram syndrome. *Am J Psychiatry* 1991;148(6):775–9. [PubMed: 2035720]
- Swift RG, Polymeropoulos MH, Torres R, Swift M. Predisposition of Wolfram syndrome heterozygotes to psychiatric illness. *Mol Psychiatry* 1998;3(1):86–91. [PubMed: 9491819]
- Swift RG, Sadler DB, Swift M. Psychiatric findings in Wolfram syndrome homozygotes. *Lancet* 1990;336(8716):667–9. [PubMed: 1975860]
- Takeda K, Inoue H, Tanizawa Y, Matsuzaki Y, Oba J, Watanabe Y, Shinoda K, Oka Y. WFS1 (Wolfram syndrome 1) gene product: predominant subcellular localization to endoplasmic reticulum in cultured cells and neuronal expression in rat brain. *Hum Mol Genet* 2001;10(5):477–84. [PubMed: 11181571]
- Tomer Y, Davies TF. Infection, thyroid disease, and autoimmunity. *Endocr Rev* 1993;14(1):107–20. [PubMed: 8491150]

- Torres R, Leroy E, Hu X, Katrivanou A, Gourzis P, Papachatzopoulou A, Athanassiadou A, Beratis S, Collier D, Polymeropoulos MH. Mutation screening of the Wolfram syndrome gene in psychiatric patients. *Mol Psychiatry* 2001;6(1):39–43. [PubMed: 11244483]
- ZatykaMRickettsCda Silva XavierGMintonJFentonSHofmann-ThielSRutterGABarrettTGSodium-potassium ATPase 1 subunit is a molecular partner of Wolframin, an endoplasmic reticulum protein involved in ER stress. *Hum Mol Genet*2008172190200 Epub 2007 Oct 18. [PubMed: 17947299]

Figure 1.

Pedigree of the five-generation American family with non-syndromic autosomal dominant LFSNHL, Graves disease (GD), Crohn's disease (CD) and Parkinson's disease (PD). Genotypes for nucleotide c.2576 in the *WFS1* gene and age (years) are shown for those individuals included in the genetic analysis. Individuals I:1 and I:2 were both reported to have hearing loss, however one of them is likely to represent a phenocopy. The lack of DNA from these individuals precluded determination of their *WFS1* genotype. Open symbols unaffected; filled black symbols affected; diagonal line deceased; question mark unknown disease status.

Figure 2. Audiograms of representative affected family members IV:2 and IV:4 at 17 and 32 years of age respectively. These individuals both exhibited similar low frequency (< 2000 Hz) moderate-to-severe SNHL, indicating that the hearing impairment is non-progressive.

Figure 3.

The novel causative mutation in the *WFS1* gene in the American family. **A** The mutation is a G-to-A nucleotide transposition in exon 8 (c.2576G→A) that results in substitution of an arginine residue for a glutamine residue at position 859 (p.R859Q). **B** Alignment of human and mouse *WFS1* proteins indicates that R859 is a highly conserved residue in mammals. **C** Hypothetical structure of wolfram protein showing the distribution of mutations known to cause non-syndromic LFSNHL. Most non-syndromic LFSNHL-causing mutations are located within the conserved C-terminal domain (box), including the novel R859Q variant.