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ORIGINAL ARTICLE

WFS1 variants in Finnish patients with diabetes mellitus, sensorineural hearing impairment or optic atrophy, and in suicide victims

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Mutations in the *wolframin* gene, *WFS1*, cause Wolfram syndrome, a rare recessive neurodegenerative disorder. The clinical features include early-onset bilateral optic atrophy (OA), diabetes mellitus (DM), diabetes insipidus, hearing impairment, urinary tract abnormalities and psychiatric illness, and, furthermore, *WFS1* variants appear to be associated with non-syndromic DM and hearing impairment. Variation of *WFS1* was investigated in Finnish subjects consisting 182 patients with DM, 117 patients with sensorineural hearing impairment (SNHI) and 44 patients with OA, and in 95 suicide victims. Twenty-two variants were found in the coding region of *WFS1*, including three novel nonsynonymous variants. The frequency of the p.[His456] allele was significantly higher in the patients with SNHI (11.5%; corrected $P=0.00008$), DM (6.6%; corrected $P=0.036$) or OA (9.1%; corrected $P=0.043$) than that in the 285 controls (3.3%). The frequency of the p.[His611] allele was 55.8% in the patients with DM being higher than that in the controls (47%; corrected $P=0.039$). The frequencies of p.[His456] and p.[His611] were similarly increased in an independent group of patients with DM ($N=299$). The results support previous findings that genetic variation of *WFS1* contributes to the risk of DM and SNHI.
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INTRODUCTION

Wolfram syndrome (WS) (OMIM 222300) is a rare neurodegenerative disorder,[1] also known as DIDMOAD (diabetes insipidus, juvenile diabetes mellitus (DM), early-onset optic atrophy (OA) and deafness). Besides the symptoms included in the acronym, the patients may have other neurological[2] and psychiatric symptoms,[3] such as paranoid delusions, psychotic behavior and suicide attempts. The estimated prevalence of WS is 1/770 000 in the United Kingdom.[4]

WS is inherited as an autosomal recessive disorder.[5] The gene involved, *wolframin* gene (*WFS1*), encodes wolframin, an 890-amino-acid transmembrane protein of the endoplasmic reticulum.[6–8] The protein is expressed ubiquitously and it is most abundant in the heart, pancreas, brain and muscle.[6,9] Wolframin participates in cellular calcium homeostasis.[10] Furthermore, it has a role in the signaling network of endoplasmic reticulum stress,[11] and suppression of *WFS1* has been shown to lead to endoplasmic reticulum stress in pancreatic β-cells.[12]

In rats, wolframin participates in the regulation of emotional behavior[13] and it may be a biomarker of post-traumatic stress.[14] Disruption of *WFS1* leads to difficulties in adaptation to stressful environment in mice[15] and can cause progressive β-cell loss and impaired glucose homeostasis.[12] In addition, it has been shown that the symptoms of *WFS1* knockout mice partly resemble a depression disorder.[16]

Mutations in the *WFS1* gene cause a loss of function of wolframin[6,7,17] by cellular depletion of the protein.[18] Patients with WS are homozygous or compound heterozygotes[17] with respect to these mutations, while heterozygotes have an increased risk to psychiatric hospitalization,[19] and an increased risk of DM and of hearing loss.[20] Indeed, some nonsynonymous variants and intronic polymorphisms in *WFS1* have shown a significant association with type II DM in several studies,[21–26] and association has been found also in the case of type I DM.[27] Many variants have been associated

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with hearing loss as well.[20,22,28–31] Some variants have been associated with suicidal behavior or severe psychiatric disorders.[32–35]

In the present study we have investigated population variation in *WFS1* in 343 Finnish patients with DM, sensorineural hearing impairment (SNHI) or OA, in 95 suicide victims (SVS), used as an approximation to psychiatric disorders, and in 285 healthy controls. The patients were ascertained in a defined population in northern Finland. In addition, some of the findings were replicated in an independent group of 299 patients with DM from southwestern Finland.

SUBJECTS AND METHODS

Subjects

The subjects included 182 patients with DM whose insulin treatment had been started between the ages 20 and 45 years, and who reported a family history with respect to any combination of DM, hearing loss or epilepsy in first- or second-degree relatives.[36] Furthermore, the subjects included 117 patients with SNHI with reported family history[37] and 44 patients with OA of unknown etiology,[38] and also included 95 SVs who were under 65 years of age. Thirty-five of the SVs had a diagnosis of psychiatric disorder. The permission for investigation of the SVs has been granted by the National Authority for Medicolegal Affairs of Finland. The controls consisted of 285 healthy volunteers free from the previously mentioned symptoms. All the subjects were residents of northern Finland. The controls were recruited from the three neighboring provinces: Northern Ostrobothnia, Kainuu and Northern Savo.

An independent group of patients ($N=299$) with DM[39] and with a family history defined as described above were screened for two variants that were detected in the primary analysis. The patients were residents of the province of Southwestern Finland.

Molecular methods

DNA was purified from blood by using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) or the standard SDS–proteinase K extraction with Phase Lock Gel (VWR International LLC, Radnor, PA, USA) from samples of the SVs. The PCR reaction was carried out with 14 separate primer pairs. Exon 8 was amplified with eight overlapping primer pairs because of its large size. The primer sequences and the conditions of amplification reactions are available on request.

Ten fragments covering five of the seven coding exons of the *WFS1* gene and part of exon 8 (amino acids from 457 to 756) were analyzed by using conformation-sensitive gel electrophoresis[40] (Figure 1). In short, amplified DNA fragments were allowed to form duplexes with themselves and with amplified control DNA fragments in a denaturating and reannealing cycle. Heteroduplexes differ from homoduplexes in mobility on a mildly denaturating gel.[40] Fragments with a known nucleotide sequence were used as controls. The beginning of exon 8 (amino acids from 288 to 456) was sequenced entirely. Three nucleotide changes were screened by restriction fragment analysis by using FastDigest restriction endonucleases (Thermo Fisher Scientific, Waltham, MA, USA). These changes included synonymous polymorphism rs1801213 in exon 6, which was analyzed using restriction fragment analysis with *Ssi*I, and two nonsynonymous nucleotide changes rs35932623 and rs71532874 in exon 8, which were analyzed using *Hha*I and *Taa*I, respectively. In addition, all the variations that were found using conformation-sensitive gel electrophoresis or restriction fragment length polymorphism was confirmed by sequencing from each reamplified sample.

Statistical analyses

Departures from Hardy–Weinberg equilibrium[41] as well as the differences in allele frequencies between the cases and controls[42] were calculated using the Arlequin 3.5.1.2 software.[43] Haplotypes were inferred and the estimated phase data was created with the ELB algorithm in the Arlequin software. In the case of each variant, four pairwise comparisons between the patient groups and controls were performed and then corrected for multiple testing by using Benjamini and Hochberg false discovery rate.[44] For all pairwise comparisons, corrected *P*-values are shown throughout. IBM SPSS Statistics 19 (IBM, New York, NY, USA) was used to calculate adjusted residuals to investigate which cells cause the difference in the allele level. Adjusted residuals were considered significant ($P<0.05$), if the value was <-1.96 or >1.96.[45] The comparisons between the patients' genotype and positive first-degree family history of similar symptoms were calculated using Pearson's χ^2-test or Fisher's exact test.

Bioinformatics

The variants were named according to the recommendations of the HGVS (Human Genome Variation Society; www.hgvs.org). Nucleotides were numbered according to the reference sequence NM_006005.3 and amino acids according to the reference protein NP_005996.2 (GeneID 7466, GenBank).

Pathogenicity of the variants was predicted by using four algorithms: PolyPhen-2,[46] SIFT,[47] SNAP[48] and MutationTaster.[49] A variant was considered a pathogenic one, if all the four predictions were against neutrality. If three of the four predictions were against neutrality, the variant was considered

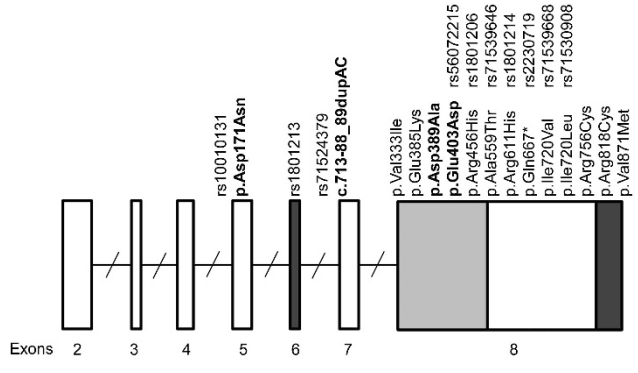

Figure 1 Coding exons of *WFS1* and exon location of identified variants. Method of sequence analysis is shown by color code. White, regions investigated by using conformation-sensitive gel electrophoresis; grey, direct sequencing; black, selected variants investigated by using restriction fragment length polymorphism. Bold font, novel variants found in this study. HGVS names are shown for nonsynonymous variants and for novel intronic variant. Rs-code is shown for synonymous and intronic variants.

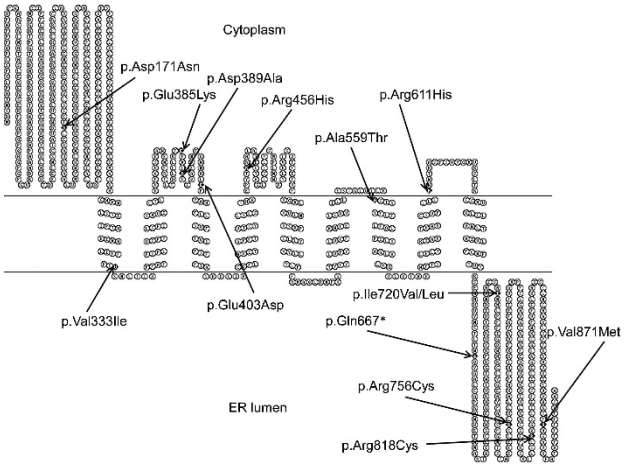

Figure 2 Structure of the wolframin protein and its membrane orientation. Positions of the 13 nonsynonymous variants and one nonsense variant found in Finnish subjects are shown. Transmembrane helices encompass the amino acids 311–333, 340–362, 405–422, 429–451, 493–515, 527–549, 559–581, 588–610 and 630–652. The figure was generated using TOPO2 (Johns SJ, TOPO2, http://www.sacs.ucsf.edu/TOPO2/).

to be possibly pathogenic. Multiple sequence alignment was done by using Clustal Omega.[50,51]

Transmembrane segments of wolframin were predicted by using TMHMM v. 2.0.[52] The figure of the protein structure (Figure 2) was drawn with TOPO2 (Johns SJ, TOPO2, Transmembrane protein display software, http://www.sacs.ucsf.edu/TOPO2/, date accessed: August 2012).

RESULTS

Genetic variation in *WFS1* in Finnish patients

We detected 22 different variants in the coding exons of the *WFS1* gene among the 438 subjects and 285 controls from northern Finland. The variants included 13 nonsynonymous, 1 nonsense and 8 synonymous variants. Three of the nonsynonymous variants were novel. In the intronic region we found one new polymorphism and several known changes.

The p.[His456] and p.[His611] alleles were associated with one or more of the clinical conditions (Table 1). Before corrections for multiple testing, four other variants suggested an association with DM, one with SNHI and DM, and a novel intronic insertion suggested an association with OA. All these variants were present also in the controls, whereas seven rare variants were detected in the patients only (Table 2). Two of the rare variants were novel nonsynonymous changes, including p.Asp389Ala and p.Glu403Asp.

Furthermore, we detected seven variants that were present in the patients and controls, but that were not associated with the clinical phenotype (Table 3), and four variants that were present only in the controls, including a novel nonsynonymous variant c.511G>A leading to p.Asp171Asn, a synonymous variant in exon 8, rs71539668, and two intronic variants, rs4688990 and rs71524379.

Differences in the frequencies of the *WFS1* alleles between patients and controls

Global test of differentiation among sample revealed that allele frequencies differed between groups with respect to the p.Arg456His variant ($P<0.000005$) and the p.Arg611His variant ($P=0.044$). The frequency of the p.[His456] allele was 3.3% (95% confidence interval (CI), 2.1–5.2%) in the controls, whereas the allele frequency was 6.6% (95% CI, 4.4–9.7%) in patients with DM ($P=0.036$ for pairwise difference), 9.1% (95% CI, 4.5–17.2%) in patients with OA ($P=0.043$ for pairwise difference) and 11.5% (95% CI, 8.0–16.3%) in the patients with SNHI ($P=0.00008$ for pairwise difference). The frequency of the p.[His611] allele was 55.8% (95% CI, 50.6–60.8%) in the patients with DM and 47.0% (95% CI, 43.0–51.1%) in the controls ($P=0.039$ for pairwise difference). Haplotype estimation suggested that the p.Arg456His and p.Arg611His variants belong to different haplotypes.

In addition, frequencies of the p.[His456] and p.[His611] alleles were examined in an independent group of patients with DM. The frequency of the p.[His456] allele was 5.9% (95% CI, 4.2–8.1%) and that of the p.[His611] allele was 53.8% (95% CI, 49.8–57.8%). The allele frequencies did not differ between the two DM groups (p.Arg456His, $P=0.85$; p.Arg611His, $P=0.76$).

Table 2 Rare heterozygous variants in the *WFS1* gene that were absent from controls and the pathogenicity predictions of the variants

Rs-code	*Amino acid change*	*EUR MAF*[a] *(Global MAF)*	*Predicted pathogenicity*[b]	*Number of heterozygotes among patients*
rs71524353	Glu385Lys	N.D.	Possibly pathogenic	1 SNHI, 1 OA
c.1166A>C	Asp389Ala	N.D.	Pathogenic	1 SNHI
c.1209G>T	Glu403Asp	N.D.	Pathogenic	1 DM, 1 SNHI
rs55814513	Ala559Thr	0.004 (0.001)	Neutral	2 DM, 1 SV
c.1999C>T	Gln667*	N.D.	WS mutation[c]	1 SV
rs138127684	Arg756Cys	N.D.	Pathogenic	1 SNHI, 1 SV
rs35932623	Arg818Cys	0.005 (0.002)	Pathogenic	2 DM

Abbreviations: DM, diabetes mellitus; EUR MAF, European minimum allele frequency; Global MAF, global minimum allele frequency; N.D., not determined; OA, optic atrophy; SNHI, sensorineural hearing impairment; SV, suicide victim; WS, Wolfram syndrome.
Rs-code is shown, if available. HGVS name is shown for novel variants that are underlined.
[a]Based on 1000 genomes database (http://www.1000genomes.org/). EUR MAF from available European populations and Global MAF from all available populations.
[b]Based on PolyPhen-2, SIFT, SNAP and MutationTaster predictions (3/4 predictions against neutrality considered as possibly pathogenic, 4/4 predictions against neutrality considered as pathogenic).
[c]Recessive WS mutation.

Table 1 Genotype percentages of the associating variants in the *WFS1* gene

			Controls N = *285*			*DM* N = *182*			*SNHI* N = *117*			*OA* N = *44*			*SV* N = *95*		
Variant	*Amino acid change*[a]	*EUR MAF*[b] *(Global MAF)*	*0/0*	*0/1*	*1/1*	*0/0*	*0/1*	*1/1*	*0/0*	*0/1*	*1/1*	*0/0*	*0/1*	*1/1*	*0/0*	*0/1*	*1/1*
Rs-code																	
(A) Associating variants with HW equilibrium with P*-value< 0.05 after correction*																	
rs1801208	Arg456His	N.D.	93.7	6.0	0.4	86.8[c]	13.2[c]	0[c]	78.6[c]	19.7[c]	1.7[c]	81.8[c]	18.2[c]	0[c]	88.4	11.6	0
rs734312	Arg611His	0.44 (0.48)	29.1	47.7	23.2	18.1[c]	52.2[c]	29.7[c]	25.6	52.1	22.2	15.9	61.4	22.7	31.6	47.4	21.1
(B) Associating variants with HW equilibrium with P*-value< 0.05 before correction*																	
rs10010131	—	0.37 (0.27)	18.6	50.2	31.2	15.4[d]	42.9[d]	41.8[d]	12.0	51.3	36.8	13.6	52.3	34.1	25.3	47.4	27.4
rs1801213	—	0.32 (0.24)	17.2	49.5	33.3	14.3[d]	40.1[d]	45.6[d]	9.4[d]	49.6[d]	41.0[d]	11.4	54.5	34.1	18.9	51.6	29.5
c.713-88_89dupAC	—		99.6	0.4	0	100	0	0	99.1	0.9	0	95.5[d]	4.5[d]	0[d]	98.9	1.1	0
rs1801212	Val333Ile	0.28 (0.14)	13.7	44.2	42.1	8.8[d]	39.0[d]	52.2[d]	6.8	46.2	47.0	9.1	43.2	47.7	14.7	48.4	36.8
rs1801206	—	0.39 (0.33)	17.2	51.6	31.2	14.3[d]	43.4[d]	42.3[d]	12.8	53.0	34.2	11.4	54.5	34.1	26.3	46.3	27.4
rs1801214	—	N.D.	17.5	51.2	31.2	14.3[d]	44.0[d]	41.8[d]	13.7	53.8	32.5	11.4	54.5	34.1	25.3	47.4	27.4

Abbreviations: DM, diabetes mellitus; EUR MAF, European minimum allele frequency; Global MAF, Global minimum allele frequency; HW, Hardy–Weinberg; N.D., not determined; OA, optic atrophy; SNHI, sensorineural hearing impairment; SV, suicide victim; 0/0, homozygote with respect to the reference allele; 0/1, heterozygote with respect to the variant; 1/1 homozygote with respect to the variant.
Rs-code is shown, if available. HGVS name is shown for novel variants that are underlined.
[a]None of the three nonsynonymous variants were predicted as pathogenic or possibly pathogenic based on PolyPhen-2, SIFT, SNAP and MutationTaster predictions (4/4 predictions against neutrality considered as pathogenic, 3/4 predictions against neutrality considered as possibly pathogenic).
[b]Based on 1000 genomes database (http://www.1000genomes.org/). EUR MAF from available European populations and Global MAF from all available populations.
[c]Pairwise differences after corrections in allele frequencies between cases and controls.
[d]Pairwise differences only before corrections in allele frequencies between cases and controls.

Table 3 Non-associating variants in the *WFS1* gene

			Controls (N = *285*)		*DM* (N = *182*)		*SNHI* (N = *117*)		*OA* (N = *44*)		*SV* (N = *95*)	
Variant	*Amino acid*[a] *change*	*EUR MAF*[b] *(Global MAF)*	N	*Allele freq.*	N	*Allele freq.*	N	*Allele freq.*	N	*Allele freq.*	N	*Allele freq.*
Rs-code												
rs56072215	—	0.053 (0.028)	6	0.0123	5	0.0137	7	0.0299	0		5	0.0263
rs71539646	—	0.007 (0.003)	2	0.0035	4	0.0110	3	0.0128	2	0.0227	3	0.0158
rs2230719	—	0.053 (0.042)	6	0.0123	5	0.0137	7	0.0299	0		5	0.0263
rs1805070	Ile720Val	0.009 (0.019)	11	0.0193	4	0.0110	10	0.0427	1	0.0114	5	0.0263
rs1805070	Ile720Leu	N.D.	3	0.0053	0		0		0		1	0.0053
rs71530908	—	0.007 (0.002)	5	0.0088	0		0		0		1	0.0053
rs71532871	Val871Met	0.009 (0.004)	7	0.0123	9	0.0247	4	0.0171	0		1	0.0053

Abbreviations: DM, diabetes mellitus; EUR MAF, European minimum allele frequency; Global MAF, global minimum allele frequency; N.D., not determined; OA, optic atrophy; SNHI, sensorineural hearing impairment; SV, suicide victim.
[a]None of the three nonsynonymous variants were predicted as pathogenic or possibly pathogenic based on PolyPhen-2, SIFT, SNAP and MutationTaster predictions (4/4 predictions against neutrality considered as pathogenic, 3/4 predictions against neutrality considered as possibly pathogenic).
[b]Based on 1000 genomes database (http://www.1000genomes.org/). EUR MAF from available European populations and Global MAF from all available populations.

Frequency of affected first-degree relatives among patients with DM and among patients with SNHI

The two groups of patients with DM ($N = 481$) were combined. Fifty-nine patients harboring the p.[His456] allele were excluded, because the p.[His611] allele and the p.[His456] allele were both associated with DM and because they were estimated to occur in separate haplotypes. In addition, eight patients were excluded because of missing family history information. Then the proportion of probands reporting a first-degree relative with DM was examined among those who harbored one or two alleles of p.[His611] or two reference alleles (Figure 3). The differences were not significant between genotypes ($P = 0.66$; between homozygotes $P = 0.23$). However, a trend-wise increase of positive family history was present in the patients who were heterozygotes or homozygotes with respect to the p.[His611].

A similar analysis among patients with SNHI, who were heterozygotes with respect to p.Arg456His, did not reveal differences in the frequency of positive family history with respect to SNHI. The p.[His456] allele homozygotes were not analyzed because of their small number.

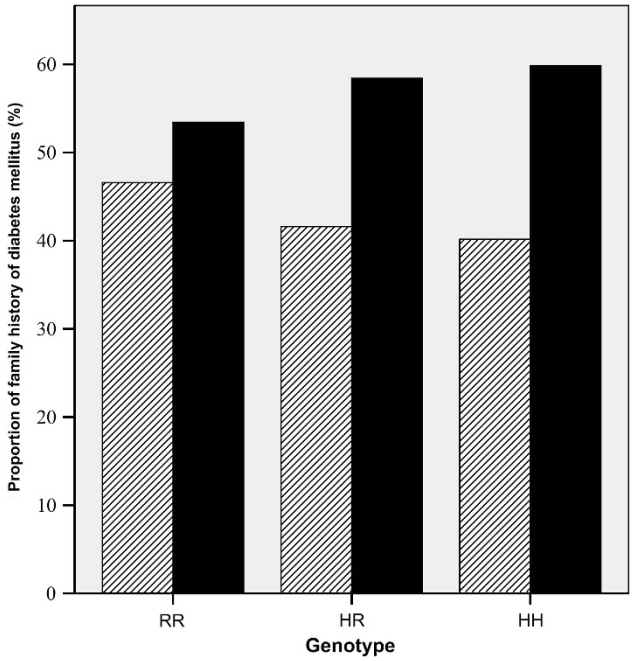

Figure 3 Proportion of patients with DM ($N = 414$) reporting family history of DM in first-degree relatives. Solid columns, DM patients with first-degree relatives with DM. Striped columns, DM patients with no first-degree relatives with DM. RR, patients homozygous with respect to p.[Arg611] allele ($N = 73$); RH, patients heterozygotes with respect to p.[Arg611] and p.[His611] alleles ($N = 214$); HH, patients homozygous with respect to p.[His611] allele ($N = 127$).

DISCUSSION

Two *WFS1* variants are associated with DM and SNHI

We found that the frequency of the p.[His456] allele was higher in patients with DM, who had started insulin therapy between the ages 20 and 45 years, than that in the controls. Previously, it has been found that the frequency of the p.[His456] allele is higher in patients with type 1 DM than that in controls.[27] A recent large-scale study recommends further investigations of p.Arg456His in the patients with type II DM.[53] We also observed an association between the p.[His611] allele and DM, supporting previous studies.[21,24–26] The increase in the allele frequencies could be observed in two independent cohorts of patients with DM. Furthermore, we pointed out that patients with DM, who were homozygotes or heterozygotes with respect to p.[His611], reported slightly more often first-degree relatives affected with DM than patients who were homozygotes with respect to the reference. This finding, however, needs to be substantiated in larger patient cohorts. These findings further support the hypothesis that the p.Arg611His variant is a risk factor for DM. Previous studies have shown a highly significant association between the rs10010131 variant and DM,[23,24,54,55] but we did not detect such an association. In addition to DM, we found association between the p.[His456] allele and SNHI and OA, respectively. The association of *WFS1* variants with hearing impairment has been shown previously,[20,22,28–31] but this is the first report where the p.[His456] allele is found to be associated with an increased risk of SNHI.

Depression and other psychiatric findings are common in WS[3] and, in addition, *WFS1* knockout mice show depression-like phenotype in behavioral tests.[16] In line with these findings, previous studies have described an association between p.Arg611His and suicide,[33,34] but we could not confirm this association. The SVs were incident cases occurring in a specific period of time, whereas the other patient

groups were prevalent cases. However, it is unlikely that the method of case finding influences the results. The factors behind suicidal behavior are heterogenic and, therefore, genetic associations are either difficult to recognize or the identified associations could be biased.

Our sample size was only modest but, even so, we could confirm an association between certain *WFS1* variants and SNHI and DM, respectively, in Finnish patients. Finns differ from Swedes, British and Germans, and, in addition, the within-population difference between Eastern Finns and Western Finns is greater than the difference between Germans and British.[56] Population structure could have a remarkable function in large, multicenter association studies.[57] Because of such differences, it is important to identify genetic variation in different ethnic groups, including populations, where the attainable sample size may remain small.

Rare heterozygous variants in *WFS1*

Two novel nonsynonymous variants were found in the patients but not in the controls (Figure 2). The p.Asp389Ala variant was found in a patient with SNHI, who did not report other health problems. An unaffected sibling of the patient was a carrier of p.[Ala389], suggesting that a single copy of this allele alone does not explain the clinical phenotype of the patient with SNHI. The p.Glu403Asp variant was found in two patients, one of whom had SNHI and DM. This patient had three siblings and mother with hearing impairment. Interestingly, the affected sister had the heterozygous p.Glu403Asp variant, whereas the unaffected brother had two reference alleles. However, more samples from the relatives should be genotyped to investigate possible pathogenicity of the variant. The other patient with p.Glu403Asp had DM, hearing impairment and impaired vision, as well as memory problems. Unfortunately, further investigation on this family was not possible. The change from aspartic acid to alanine is a nonconservative one, whereas the change from glutamic acid to aspartic acid is a conservative one. Despite the lack of clear evidence of pathogenicity, four algorithms predicted the variants p.Asp389Ala and p.Glu403Asp to be pathogenic.

Furthermore, five previously reported rare variants were found in the patients but not in the controls (Figure 2). The p.Glu385Lys variant was found in a patient with OA and in a patient with SNHI. The patient with OA had had epilepsy in her childhood, whereas the patient with SNHI was otherwise healthy. This variant has previously been described as a polymorphism,[58] but was predicted to be possibly pathogenic in our study. We found the p.Ala559Thr variant in an SV and in two patients with DM free from other health problems. This variant has been suggested to be related to psychiatric disorders[32,59] and, furthermore, this variant has been found in a patient with WS in heterozygous form.[60] The contribution of the p.Ala559Thr remains unclear, however, as the WS patient was a compound heterozygote for an in-frame deletion and a frameshift insertion in *cis* with p.Ala559Thr. In addition, the variant has been found in patients with DM, and also in their unaffected relatives.[61] Predictions of pathogenicity suggested p.Ala559Thr to be a polymorphism. The third previously described rare variant, p.Arg818Cys, was found in two patients with DM free from other health problems. This variant has been found in psychiatric patients[62] and in homozygous form in two Spanish WS patients.[63] However, these WS patients were homozygotes also with respect to the p.Glu737Lys mutation in *WFS1* and, in addition, they had a possibly pathogenic point mutation and multiple deletions in their mitochondrial DNA. It has been suggested that p.Arg818Cys is a polymorphism.[30] The p.Arg756Cys variant was found in a patient with SNHI, whose mother had hearing impairment, and in an SV. This variant was found in the single-nucleotide polymorphism database of the National Institute of Biotechnology Information (http://www.ncbi.nlm.nih.gov/projects/SNP/), but no functional data are available. Both p.Arg756Cys and p.Arg818Cys were predicted to be pathogenic. In our study, however, the functional role of these six variants remains unclear because of the heterozygosity and rarity of the variants. Interestingly, each of these six rare amino acid variants occurred in a more conserved segment of the wolframin protein than the p.Arg456His and p.Arg611His variants (Supplementary File S1). The last previously described rare variant was the nonsense mutation p.Gln667*, which was found in one SV. This mutation has been previously found in three WS patients, whose symptoms did not include psychiatric findings.[17,64] Heterozygotes of WS mutations have been estimated to require 26-fold more likely psychiatric hospitalization.[19]

In conclusion, we suggest that p.Arg456His and p.Arg611His variants of *WFS1* carry an increased risk of DM and SNHI. These variants may be harmful by themselves, be markers for linked harmful mutations or their effect could be additive to other genetic factors.

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