SHORT REPORT

Autosomal dominant optic atrophy associated with hearing impairment and impaired glucose regulation caused by a missense mutation in the *WFS1* gene

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Autosomal dominant optic atrophy (ADOA) is genetically heterogeneous, with *OPA1* on 3q28 being the most prevalently mutated gene. Additional loci are *OPA3*, *OPA4*, and *OPA5*, located at 19q13.2, 18q12.2, and 22q12.1–q13.1, respectively. Mutations in the *WFS1* gene, at 4p16.3, are associated with either optic atrophy (OA) as part of the autosomal recessive Wolfram syndrome or with autosomal dominant progressive low frequency sensorineural hearing loss (LFSNHL) without any ophthalmological abnormalities. Linkage and sequence mutation analyses of the ADOA candidate genes *OPA1*, *OPA3*, *OPA4*, and *OPA5*, including the genes *WFS1*, *GJB2*, and *GJB6* associated with recessive inherited OA or dominant LFSNHL, were performed. We identified one novel *WFS1* missense mutation E864K, c.2590G→A in exon 8 that co-segregates with ADOA combined with hearing impairment and impaired glucose regulation. This is the first example of autosomal dominant optic atrophy and hearing loss associated with a *WFS1* mutation, supporting the notion that mutations in *WFS1* as well as in *OPA1* may lead to ADOA combined with impaired hearing.

Hereditary optic neuropathy occurs with a prevalence between 1:10 000 and 1:50 000, and the most common type is autosomal dominant optic atrophy, ADOA (MIM #165500). This disease usually starts in childhood and is characterised by a progressive decline in visual acuity and temporal optic nerve pallor.[1] Optic atrophy (OA) is genetically heterogeneous,[2] and four autosomal loci have been identified: *OPA1* on chromosome 3q28,[3] *OPA3* on 19q13.2–q13.3.[4] *OPA4* on chromosome 18q12.2–q12.3,[5] and recently, *OPA5* on chromosome 22q12.1–q13.1 in two unrelated French families with ADOA.[6] In addition, juvenile optic atrophy is a key component of the autosomal recessive neurodegenerative disorder Wolfram syndrome (WS), caused by mutations in the *WFS1* gene on chromosome 4p16.3.[7]

OPA1 seems most frequently involved in OA, and one particular mutation, c.2826delT, explains the high prevalence of ADOA, Kjer type, in Denmark.[3 8] *OPA3* or Costeff optic atrophy syndrome (MIM #258501) is an autosomal recessive disorder associated with neurological abnormalities, and recently, mutations in the *OPA3* gene were shown to segregate with ADOA associated with cataract.[4] *OPA4* (MIM #605293) designates a locus for autosomal recessive optic atrophy, but the corresponding gene remains to be identified.[5]

ADOA associated with hearing impairment not linked to *OPA1* has been described in several instances.[9–15] One specific missense mutation, R445H, in *OPA1* has been identified in four sporadic/familial cases with dual sensory impairment, and furthermore, in two families with ptosis, ophthalmoplegia, and movement disorders.[16]

Autosomal recessive WS is characterised by juvenile onset diabetes mellitus and optic atrophy, with 60% of the patients showing various degrees of hearing impairment by 20 years of age,[17] and frequently endocrinological, psychiatric, urological, and neurological symptoms. Mutations in the *WFS1* gene in WS are distributed over the entire coding region and result in loss of function of the encoded protein.[18] *WFS1* has also been implicated in families with autosomal dominant isolated low frequency sensorineural hearing loss (LFSNHL) mapping to 4p16.[7 19] The corresponding mutations are mainly missense mutations in exon 8, probably resulting in gain of function of the protein.[20 21] Optic atrophy has not been reported as a concomitant feature in families with autosomal dominant low frequency hearing loss associated with *WFS1* mutations. The fact that carriers of *WFS1* mutations associated with WS do not have hearing impairment and heterozygosity for other *WFS1* mutations resulting in non-syndromic autosomal dominant LSFHNL illustrates our incomplete knowledge of the function of the WFS1 protein and the consequences of the different types of *WFS1* mutations.[21]

In this study, we performed extended linkage and mutation analyses in one Danish family with ADOA in combination with hearing impairment and identified the novel disease causing mutation.

METHODS

A three generation family (fig 1) was followed for 20 years at the National Eye Clinic for the Visually Impaired (NEC). Patient histories were recorded and the subjects underwent ophthalmological and optometric examinations at the NEC at regular intervals. Audiological and other medical information was also collected from hospital departments. The definitions used for the classification of hearing impairment are given by European Working Group on Genetics of Hearing Impairment.[22] After an overnight fast, the members V:3, IV:4, and IV:6 underwent a standard 75 g oral glucose tolerance test (OGTT). Plasma glucose, serum specific insulin (excluding des(31, 32)– and intact proinsulin), serum C-peptide and HbA1C were analysed using standard methods (Steno Diabetes Center). The insulinogenic index was calculated as the 30 minute post-OGTT serum insulin (pmol/l) minus the fasting serum insulin (pmol/l) and divided by the 30 minute post-OGTT plasma glucose level (mmol/l). Results obtained from previous studies performed

Abbreviations: ADOA, autosomal dominant optic atrophy; LFSNHL, low frequency sensorineural hearing loss; NEC, National Eye Clinic for the Visually Impaired; OA, optic atrophy; OGTT, oral glucose tolerance test; WS, Wolfram syndrome

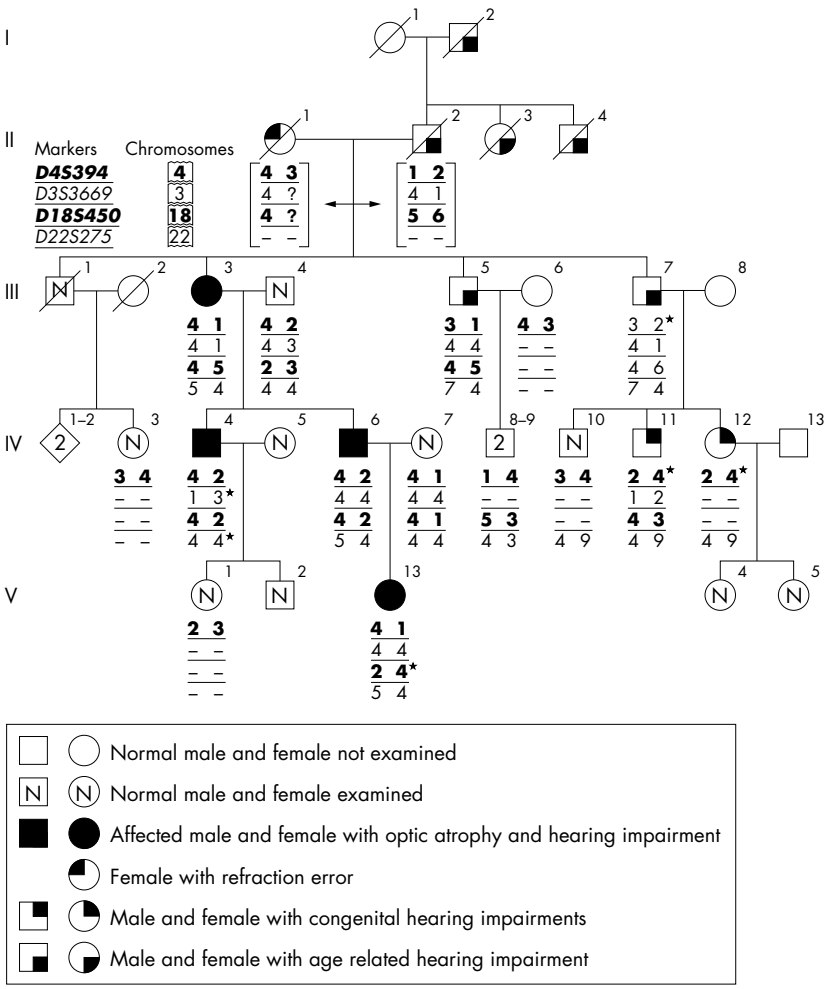

Figure 1 Five generation pedigree for family 148. The STS markers on chromosome 3, 4, and 18 each follow a haplotype map flanking candidate genes: *D4S394* (*WSF1*), *D3S3669* (*OPA1*), *D18S450* (*OPA4*), and *D22S275* (*OPA5*). An asterisk denotes STS marker results that exclude ADOA. STS marker D4S394 segregates together with the combined sensory affections in the members III:3, IV:4, IV:6, and V:3, and does not segregate together with the hearing impairment in III:5, III:7, IV:11, and IV:12. Individuals III:5 and III:7 had age related HI presenting after the age of 70 years (audiological data not shown) preferentially affecting the higher frequencies. V:4 had normal hearing. It was reported that II:2, born in 1881, who died at 78 years of age, had HI at old age. He was one of 10 siblings, of whom a sister (II:3) and a brother (II:4) had HI, as did as their father (I:2).

on age and sex matched glucose tolerant subjects at Steno Diabetes Center were used as control data. The investigations adhered to the tenets of the declaration of Helsinki and all participants gave informed consent for the investigations.

Blood samples were obtained from all available informative family members, and DNA was extracted from whole blood using standard procedures. DNA from 60 unrelated normal individuals from the Copenhagen Family Bank (families 604–1505)[23] were used as controls. Ten closely linked polymorphic markers (*D3S1601, D3S2418, D3S3669, D3S1523, D3S3642, D3S2305, D3S3562, D3S2748*, and *D3S1265*) flanking the *OPA1* gene on 3q28, four closely linked markers (*D18S66*, *D18S450*, *D18S851*, and *D18S68*) flanking *OPA4* on 18q12.2–q12.3, and three closely linked markers (*D4S412*, *D4S394*, and *D4S403*) on 4p16.3, enclosing the *WFS1* gene, were analysed. Furthermore, four closely linked markers (*D13S1316*, *D13S175*, *D13S1275*, and *D13S787*) flanking *GJB3* and *GJB6* on 13q12.11 were tested. One closely linked marker to *OPA5*, *D22S275*, was used to investigate for segregation with ADOA. All marker systems were typed by standard methods. Primers were radioactively end labelled, PCR was carried out under standard conditions and bands were separated by electrophoresis under denaturing conditions on polyacrylamide gels, and autoradiography was carried out.

Two point linkage analyses using the MLINK subroutine of FASTLINK package (version 5.1) were performed.[24] Optic atrophy was modelled as a fully penetrant autosomal trait with a frequency of 0.001 and haplotype, and analyses were done for all chromosome regions.

PCR and sequencing primers were designed using the software Primer3[25] and intron located primers were designed to be a minimum of 100 bp from the intron−exon splice sites (table 1). Primers for sequencing of *GJB6* exon 3, *GJB2* exon 1 and exon 2 ,and PCR primers for detection of the 342 kbp (del-*GJB6*-D131830) large deletion of *GJB6*[26 27] are shown in table 1. Coding sequence exons in all four genes were sequenced in the affected people IV:4, IV:6, IV:11, and IV:12 (fig 1), and in addition IV: 6 was tested for the common Danish *OPA1* founder mutation c.2826delT in exon 28.[8]

PCR was carried out under standard conditions according to the enzyme manufactures protocols (*Taq* DNA polymerase; Amersham Biosciences, Amersham, Bucks, UK). Reactions were carried out in 15 µl volumes containing the buffer, 2.5 µmol/l dNTP, 10 µmol/l of each primer, 0.008% cresol red (Sigma-Aldrich Co., St. Louis, MO, USA), 12% sucrose (w/v), and 50–100 ng templates DNA. Standard reaction conditions for all primers were: 95°C for 5 minutes; 40 cycles of 95°C for 30 seconds, 56.4°C for 30 seconds and 72°C for 1 minute; followed by 5 minutes at 72°C. PCR reactions were separated in a 2% agarose gel and stained with ethidium bromide in 1× TBE buffer before sequencing. Both strands were sequenced directly using the PCR primers and a commercial kit (BigDye version 1.0, Applied Biosystems, Foster City, CA, USA) without further modifications and run on an ABI 370 sequencer (Applied Biosystems). Sequence data were analysed using standard software (Chromas, version 2.1; Australia) and sequence alignments were carried out using ClustalW[28] or BLAT[29] respectively. All exons were completely

Table 1 Primer information for PCR amplification and sequencing of *WFS1*

Exon	Forward primers	Reverse primers	Size (bp
1	CGGAGATGTGGAGTGATTGG	GACCTTGTCGGGACTTTGG	432
2	GCATGTGCAGGTCTGAGATT	ACAAAGGTGACAGGTGCAAG	679
3	CACCTCACCCGCATAGAGTT	GCCCAAGATGAGGTGTGAGT	571
4	CACCTTCCTCACCGTGTTTT	ACCACACAAGCCAAAGAGAA	578
5	CGTCTGGTGGGAGACCAGT	GGTCTCTACAGGAAGGTTCTGG	576
6	CTGAGTCCACCCCAGCTACT	GGTCTACTCCCAGCGTCCA	500
7	CCTGGTCCTCAACCCTCAG	CTCCCGAGGACACATCCTTA	499
8:1	AGGGTAGGACCACTAGGATGG	CTTCAGGTAGGGGCAATTCA	735
8:2	TATGCCCATTTCCTGCTCTC	TACTGCTGCCAGGTCAGTGT	794
8:3	GTCTGTAGTGTGCCCCTGCT	GATGGTGCTGAACTCGATGA	675
8:4	TTCGACCGCTACAAGTTTGA	TGTGGAGGTTACACAGATGCTT	783
Primer information for PCR amplification and sequencing of *GJB2*			
1	GCACTTGGGGAACTCATGG	GCAACCGCTCTGGGTCTC	466
2	TCAGAGAAGTCTCCCTGTTCTGTCC	TGAGCACGGGTTGCCTCATC	833
	CTGGCTCACCGTCCTCTTCATTTATGGGCCCTGCAGCTGATC	CGCATCGAAGGCTCCCTGT	†
Primer information for PCR amplification and sequencing of *GJB6* exon 3			
3:1	AGACTAGCAGGGCAGGGAGT	CCCCTCTATCCGAACCTTCT	526
3:2	TGCATGTGGCCTACTACAGG	GGTTGGTATTGCCTTCTGGA	577
Primer information for PCR amplification of del(*GJB6*-D131830) large deletion*			
Deletion	TTTAGGGCATGATTGGGGTGATTT	CACCATGCGTAGCCTTAACCATTTT	460
Normal		AGCCTTTATGTATGTGTTTTACTTCCTT	267
Primer information for PCR amplification and sequencing of *OPA3*			
1	AGGATGCGCCTCTGAAGTT	CCACTATTGGCCACTGGACT	415
2	TACCCAGGCTAGGTGACTGC	GCATCAAGATCCTGGTGGTT	604

*Primers identical to Del Castillo *et al* 2002[25] ; †Internal sequencing primers.

sequenced for two affected people, IV:6 and IV:12. PCR products of WFS1 exons 1, 2, 5, 8-2, 8-3, and 8-4 were generated with Platinum *Taq* DNA polymerase (Invitrogen A/S, Denmark) using 1.5 mmol/l Mg^{2+} under the same PCR conditions as above. Diagnostic restriction enzyme digest of the mutation c.2590G→A was carried out by PCR of exon 8 resulting in amplification of a 783 bp PCR product using primer pair 8:4F and 8:4R (table 1). Digestion with the restriction enzyme *Bsp*1286I resulted in four DNA fragments (69, 177, 218, and 319 bp), representing the wild type allele, and one additional 246 bp fragment (69 plus 177 bp) due to the mutation in one *Bsp*1286I site in affected heterozygotes. The digested PCR products were separated by 2% agarose gel electrophoresis with 1×TBE buffer.

RESULTS

The main clinical characteristics (table 2), and the audiometric pattern and course of the hearing impairment are shown (fig 2, table 3). An OGTT showed that IV:6 had undiagnosed diabetes, IV:4 had impaired glucose tolerance, and V:3 had normal glucose tolerance according to the WHO 1999 definition. Furthermore, IV:6 and V:3 had poor pancreatic β-cell function as evaluated by the insulinogenic index (0.7 and 2.1, respectively, compared to age and sex matched glucose tolerant control subjects; reference insulinogenic index: mean (SD) males 27.0 (11.2), females 25.0 (13.5)).

Haplotype analyses with markers flanking the genes *OPA1*, *OPA4*, *OPA5*, *GJB2*, and *GJB6* did not support those loci on

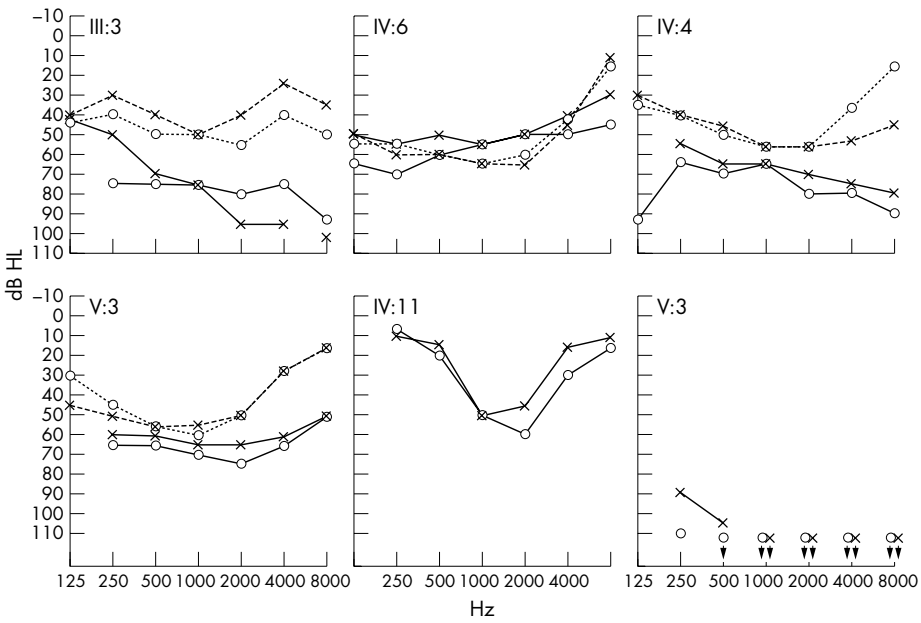

Figure 2 Audiograms showing the six affected people in family 148. Four members (III:3, IV:4, IV:6, and V:3) are heterozygous for the *WFS1* mutation and two (IV:11 and IV:12) are homozygous normal. Broken lines indicate the first examination and solid lines the latest examination (age given below each audiogram). Circles and crosses represent air conduction thresholds for the right and left ear, respectively. Audiological information about mutation carriers is summarised in the text (table 2). The audiograms from the WFS1 mutation carriers are described (table 2), and in all instances affect the low frequencies. IV:11 (male, aged 55 years) had a deeply U-shaped audiogram and progressive HI, and used a hearing aid from 11 years of age. IV:12 (female, aged 44 years) had congenital profound HI and used sign language. She also had cleft palate.

Table 2 Clinical characteristics, audiometric pattern, and course of the hearing impairment in patients

	People in the pedigree (sex; age in years) III:3 (F; 89)	IV:4 (M; 70)	IV:6 (M; 67)	V:3 (F; 32)
Visual impairment				
Age of onset (years)	6–7	35	Youth	9
Age at last examination	66	55	56	26
Optic atrophy	Severe	Moderate	Moderate	Mild
Optic discs	Greyish-white	Pale	Pale	Pale
VA (OD)	4/60−4.50 sph, 1.50×5°	0.25−4.75 sph, 0.50×105°	0.25+1.00 sph, 1.25×115°	0.3−6.50 sph
VA (OS)	4/36−3.00 sph, 3.50×0°	0.3−4.75 sph, 1.00×85°	0.3+0.75 sph, 1.00×75°	0.3−5.00 sph
Cataract (age of onset)	Bilateral mild (66 years)			
Colour vision	Failed	Normal	Normal	
Goldman perimetry	Abnormal	Normal	Normal	Normal
VEP	Extinct monocular		Normal	
Hearing impairment				
Age of onset	?	Childhood	Childhood	4 years
Degree of HI	Severe	Moderate	Moderate	Moderate
PTA right/left at latest examination, dB (age at examination, years)	76.3/73.8 (83 years)	73.8/68.8 (64 years)	53.8/48.8 (58 years)	68.8/62.5 (27 years)
Audiometric configuration, right/left at first examination	Flat/U-shaped	U-shaped/U-shaped	LF asc./LF asc	U-shaped/U-shaped
PTA progression right/left, dB, (period of observation)	27.5/45.0 (44 years)	25.0/18.8 (44 years)	−3.8/−10 (12 years)	21.3/16.3 (19 years)
Audiometric configuration (ight/left at latest examination	Flat/gently sloping	Gently sloping/flat	LFasc./LF asc.	Flat/Flat
CT scan (temporal bone)	–	–	–	Normal
Glucose tolerance	–	Impaired (70 years)	Diabetes (67 years)	Normal (32 years)
Fasting P-glucose (mmol/l)*	–	5.9	16.6	5.1
2h-P-glucose (mmol/l)	–	7.8	28.0	5.3
Psychiatric	–	Normal	Anxiety and sleeping disorder	Normal

F, female; M, male; VEP, visual evoked potential; VA, visual acuity. OD, right eye; OS, left eye; LF, low frequency; Asc., ascending; PTA, pure tone threshold average at last audiological examination; HI, hearing impairment. Hearing levels are averaged across 0.5, 1, 2, and 4 kHz. PTA progression is the deterioration in PTA between the first and the last audiological evaluation, spanning up to 44 years. Audiometric configurations (as shown in fig 2) at the first and the latest audiometry performed are stated. *Normal (F/M) 5.1 (0.5)/5.6 (0.2) (mean (SD)). Additional family information: III:1 and his three children never complained of visual or hearing difficulties; II:2 had a driver's licence; II:1 was born in 1883, and died at 77 years of age. She had ''a refraction error'' and wore glasses in old age, but did not have severe visual impairment.

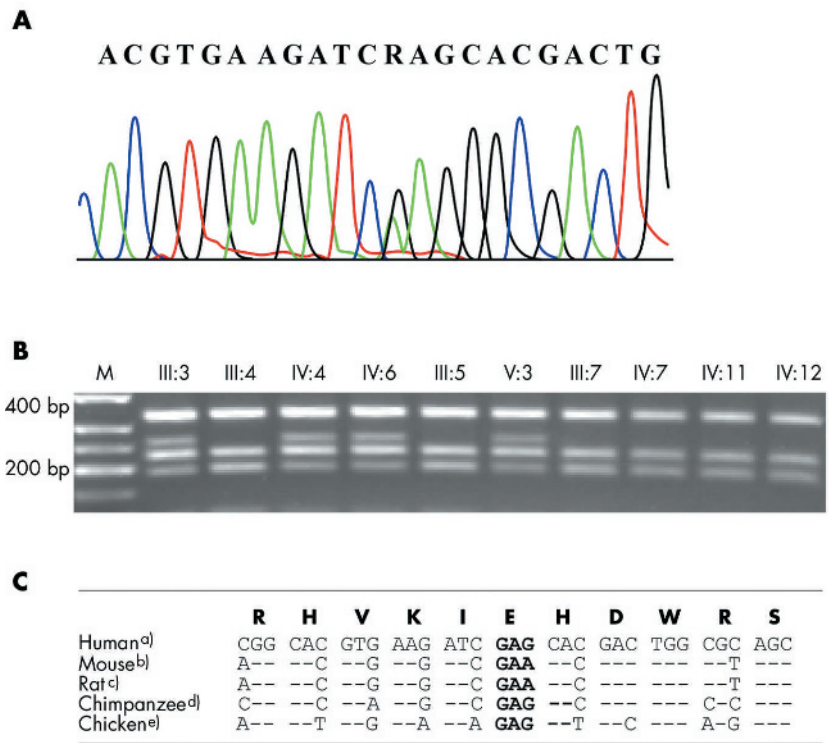

Figure 3 (A) Sequencing chromatogram of the mutation c.2590 G→A in IV:6. The affected person is heterozygous for the missense mutation E864K and the G→A substitution is denoted by an "R". (B) The mutation segregates with sensorineural hearing loss in family #148. The 783 bp PCR product digested by *Bsp*1286I presents a 246 bp fragment (69 plus 177 bp) in heterozygotes in addition to the wild type fragments (69, 177, 218, and 319 bp) after separation in 2% agarose gel. Roman numbers above the gel refer to the family pedigree (fig 1). M is the DNA ladder (150, 200, 250, 300 and 400 bp) (C) Alignment of the 11 amino acid codons around the E864K mutation demonstrate conservation of the protein sequence and redundancy in the DNA sequence (sequence data: UCSC Genome Browser;[34] (a) *Homo sapiens* chr4:6368639–6368671, NCBI build 34 July 2003; (b) *Mus musculus* chr5:35260341–35260373, NCBI build 30 February 2003; (c) *Rattus norvegicus* chr14:79406244–79406276, HGSC version3.1, Jun 2003; (d) *Pan troglodytes* chr3:6485955–6485987, NCBI build 1, version 1, November 2003, (e) *Gallus gallus* chr4:79834614–79834646, galGal, February 2004).

chromosomes 3q28, 18q12.2–q12.3 and 22q12.1–q13.1, because of lack of co-segregation with ADOA and negative LOD scores for close linkage (fig 1, data not shown). Marker *D4S394* close to the *WFS1* gene on 4p16.3 gave a positive LOD score ($Z = 1.61$, $\theta = 0.0$) for this area.

Mutation analysis of all coding exons in *OPA3*, *GJB2*, and *GJB6* in IV:6, IV:11, and IV:12 gave negative results. The gene *OPA1* did not have the common Danish founder mutation c.2826delT (frameshift mutation in exon 28) in IV:6 and IV:12. The *OPA3* gene was excluded based on sequencing in IV:6 and additionally, the single nucleotide polymorphism rs3826860 in exon 2 found in IV:6 showed heterozygosity, which excluded the presence of a large deletion of the gene. Mutation analysis of exons 1–8 of the *WFS1* gene in IV:6, IV:11, and IV:12 revealed one novel missense mutation (c.2590G→A in exon 8, only in person IV:6; fig 3A).

The disease phenotype segregated with this *WFS1* mutation demonstrated by the restriction enzyme *Bsp*1286I digest of the PCR amplicons (fig 3B) and the mutation was absent in unaffected family members and in those with isolated hearing impairment (IV:11 and IV:12). The *WFS1* mutation was not found among 60 normal control individuals.

DISCUSSION

A Danish family with juvenile onset of optic atrophy and associated hearing impairment was investigated. Haplotype and sequence analysis excluded mutations in *OPA1*, *OPA3*, *OPA4*, *OPA5*, *GJB2*, and *GJB6*. A positive LOD score of $Z = 1.61$ for 4p16.3 followed by sequencing of the *WFS1* gene led to identification of a novel *WFS1* missense mutation, E864K.

Four individuals carrying this mutation had optic atrophy and hearing impairment. The three with the most extended observation periods (III:3, IV:4, V:3) presented initially with a pure sensorineural moderate hearing impairment with shallow mid frequency U-shaped audiograms (fig 2, tables 2 and 3). The hearing impairment was progressive, becoming moderate to severe with flat or gently sloping audiogram configurations. The hearing impairment found in other family members was phenotypically different. In III:5 and III:7, the hearing impairment was age related, presenting in old age. The profound congenital hearing impairment in IV:12 and the more moderate deeply U-shaped hearing loss in her brothe (IV:11) are so far unexplained, as we excluded mutations in the coding regions of *GJB2*, and *GJB6*.

The type of hearing impairment in V:3, IV:6, IV:4, and III:3 significantly affected the lower frequencies, although only in IV:6 did they fulfill the stringent definition of LFSNHL.

The metabolic examinations of three individuals with the WFS1 mutation revealed that two of them had overt but undiagnosed diabetes (IV:6) or impaired glucose tolerance (IV:4) and that two mutation carriers (IV:6 and V:3) had impaired insulinogenic index. Thus, these data of impaired glucose regulation indicate that the *WFS1* mutation may affect the pancreatic β-cell function as well.

Altogether, at least 20 different missense mutations in *WFS1* have been reported to associate with autosomal dominant LFSNHL; 19 in exon 8 and 1 in exon 5.[30 31] Seventeen cluster in the C-terminus of the WFS1 protein, one is located in the transmembrane domain 9, one in the cytoplamatic domain 5, and one sporadic case in the N-terminus.[20 21 32 33] It is well known that mutations in the *WSF1* gene can cause variable expression, giving rise to different clinical complications such as hearing, vision, diabetes, and depression. In a large non-syndromic autosomal dominant LFSNHL family reported by Young *et al*[21], one individual who was homozygous for a A716T mutation in the C-terminus had partial WS features (diabetes mellitus from 3 years of age, 20–30 dB HL in the frequency range 0.5–4 kHz and cataract, but normal renal ultrasound and no optic atrophy).[21] These findings clearly demonstrate that access to in vitro functional tests of the WFS1 protein is crucially wanted in order to assess the implications of the individual mutations.

In conclusion, this is the first report of a missense mutation (c.2590G→A, E864K) in the *WFS1* gene in a family with ADOA in combination with hearing impairment. This mutation changes the C-terminus of the protein by substitution of the non-charged amino acid glutamine by the positively charged lysine and is predicted to causes a critical change of the part of the WFS1 protein that is at the endoplasmic reticulum lumen. The glutamine E864 is conserved in the WFS1 protein in human, mouse, rat, chimpanzee, and chicken (fig 3C), supporting our interpretation that the mutation is causative. Our findings illustrate that mutations in *WFS1* may be associated with a broader clinical spectrum of phenotypes than previously reported, in terms of ADOA, impaired glucose regulation, and audiological characteristics. The increasing recognition of different inheritance patterns and the spectrum of a broad clinical presentation associated with mutations in the *OPA1*, *OPA3*, and *WFS1* genes are striking. Such observations warrant a strong awareness of the possibility of multiple organs being affected in patients seen by both ophthalmologists and audiologists. Through meticulous characterisation of the clinical implications of mutations in these genes, we may obtain a deeper understanding of the normal biological functions of *WSF1* as well as other candidate genes involved in hearing and vision disorders.

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