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Clinical and Molecular Genetic Analysis of 19 Wolfram Syndrome Kindreds Demonstrating a Wide Spectrum of Mutations in WFS1

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Summary

Wolfram syndrome is an autosomal recessive neurodegenerative disorder characterized by juvenile-onset diabetes mellitus and progressive optic atrophy. mtDNA deletions have been described, and a gene (WFS1) recently has been identified, on chromosome 4p16, encoding a predicted 890 amino acid transmembrane protein. Direct DNA sequencing was done to screen the entire coding region of the WFS1 gene in 30 patients from 19 British kindreds with Wolfram syndrome. DNA was also screened for structural rearrangements (deletions and duplications) and point mutations in mtDNA. No pathogenic mtDNA mutations were found in our cohort. We identified 24 mutations in the WFS1 gene: 8 nonsense mutations, 8 missense mutations, 3 in-frame deletions, 1 in-frame insertion, and 4 frameshift mutations. Of these, 23 were novel mutations, and most occurred in exon 8. The majority of patients were compound heterozygotes for two mutations, and there was no common founder mutation. The data were also analyzed for genotype-phenotype relationships. Although some interesting cases were noted, consideration of the small sample size and frequency of each mutation indicated no clear-cut correlations between any of the observed mutations and disease severity. There were no obvious mutation hot spots or clusters. Hence, molecular screening for Wolfram syndrome in affected families and for Wolfram syndrome–carrier status in subjects with psychiatric disorders or diabetes mellitus will require complete analysis of exon 8 and upstream exons.

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Introduction

Wolfram syndrome (MIM 222300) is the inherited association of juvenile-onset diabetes mellitus with optic atrophy (Wolfram and Wagener 1938). It is also known by the acronym "DIDMOAD" (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness). It is a progressive neurodegenerative disorder, and many patients also develop urinary-tract atony, ataxia, peripheral neuropathy, and psychiatric illness. We previously characterized the natural history of this condition in a U.K. nationwide series of 45 patients (Barrett et al. 1995). The minimum diagnostic criteria for this condition are juvenile-onset diabetes mellitus and optic atrophy, which presented at median ages of 6 years and 11 years, respectively. Of these, 33 patients (73%) developed cranial diabetes insipidus, and 28 patients (62%) presented with sensorineural deafness in the 2d decade. Renal tract abnormalities were observed in 26 patients (58%) in the 3d decade, and neurological complications (cerebellar ataxia, myoclonus) presented in 28 patients (62%) in the 4th decade. The median age at death was 30 years (range 25–49 years). On the basis of this study, the prevalence of this autosomal recessive syndrome was estimated at ~1/770,000 in the United Kingdom, with a carrier frequency of 1/354. The prevalence has been estimated at ~1/100,000 in a North American population, on the basis of the 1/175 occurrence of optic atrophy in a juvenile-onset diabetes clinic (Fraser and Gunn 1977). Although Wolfram syndrome is rare, heterozygote carriers have been reported as 26 times more likely to require hospitalization for psychiatric illness, leading to the hypothesis that heterozygosity for Wolfram syndrome gene mutations may be a significant cause of psychiatric illness in the general population (Swift et al. 1998). An increased prevalence of diabetes mellitus in first-degree relatives has also been reported (Fraser and Gunn 1977).

A search for mutations in the mitochondrial genome,

initiated as a result of the observed similarity in phenotype to patients with defects of oxidative phosphorylation, yielded contradictory results (Bundey et al. 1992; Jackson et al. 1994; Barrientos et al. 1996). Genetic-linkage studies linked Wolfram syndrome to the short arm of chromosome 4 (4p16) (Polymeropoulos et al. 1994; Collier et al. 1996). The gene, WFS1/Wolframin, was recently identified by Inoue et al. (1998) and Strom et al. (1998) and was found to consist of eight exons encompassing 33.4 kb genomic DNA encoding a polypeptide of 890 amino acids with an apparent molecular mass of 100 kD. Hydrophobicity analysis suggested a transmembrane protein comprising three structural domains; a hydrophilic amino terminus separated from a hydrophilic COOH tail by a hydrophobic region containing nine predicted transmembrane segments (fig. 1; TMpred). These two studies identified loss-of-function mutations in affected individuals in a total of 12 families screened, with no apparent phenotype-genotype correlation and an absence of mitochondrial genome abnormalities.

In the present study, we performed mutational analysis of the WFS1 gene and mitochondrial genome in 30 patients from 19 British Wolfram kindreds, the largest cohort of patients studied to date. We aimed to define the spectrum of WFS1 mutations in our population, to correlate these mutations with the clinical findings in our patients, and to determine whether a molecular genetic screening strategy for predictive use in apparently unaffected siblings from Wolfram syndrome families would be feasible.

Patients and Methods

Patients

Patients for this study were selected on the basis of (a) availability of DNA, (b) previous inclusion in a U.K. national cohort (Barrett et al. 1995), and (c) inclusion in a previous genetic linkage study (Collier et al. 1996). Patients were recruited from major referral centers, a national DIDMOAD register set up under the auspices of The British Diabetic Association, and The British Neurological Surveillance Unit. Minimal ascertainment criteria were juvenile-onset (age <30 years) diabetes mellitus and optic atrophy. These criteria were chosen because they were the only features consistently present and were the earliest to develop in 166 of 168 case

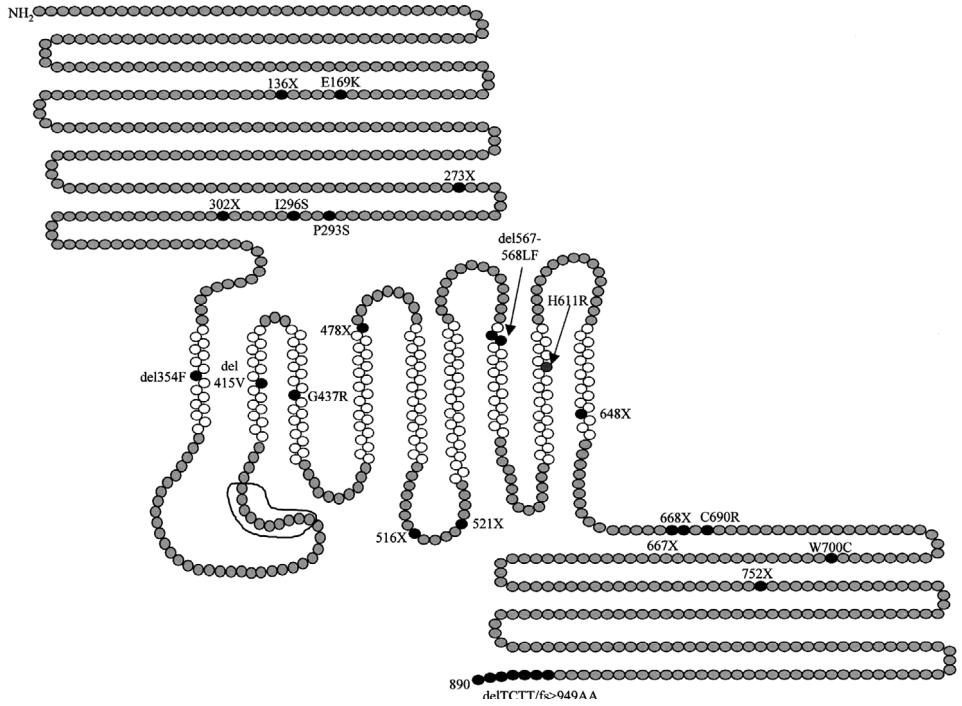


Figure 1 Hypothetical structure of the Wolframin protein, and positions of mutations detected in British families. The amino acid sequence of Wolframin (GenBank accession number Y18064) was analyzed for hydrophobicity with the transmembrane prediction program, TMpred. The best prediction for the sequence is schematically represented in the figure above. Grey circles denote hydrophilic domains, and the unblackened circles denote the best-predicted transmembrane domains. Blackened circles denote amino acid residues that were deleted or mutated to STOP codons, or sites of missense mutations in the present study. Residue 611 is highlighted by dark grey. The region of Wolframin that was found to have homology to the prenyltransferase α-subunit repeat structure has been circled (residues 393–402).

reports (Blasi et al. 1986). All patients were visited at home, for clinical history taking, examination, and blood sampling for DNA extraction. Each patient's hospital record was also examined, then follow-up visits were arranged to initiate further investigations. All patients were examined, with pupils dilated, by experienced ophthalmologists. Studies were carried out with the informed consent of each patient and with the approval of the regional ethics committee. Blood was drawn from a member of staff, for DNA extraction, to act as a normal control for sequencing analysis. Polymorphisms and missense mutations were screened against a panel of 128 CEPH normal alleles.

DNA Extraction

Blood samples were obtained from all available family members after informed consent was given. Genomic DNA was extracted from whole blood with use of Puregene DNA extraction kits (Gentra Systems), according to the manufacturer's instructions, and diluted to stock solutions of 500 ng/μl.

PCR Amplification and Sequencing of the WFS1 Gene

Exons 2–8 of the WFS1 gene were amplified with a combination of 11 primer pairs (Inoue et al. 1998). PCR reactions were performed in a 20-μl vol containing 200–500 ng DNA, 6 pmol each of forward and reverse primer, 200 μM dATP, dTTP, dGTP, and dCTP (Pharmacia), 1 × PCR buffer (20 mM Tris-HCl [pH 8.4] and 50 mM KCl) (Gibco-BRL), 1.5 mM $MgCl_2$, and 1 U of Ampli*Taq* polymerase (PE Biosystems). After an initial denaturation step of 94°C for 5 min, reactions were cycled at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s for 35 cycles, with a final extension step of 72°C for 10 min in a Hybaid Omnigene PCR machine. Unincorporated dNTPs and primers were removed by treatment of 5–10 μl PCR reactions with 10 U of exonuclease I and 2 U of shrimp alkaline phosphatase (PCR Product Pre-Sequencing kit, Promega) for 15 min at 37°C, followed by 80°C for 15 min. Sequencing reactions were performed with either the original amplification primers or with M13–21 forward primer (5′-TGTAAAACGACGGCCAGT-3′) or M13 reverse primer (5′-CAGGAAACAGCTATGACC-3′), which had been engineered into the 5′ ends of the PCR primers (Inoue et al. 1998). Reactions containing 2 μl purified PCR product, 5 pmol primer, and 4 μl dRhodamine dye terminator reaction mix (PE Biosystems), in a final vol of 10 μl, underwent 25 cycles each, consisting of 96°C for 30 s, 50°C for 15 s, and 60°C for 4 min. PCR DNA was precipitated with 0.1 vol of 3M sodium acetate (pH 4.6) and 2.5 vol of 95% ethanol on ice for 10 min, followed by centrifugation at 13,000 rpm for 30 min. DNA pellets were washed with 70% ethanol, dissolved in 2 μl loading buffer (83% deionized formamide, 4.1mM EDTA [pH 8.0], and 8.3 mg/ml dextran blue), and sequences were generated and analyzed on an ABI 377 Gene Sequencer according to the manufacturer's instructions (PE Biosystems).

Screening of Control Alleles

PCR reactions were performed in a 100-μl vol containing 50 ng of DNA from CEPH controls, 400 ng each of forward and reverse primers, 200 mM dATP, dTTP, dGTP, and dCTP (Pharmacia), 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, and 0.6 U of Ampli*Taq* polymerase (PE Biosystems). After an initial denaturation step of 94°C for 5 min, reactions were cycled at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s for 35 cycles, with a final extension step of 72°C for 10 min in a PE Biosystems 9600 thermal cycler.

For the digestions, 5 μl of the PCR product was digested with an enzyme, according to the protocol supplied with each enzyme, in a 15-μl vol. Ten microliters of the restriction digestion were loaded onto a 3% agarose gel and electrophoresed for 2 h.

Mitochondrial Genome Analysis

A PCR *Apa*I restriction-site assay was used to screen for the mitochondrial tRNA Leu (UUR) A→G (nucleotide 3243) mutation (Goto et al. 1990), which has been associated with maternally transmitted diabetes and deafness (van den Ouweland et al. 1992). The final cycle of PCR was labelled with P^{33} dATP, permitting detection of the mutation at levels of heteroplasmy <1%. Blood DNA was also screened for the 11778A→T and 3460A→T mutations associated with Leber's hereditary optic neuropathy (LHON) with use of allele-specific PCR (Norby 1993).

mtDNA was also analyzed for major rearrangements by long-range PCR (Li et al. 1995). PCRs were performed in a final vol of 50 μl with 5 pmol each of primers L1 (nucleotides 2695–2720) and H3 (nucleotides 16459–16436), 2.5 mM $MgCl_2$, 250 μM of each dNTP, 1 × Bio-Optiform 111 buffer (Bioline), and 1.5 of U Bio-X-ACT *Taq* polymerase (Bioline). PCRs were hot started at 80°C with the addition of 20–50 ng of DNA and were denatured at 95°C for 10 s and at 68°C for 10 min, plus 30 s for each subsequent cycle (25 cycles). PCR products were electrophoresed on 0.7% Seakem agarose gels.

Results

Clinical Data

The clinical features of our cohort have been described elsewhere by Barrett et al. (1995), and family trees and linkage data have been presented by Collier et al. (1996). Ascertainment bias was negated by use of the minimum

ascertainment criteria of juvenile-onset diabetes mellitus and optic atrophy. The ascertainment criteria were validated by comparison of index with secondary patients. All secondary patients also had diabetes mellitus and optic atrophy; there was no significant difference in the ages at onset or prevalence of complications between index and secondary patients ($P > .02$). It was, therefore, concluded that intrafamilial variability was not significant in our cohort. Thirty subjects from 19 families in this cohort were investigated for the current study. The clinical details are summarized intable 1. The families included in our genetic-linkage study are indicated by the original family letter (Collier et al. 1996). All families were of white U.K. origin except one family from Pakistan (family 11) and one family of mixed African/Arabic descent (family 1[B]). This last family was the only one that showed psychiatric signs; these included short-term memory loss, hallucinations, endogenous depression, emotional lability, and presenile dementia reported in a deceased affected sibling. There was no evidence of psychiatric illness in the other patients as judged from the patient history, formal examination, or hospital records.

Magnetic-resonance imaging (MRI) scans were available for six patients, including the two siblings with psychiatric signs (patients 2016, 2017, 2054, 7508, 7509, and 1945). In all six cases, the MRI scans revealed generalized brain atrophy, especially in the cerebellum, medulla, and pons; absence of signal in the posterior pituitary; and reduced signal in the optic nerve.

Mutation-Analysis Data

The WFS1 cDNA sequence (GenBank accession number AF084481) was compared with and found to be almost identical to the Wolframin cDNA sequence (GenBank accession number Y18064). Eight differences were found: seven were silent mutations, and the remaining G→A conversion resulted in a conservative substitution of 611H→R (fig. 1). In our study, sequences obtained from patients were compared with the WFS1 sequence and with normal control chromosomes. The A of the ATG of the initiator Met codon was denoted as "nucleotide +1." Sequencing of exons 2–8 (exon 1 is noncoding) revealed mutations in all the familial patients and in eight of the nine sporadic patients (table 2). In one of the singleton patients (patient 1519), only one mutation was identified. The majority of mutations were nonsense or frameshift mutations (fig. 1) and suggested WFS1 loss-of-function mutations as the cause of Wolfram syndrome. The effect, if any, of these mutations on mRNA transcript stability is not known.

Deletions

Single-bp deletions resulting in frameshifts and premature STOP codons were detected in five patients (table 2). A single-bp deletion of nucleotide 1549 in exon 8 was found as a heterozygous mutation in two families (family 2[D] and family 10[W]), resulting in a frameshift at codon 517 (Arg) and an early STOP codon, predicting a truncated protein of only 521 amino acids (aa). Nucleotide 2433 in exon 8 was deleted from both alleles in family 6(I), resulting in a frameshift and a truncated protein of 861 aa. The parents of this affected subject were known to be consanguineous. Family 4(F) was found to have a 14-bp deletion, resulting in 537Cys→X and early termination.

In-frame deletions were also detected: a 3-bp deletion, nucleotides 1060–1062, in exon 8, in family 16 resulted in the heterozygous loss of Phe354. Another in-frame deletion (loss of Val415, nucleotides 1243–1245 in exon 8) was found in family 9(S). A heterozygous 6-bp deletion was found in exon 8 of family 3(E) (nucleotides 1698–1703) with the loss of codons 567–568 (Leu and Phe). A 4-bp heterozygous deletion of nucleotides 2648–2651 was found in families 8(T), 10(W), 12, and 18 (fig. 2*A* and 2*B*). This deletion resulted in a frameshift, a readthrough of the normal STOP codon, and termination at a downstream UAG at nucleotide 2850 (normal sequence) (fig. 2*A*). The mutant protein encoded by this sequence is 949 aa long, with a predicted molecular mass of 106 kD (fig. 2*B*) (calculated with program pI/MW [ExPASy web site]).

Substitutions

Several nonsense mutations were detected in the families studied. A nonsense mutation in exon 4, 136Glu→X (CAG→UAG), was found in family 1(B) (fig. 3*A*). This mutation was present in all three affected patients as a homozygous mutation and predicted a severely truncated protein product of 135 aa, which lacked the transmembrane domains and the hydrophilic carboxy tail. Affected members of family 2(D) were found to be heterozygous for the 1944G→A substitution, which resulted in 648Trp→X and loss of the hydrophilic carboxy terminus (fig. 1). A G→A substitution at nucleotide 1,433, resulting in 478Trp→X, was found in families 4(F), 5(H), and 16 (fig. 3*B*). The resulting predicted truncated protein product encodes for the hydrophobic amino terminus and for only four of the predicted transmembrane-spanning domains (fig. 1). Family 5(H) was found to harbor a second nonsense mutation, 2002C→T, which resulted in 668Glu→X (fig. 1). Patients from family 7(J) had a 906C→A substitution that resulted in 302Tyr→X (fig. 1). A substitution of 1999C→T in family 8(T), and the corresponding Gln→X mutation at aa residue 667, predicted a protein product with the majority of the carboxy tail deleted (fig. 1). The affected patient in family 12 was found to be heterozygous for a 2254T→G substitution that converted 752Glu→X. This mutation

Table 1

Clinical Features of Patients Analyzed in Mutation Analysis

Family and Patient No.[a]	Age (Years)[b]	Sex	Consanguinity[c]	Diabetes Mellitus (Age at Onset, in Years)	Optic Atrophy (Age at Onset, in Years)	Diabetes Insipidus (Age at Onset, in Years)[d]	Deafness (Age at Onset, in Years)[d]	Renal Tract Abnormalities (Age at Onset, in Years)[d]	Neurological Abnormalities (Age at Onset, in Years)[d]	Other Complications (Age at Onset, in Years)
1(B):										
IV 71558	34	M	+	5	11	+	+	+	+	Psychiatric
IV 10 7508	43	M	+	4	17	17	18	23	38	Psychiatric
IV 15 7509	34	M	+	4	12	11	11	11	30	Psychiatric
2(D):										
II 1 1270	11	M	−	3	5	3	9	−	9	
II 2 1271	9	F	−	4	4	4	6	−	−	
3(E):										
II 1 1547	48 RIP	F	−	10	10	32	32	46	44	
II 3 1588	42	F	−	10	15	40	−	−	−	
4(F):										
II 1 5533	40	M	−	7	10	12	30	34	36	Gonadal
II 2 5534	39	M	−	12	15	12	15	33	36	Gonadal
5(H):										
II 2 2781	38	F	−	12	12	16	16	−	35	
II 3 3304	35	M	−	6	6	11	6	20	30	
6(I):										
II 1 5284	38	M	+	2	8	15	9	18	32	
7(J):										
II 1 2016	29	M	−	8	8	12	−	−	28	
II 3 2017	25	F	−	11	9	11	−	−	−	
8(T):										
II 1 5216	26	F	−	7	11	13	−	−	−	
II 2 8277	24	M	−	7	11	13	−	−	22	

9(S):										
II 1 7087	34	F	−	4	7	−	−	−	−	
II 2 7669	32	F	−	6	8	24	8	−	30	
II 3 7668	31	M	−	6	8	18	7	28	−	
10(W):										
II 3 6031	48	F	−	4	12	14	−	20	34	
II 5 6032	31	F	−	5	12	2.5	−	27	29	
11 :										
1709	18	M	+	5	9	−	12	−	−	
12:										
3328	23	M	−	4	11	14	−	−	−	
13:										
7944	30	M	−	16	16	−	16	−	−	
14:										
1519	33	F	−	5	13	11	22	24	30	
15:										
5461	37 RIP	F	−	11	7	23	32	30	32	
16:										
5514	39	M	−	3	11	24	16	34	36	
17:										
4815	9	M	−	2	7	7	5	−	−	
18:										
1945	29	M	−	5	9	−	−	10	26	Respiratory
19:										
2054	34 RIP	M	−	1.5	8	18	9	20	29	

[a] Family letters refer to those in the study by Collier et al. (1996).

[b] RIP: Rest in peace (patient died).

[c] A plus sign (+) denotes consanguinuity within a family, and a minus sign (−) denotes absence of consanguinuity.

[d] A plus sign (+) denotes complication present, age of onset unknown. A minus sign (−) denotes complication absent.

Table 2

Mutations in the WFS1 Gene

Family (Patient No[s].)[a]	Exon	Nucleotide Change	Amino Acid Change[b]	Type of Mutation
1(B)[a] (7509, 7508, 1558)	4	406C→T	Q136X	Nonsense
2(D) (1270, 1271)	8	1549del (C); 1944G→A	Del517fs/ter521; W648X	Frameshift/truncation; nonsense
3(E) (1547, 1588)	8	1698-1703del (CTCTTT); 1309G→C	Del567-568LF; G437R	Deletion; missense
4(F) (5534, 5533)	8	1611-1624del (CTTCATGTGGTGTG); 1433G→A	Del538-542fs/ter537; W478X	Frameshift/truncation; nonsense
5(H) (2781, 3304)	8	1433G→A; 2002C→T	W478X; Q668X	Nonsense; nonsense
6(I) (5284)	8	2433del (A)	Del812fs/ter861	Frameshift/truncation
7(J) (2016, 2017)	8	906C→A; 2206G→A	Y302X; G736S	Nonsense; missense
8(T) (5216, 8277)	8	1999C→T; 2648-2651del (TCTT)	Q667X; Del883fs/ter949	Nonsense; frameshift/elongation
9(S) (7087, 7668, 7669)	8	1243-1245del (GTC)	Del415V	Deletion
10(W) (6032, 6031)	8	1549del (C); 2648-2651del (TCTT)	Del517fs/ter521; Del883fs/ter949	Frameshift/truncation; frameshift/elongation
11 (1709)	8	2654C→T	P885L	Missense
12 (3328)	8	2648-2651del (TCTT);2254G→T; 2100G→T	Del883fs/ter949; E752X; W700C	Frameshift/elongation; nonsense; missense
13 (7944)	8	874C→T; 817G→T	P292S; E273X	Missense; nonsense
14 (1519)	8	2254G→T; ND	E752X; ND	Nonsense: ND
15 (5461)	8	505G→A; 2068T→C; 887T→G	E169K; C690R; I296S	Missense; missense; missense
16 (5514)	8	1060-1062del (TTC); 1433G→A	Del354F; W478X	Deletion; nonsense
17 (4815)	8	817G→T; 1504ins24bp	E273X; 502Sins/8aa	Nonsense; in-frame insertion
18 (1945)	8	2648-2651del (TCTT)	Del883fs/ter949	Frameshift/elongation
19 (2054)	ND	ND	ND	ND

NOTE.—Abbreviations: del = deletion, fs = frameshift, ter = termination, and ND = none detected.

[a] Family letters refer to those in Collier et al. (1996).

[b] Amino acids are given in single-letter code.

was also found in family 14 (table 2). A 273Glu→X, caused by the 812T→G mutation, was also found in two of the families studied (families 13 and 17).

A homozygous missense mutation was found in the affected patient from family 11 (885Pro→Leu [CCA→CUA]). This patient had consanguineous parents. Other missense mutations were present as heterozygous mutations in several families: 437Gly→Arg in family 3; 700Trp→Cys in family 12; 292Pro→Ser in family 13; and 169Glu→Lys, 296Ile→Ser, and 690Cys→Arg in family 15. These missense mutations were not present in 128 CEPH control chromosomes.

Noncoding Polymorphisms

Polymorphisms that did not code for an aa change or that resulted in conservative aa changes are summarized in table 3. A substitution, 1832→G, resulted in a conservative 611His→Arg change in 54% of the individuals analyzed. Another polymorphism, 997A→G with a resulting 333Ile→Val, was detected in 41% of individuals. The functional significance of these changes is not known at present.

Genotype-Phenotype Analysis

The data were analyzed for genotype-phenotype relationships, and several interesting cases were noted. The three affected subjects analyzed in family 1(B) carried a homozygous nonsense mutation in exon 4. This predicted a severely truncated protein of 135 aa, deleting the transmembrane and carboxy-terminal domains. The affected individuals in this family suffered severe psychiatric complications. These complications developed when the patients were in their 30s and included short-term memory loss, emotional lability, hallucinations, and endogenous depression. This family was a large consanguineous pedigree of African/Arabic origin, with affected individuals in two separate branches of the family. An affected older sister of the affected brothers in this study had died at age 39 years, after admission, in her 20s, to hospital when she was given a diagnosis of presenile dementia, endogenous depression, and frequent hallucinations.

The affected subject in family 18 (patient 1945) developed central respiratory failure at age 16 years and required a permanent tracheostomy. MRI demonstrated severe brain-stem atrophy. Sequencing analysis revealed a homozygous 4-bp deletion, resulting in a frameshift

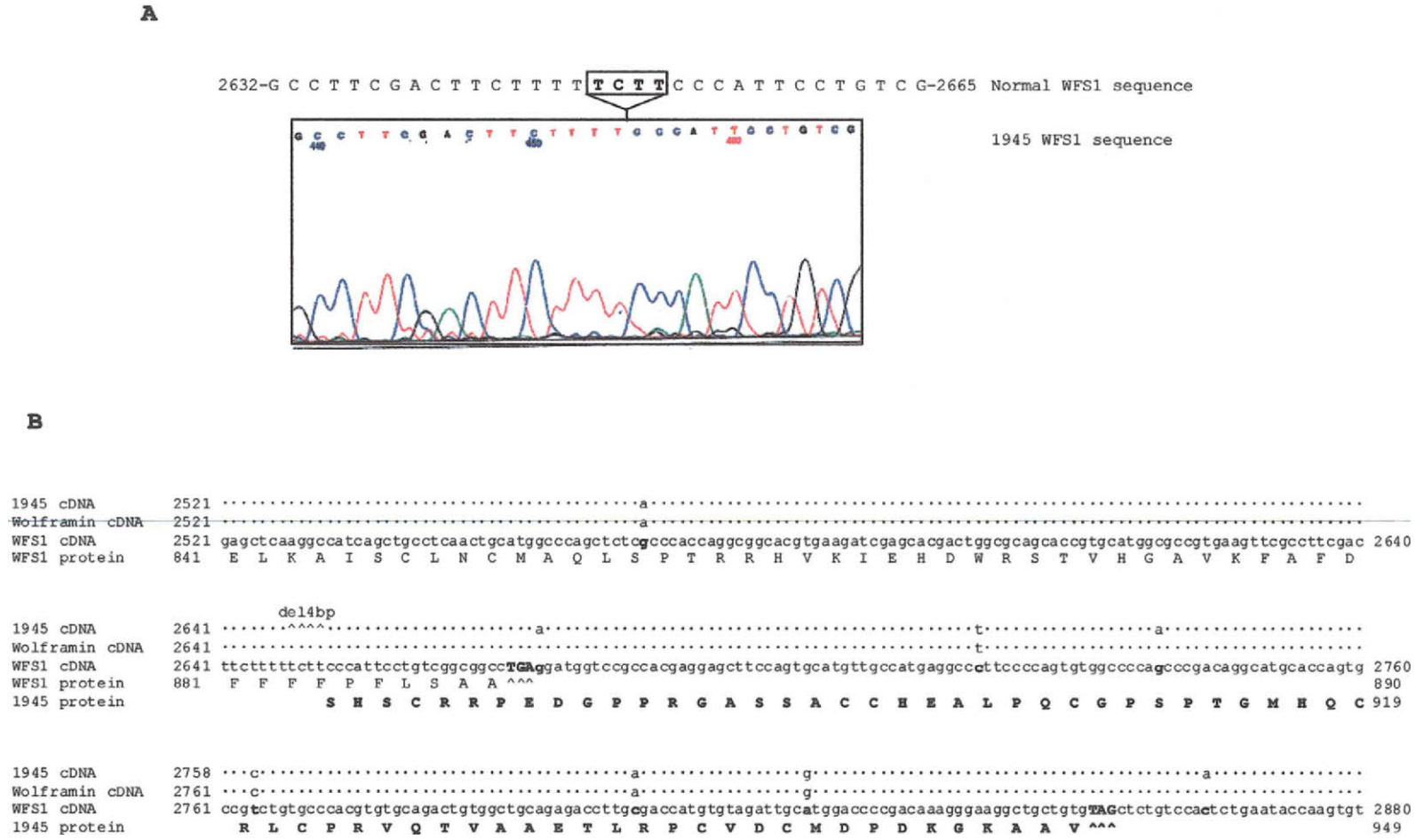
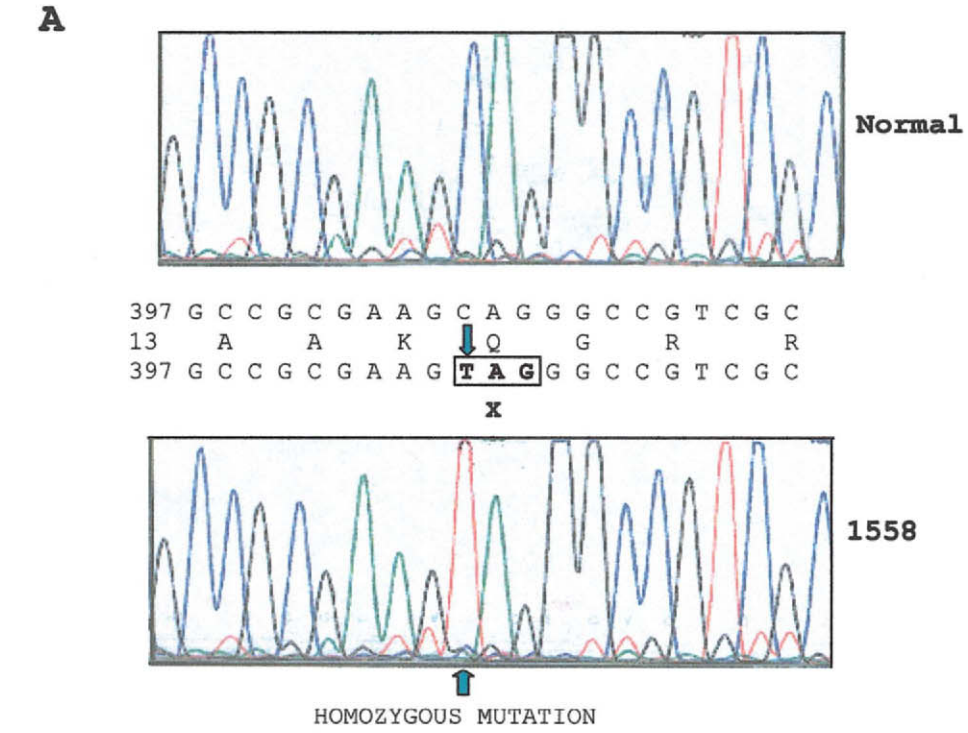


Figure 2 A 4-bp deletion and the resultant predicted chimeric 949-aa protein in the patient from family 18. DNA from exon 8 was amplified, purified, and sequenced with M13-21 forward primer. *A*, representative electropherogram of a homozygous 2648TCTT deletion in patient 1945 from family 18. The wild-type sequence is shown above the deleted sequence. *B*, depiction of the predicted aa sequence of the mutant protein, which is 59 aa longer than the wild-type protein. The deleted nucleotides are boxed, and the stop codons are shown in boldfaced capital letters. A single-letter code is used to denote aa.



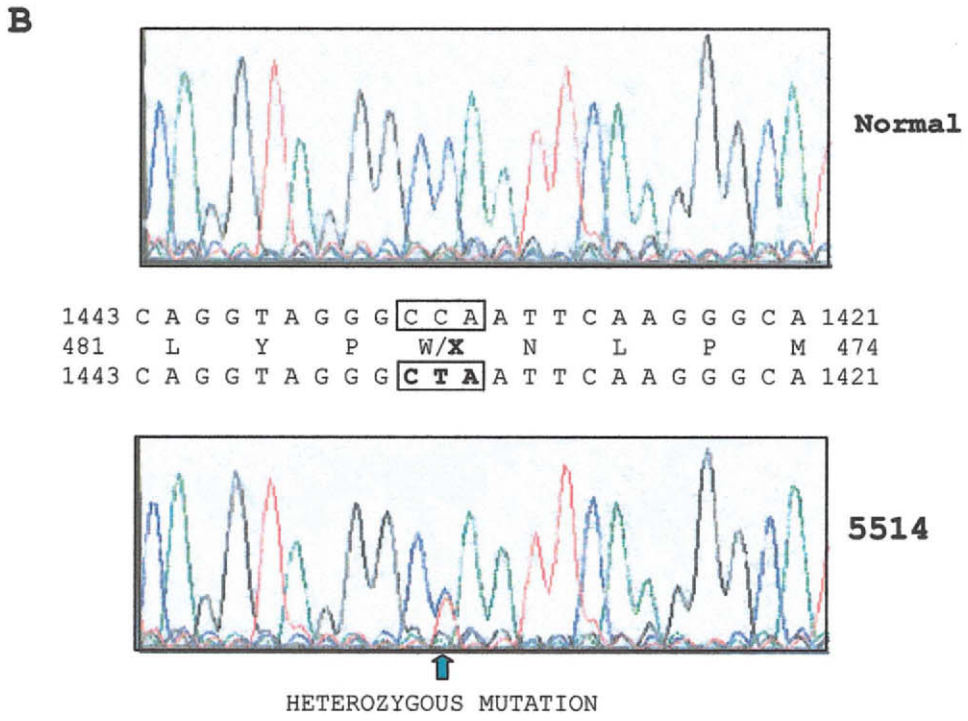


Figure 3 Nonsense mutations in patients 1558 and 5514. Exon 8 DNA was amplified by PCR, purified, and sequenced on an ABI 377 automated sequencer. *A,* representative electropherograms from a normal control and from patient 1558 (family 1[B]) harboring a homozygous C→T substitution that results in Q136X and premature termination of the protein. *B,* representative electropherograms of the reverse complement sequence of residues 1421–1443. Patient 5514 carries the heterozygous nonsense mutation, 1433C→T, and W478X, compared to the normal control.

Table 3

Polymorphisms

Nucleotide Change	Amino Acid	No. of Alleles (%)[a]
1831A→G	H611R	29/54 (54)
1185T→C	V395V	27/54 (50)
2433A→G	K811K	24/54 (44)
997A→G	I333V	22/54 (41)
2565G→A	S855S	19/54 (35)
684G→C	R228R	14/54 (26)
1500T→C	D500D	12/54 (22)
1023C→T	F341F	7/54 (13)
1725C→T	A575A	6/54 (11)
2322G→A	K774K	5/54 (9)
2292C→T	P764P	2/54 (4)
1761G→T	R587R	2/54 (4)
1367C→A	R456R	1/54 (2)
993C→T	F331F	1/54 (2)

[a] Frequency of polymorphism is calculated as the number of affected alleles, as a percentage of the total number of alleles.

and readthrough, predicting a chimeric protein of 949 aa (fig. 2). The patient had an older sister with Wolfram syndrome who had died at age 28 years from brain-stem atrophy and central respiratory failure. Five other patients from three families (families 8, 10, and 12) in our cohort were heterozygous for the 4-bp deletion; however, central respiratory failure was not observed in these patients.

The affected individual in family 11 had a homozygous missense mutation at codon 885 (Pro→Leu), 5 aa from the carboxy terminus. This patient has a mild phenotype and has not yet developed diabetes insipidus, renal involvement, or neurological abnormalities. His deafness is mild and he does not require a hearing aid.

The spread of mutations was compared between families in which symptoms developed at an earlier age ($\leq$5 years) and those in which symptoms developed at a later age ($\geq$6 years). There was no obvious difference in the proportion of different mutations between the two groups.

mtData

Lymphocyte-derived DNA from all patients was screened for the mitochondrial tRNA Leu 3243 mutation and for the 11778 and 3460 mutations associated with LHON. No mutations were found in this cohort. In addition, lymphocyte-derived DNA was examined for the presence of major mitochondrial rearrangements, including deletions, as outlined above. No rearrangements were found.

Discussion

Our study presents detailed clinical and mutation-analysis data on the largest cohort of patients with Wolfram syndrome studied to date. A total of 30 patients from 19 kindreds were screened for mutations in the WFS1 gene and in their mtDNA. WFS1 gene mutations were identified on both alleles in 28 of 30 patients and on one allele of 1 patient (patient 1519). This confirms the genetic homogeneity of this disorder in our population. We identified 24 different mutations, 23 of which were novel, comprising 8 missense mutations, 8 nonsense mutations, 3 in-frame deletions, 1 in-frame insertion, and 4 frameshift mutations. A nonsense mutation, W648X, identified in affected individuals from family 3(E), had been previously reported (Inoue et al. 1998). There was only one patient (patient 2054, family 19) in whom no mutations were identified. The possibility of mutations in the promoter or intronic sequences was not addressed, and this possibility may explain the disease manifestation in this patient. Of the 28 patients with Wolfram syndrome for whom both mutations were identified, 19 were compound heterozygotes for two different mutations. This has been reported in many other autosomal recessive conditions, including ataxia-telangiectasia, in which most patients are compound heterozygotes for truncated proteins (Concannon and Gatti 1997). No evidence of pathogenic mtDNA mutations was detected in our cohort of patients. The mt3243 mutation in tRNA Leu (UUR), associated with diabetes mellitus and deafness, was absent, and no major rearrangements of mtDNA were found in lymphocyte DNA.

To identify any possible mutational hot spots in the WFS1 protein sequence, we combined our data with results from two other reports (Inoue et al. 1998; Strom et al. 1998). The frequency of mutations was plotted against aa residues (fig. 4). Although the absolute numbers of patients examined is still small, we noted that the majority of frameshifts and nonsense mutations localized to the predicted transmembrane domains of the protein. Mutations resulted in loss of the carboxy tail in 28 (47%) of 60 chromosomes analyzed, and an additional 7 (12%) of 60 chromosomes had a 4-bp deletion resulting in a predicted protein of 949 aa with a chimeric carboxy terminus. Missense mutations were identified on 13 (22%) of 60 chromosomes and were not observed in a panel of 128 CEPH chromosomes. There was no obvious common mutation in our families or in those reported elsewhere. A 4-bp deletion at position 2648 was found in four of the U.K. families (families 8[T], 10[W], 12, and 18); however, genotyping across the WFS1 region on 4p did not reveal a common haplotype in the two families reported elsewhere (families T and W; Collier et al. 1996). The lack of common mutations and the presence of multiple private mutations imply that screening of the WFS1 gene will not be straightforward and will require sequence analysis of exon 8 and, possibly, of other exons if mutations are not identified.

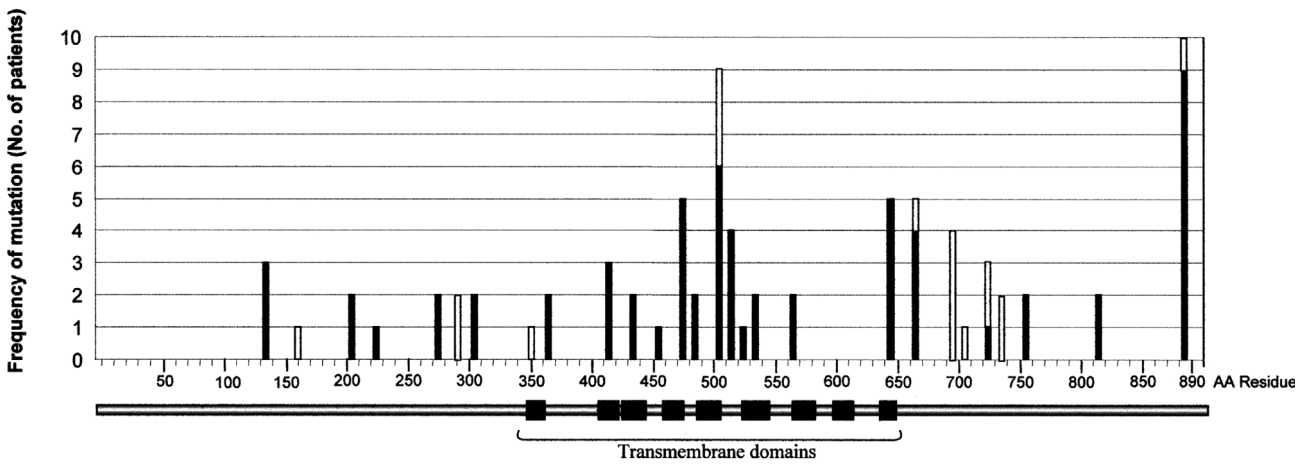


Figure 4 Clustering of deletions and nonsense mutations in the transmembrane domain of WFS1. Mutation-screening data from the present study and two other studies (Inoue et al. 1998; Strom et al. 1998) were combined and plotted as frequency (number of patients) of mutation against aa residue. Blackened bars represent deleted residues or positions of nonsense mutations, and unblackened bars represent missense mutations. A linear schematic of the Wolframin protein is depicted below the graph, with the transmembrane domains denoted by blackened boxes.

The detailed clinical data on our cohort of patients enabled us to investigate whether particular mutations were associated with common clinical patterns. Family 1(B) carried a homozygous mutation predicting a severely truncated protein. This family has all the main complications of Wolfram syndrome, including psychiatric complications. This phenotype may be due to complete absence of any residual WFS1 activity or to aberrant function of the short 136 aa mutant protein. Alternatively, the affected members may have all the complications because they have lived long enough to develop them. It is also possible that the mutation may be in linkage disequilibrium with a locus for bipolar affective disorder on chromosome 4p (Blackwood et al. 1996). This has been observed for the bipolar locus and the gene for Darier disease on chromosome 12q (Craddock et al. 1993).

The affected subject from family 18 (patient 1945), with severe brain-stem atrophy, was homozygous for a 4-bp deletion, predicting an elongated protein (949 aa). Given that severe respiratory involvement was observed only in this patient and his sister, we speculate that this phenotype is associated with aberrant function of the chimeric 949 aa WFS1 protein; however, at present there are no functional data to support this speculation. The affected individual from family 11 had a homozygous missense mutation at codon 885 and a mild phenotype. He may be too young to have developed other complications. Alternatively, this carboxy-terminal missense mutation may disrupt only a limited spectrum of WFS1 function.

Our characterization of the spectrum of mutations in Wolfram syndrome now allows us to offer genetic testing to unaffected younger siblings in Wolfram families. We previously estimated the chance of Wolfram syndrome manifesting itself in an affected individual by their 15th birthday at ~95% (Barrett et al. 1995). We now have the potential to provide reassurance for siblings of affected patients at a much earlier age. Heterozygote carriers of the Wolfram gene are reported to be 26 times more likely to require hospitalization for psychiatric illness (Swift et al. 1998) and to have an increased prevalence of diabetes mellitus (Fraser and Gunn 1977). It may be possible to screen populations with psychiatric illness and diabetes mellitus for carriers of these mutations. The obvious candidates include patients with bipolar depression, for which a locus has been mapped to chromosome 4p (Blackwood et al. 1996), and patients with type 2 diabetes mellitus. Our cohort includes an excess of families in which relatives of affected patients have type 2 diabetes. Patients with type 2 diabetes share two characteristics with Wolfram diabetics: they do not usually have evidence of autoimmune insulitis, and they usually have an element of insulin deficiency as well as insulin resistance.

The aim of the present study was to sequence and characterize the WFS1 gene in a large cohort of Wolfram patients and to determine any genotype-phenotype relationships. All except two patients had mutations on both alleles of their WFS1 gene. Even though the function of the WFS1 protein is not yet known, it is likely that the cytoplasmic carboxy terminus of WFS1 is interacting with other, as yet unidentified, proteins. Mutation or deletion of the carboxy-terminal domain could disrupt these interactions and, hence, protein function.

Studies are currently under way to investigate the effects of these mutations on protein function.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

ExPASy, http://www.expasy.ch/ (for pI/MW program)
Fondation Jean Dausset–CEPH, http://ceph-genethon-map.cephb.fr/ (for control alleles)
GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank/index.html (for WFS1 cDNA sequence [AF084481] and Wolframin cDNA sequence [Y18064])
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for Wolfram syndrome [MIM 222300])
TMpred, http://www.ch.embnet.org/software/TMPRED_form.html (for TMpred software)

References

Barrett TG, Bundey SE, Macleod AF (1995) Neurodegeneration and diabetes: UK nationwide study of Wolfram (DIDMOAD) syndrome. Lancet 346:1458–1463

Barrientos A, Casademont J, Saiz A, Cardellach F, Volpini V, Solans A, Tolosa E, et al (1996) Autosomal recessive Wolfram syndrome associated with an 8.5-kb mtDNA single deletion. Am J Hum Genet 58:963–970

Blackwood DH, He L, Morris SW, McLean A, Whitton C, Thomson M, Walker MT, et al (1996) A locus for bipolar affective disorder on chromosome 4p. Nat Genet 12: 427–430

Blasi C, Pierelli E, Rispole E, Saponara M, Vingolo E, Andreani D (1986) Wolfram's syndrome: a clinical, diagnostic, and interpretive contribution. Diabetes Care 9:521–528

Bundey S, Poulton K, Whitwell H, Curtis E, Brown IA, Fielder AR (1992) Mitochondrial abnormalites in the DIDMOAD syndrome. J Inher Metab Dis 15:315–319

Collier DA, Barrett TG, Curtis D, Macleod A, Arranz MJ, Maassen JA, Bundey S (1996) Linkage of Wolfram syndrome to chromosome 4p16.1 and evidence for heterogeneity. Am J Hum Genet 59:855–863

Concannon P, Gatti RA (1997) Diversity of ATM gene mutations detected in patients with ataxia-telangiectasia. Hum Mutat 10:100–107

Craddock N, Dawson E, Burge S, Parfitt L, Mant B, Roberts Q, Daniels J, et al (1993) The gene for Darier's disease maps to chromosome 12q23-q24.1. Hum Mol Genet 2: 1941–1943

Fraser FC, Gunn T (1977) Diabetes mellitus, diabetes insipidus, and optic atrophy. An autosomal recessive syndrome? J Med Genet 14:190–193

Goto Y, Nonaka I, Horai S (1990) A mutation in the tRNA Leu (UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature 348:651–653

Inoue H, Tanizawa Y, Wasson J, Behn P, Kalidas K, Bernal-Mizrachi E, Mueckler M, et al (1998) A gene encoding a transmembrane protein is mutated in patients with diabetes mellitus and optic atrophy (Wolfram syndrome). Nat Genet 20:143–148

Jackson MJ, Bindoff LA, Weber K, Wolson JN, Ince P, Alberti KG, Turnbull DM (1994) Biochemical and molecular studies of mitochondrial function in diabetes insipidus, diabetes mellitus, optic atrophy and deafness. Diabetes Care 17: 728–733

Li YY, Hengstenberg C, Maisch B (1995) Whole mitochondrial genome amplification reveals basal level multiple deletions in mtDNA of patients with dilated cardiomyopathy. Biochem Biophys Res Comm 210:211–218

Norby S (1993) Screening for the two most frequent mutations in Leber's hereditary optic neuropathy by duplex PCR based on allele specific amplification. Hum Mutat 2:309–313

Polymeropoulos MH, Swift RG, Swift M (1994) Linkage of the gene for Wolfram syndrome to markers on the short arm of chromosome 4. Nat Genet 8:95–97

Strom TM, Hortnagel K, Hofmann S, Gekeler F, Scharfe C, Rabl W, Gerbitz KD, et al (1998) 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 7: 2021–2028

Swift RG, Polymeropoulos MH, Torres R, Swift M (1998) Predisposition of Wolfram syndrome heterozygotes to psychiatric illness. Mol Psychiatry 3:86–91

van den Ouweland JMW, Lemkes HHP, Ruitenbeek W, Sandkuijl LA, de Vijlder MF, Struyvenburg PA, van de Kamp JJ, et al (1992) Mutation in mitochondrial $tRNA^{Leu(UUR)}$ gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. Nat Genet 1:368–371

Wolfram DJ, Wagener HP (1938) Diabetes mellitus and simple optic atrophy among siblings: report of four cases. Mayo Clin Proc 13:715–718