# Diabetes and Neurodegeneration in Wolfram Syndrome

## A multicenter study of phenotype and genotype

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**OBJECTIVE**—To describe the diabetes phenotype in Wolfram syndrome compared with type 1 diabetes, to investigate the effect of glycemic control on the neurodegenerative process, and to assess the genotype-phenotype correlation.

**RESEARCH DESIGN AND METHODS**—The clinical data of 50 patients with Wolfram syndrome-related diabetes (WSD) were reviewed and compared with the data of 24,164 patients with type 1 diabetes. Patients with a mean $HbA_{1c}$ during childhood and adolescence of ≤7.5 and >7.5% were compared with respect to the occurrence of additional Wolfram syndrome symptoms. The wolframin (*WFS1*) gene was screened for mutations in 39 patients. *WFS1* genotypes were examined for correlation with age at onset of diabetes.

**RESULTS**—WSD was diagnosed earlier than type 1 diabetes (5.4 ± 3.8 vs. 7.9 ± 4.2 years; $P < 0.001$) with a lower prevalence of ketoacidosis (7 vs. 20%; $P = 0.049$). Mean duration of remission in WSD was 2.3 ± 2.4 vs. 1.6 ± 2.1 in type 1 diabetes (NS). Severe hypoglycemia occurred in 37 vs. 7.9% ($P < 0.001$). Neurologic disease progression was faster in the WSD group with a mean $HbA_{1c}$ >7.5% ($P = 0.031$). Thirteen novel *WSF1* mutations were identified. Predicted functional consequence of *WFS1* mutations correlated with age at WSD onset ($P = 0.028$).

**CONCLUSIONS**—Endoplasmic reticulum stress–mediated decline of β-cells in WSD occurs earlier in life than autoimmune-mediated β-cell destruction in type 1 diabetes. This study establishes a role for *WFS1* in determining the age at onset of diabetes in Wolfram syndrome and identifies glucose toxicity as an accelerating feature in the progression of disease.

Wolfram syndrome, also referred to as DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness), is a rare autosomal recessive syndrome (OMIM #222300) characterized by juvenile-onset diabetes, progressive neurologic degeneration, and endocrine dysfunction. Wolfram syndrome is caused by biallelic mutations of the *WFS1* gene encoding wolframin (1,2), a transmembrane glycoprotein that localizes in the endoplasmic reticulum (ER). *WFS1* has been implicated in the unfolded protein response, a cellular stress response induced by the accumulation of unfolded proteins within the ER lumen, which is pivotal to cellular homeostasis and integrity (3,4). Loss of *WFS1* function is thought to result in chronic ER stress–mediated apoptosis of pancreatic β-cells, neuroendocrine, and neuronal cells, leading to a progressive decline of endocrine function and neurodegeneration (5).

Clinically, Wolfram syndrome is suspected in a patient presenting with the minimal criteria of juvenile-onset diabetes and bilateral progressive optic atrophy (6). Additional clinical features include sensor neuronal hearing loss; neurogenic bladder; ataxia; dysarthria; dysphagia; dementia; psychiatric disease; and endocrine dysfunction, such as diabetes insipidus, hypogonadism, hypothyroidism, and growth retardation (7–9).

To date, little is known about Wolfram syndrome–related diabetes (WSD) features compared with autoimmune type 1 diabetes (10), and the contribution of glycemic control to the observed decline in neurologic and endocrine function remains undefined. Previous studies in small patient cohorts have provided some evidence for a genotype–phenotype correlation in Wolfram syndrome (11–13). To gain further insight into the natural history of Wolfram syndrome, we conducted a large-scale phenotypic study of WSD compared with type 1 diabetes, delineated the role of glycemic control in disease progression, and examined genotype–phenotype relationships.

## RESEARCH DESIGN AND METHODS

### Patient cohorts and study design

The study enrolled 50 patients with Wolfram syndrome from 40 families between 2004 and 2010. Patients were identified through the German Diabetes Prospective Documentation (DPV) database, a nationwide prospective registry that evaluates

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type 1 diabetes, and inquiries at scientific meetings. Overall, 24 centers from Germany and Southeastern European countries participated in this study (Wolfram Syndrome Diabetes Writing Group).

Criteria for patients being included in the study were juvenile-onset diabetes and optic atrophy or juvenile-onset diabetes and molecular evidence of *WFS1* mutations. Detailed clinical and laboratory data were recorded by referring physicians in a standardized questionnaire. Blood samples were taken from a subset of patients for mutation analysis. For comparison of WSD with type 1 diabetes, data of 24,164 patients with type 1 diabetes aged <21 years from the DPV database were used. The local ethics committee approved the study.

### Mutation analysis

Thirty-nine patients with unknown *WFS1* genotype were screened for mutations. Genomic DNA was extracted from peripheral blood lymphocytes according to standard procedures. Exon 1 to 8 of the *WFS1* gene, including flanking intronic regions, were amplified by PCR and sequenced on an automated sequencer (ABI 3730, Applied Biosystems, Foster City, CA). Identified mutations were compared with the Human Gene Mutation Database and the *WFS1* Gene Mutation and Polymorphism Database of the Kresge Hearing Research Institute.

### Characterization of WSD and comparison with type 1 diabetes

$HbA_{1c}$ was measured by standardized methods, as established in the referring physician's hospital, and the patient's individual mean $HbA_{1c}$ during the observation period during childhood and adolescence was determined. Data on diabetes onset, remission phase, insulin requirements, glycemic control, and complications in WSD were compared with the corresponding data of 24,164 patients with type 1 diabetes aged <21 years from the DPV database. Differences between WSD and type 1 diabetes data were statistically analyzed using the one-sample *t* test and the Fisher exact test. Normal distribution was assessed by the Kolmogorov-Smirnov test.

### Analysis of neurodegenerative and endocrine disease in WSD and effect of diabetes control on disease progression

All patients with WSD were examined for additional symptoms such as diabetes insipidus, deafness, bladder dysfunction, neurologic or psychiatric symptoms, puberty disorders, hypogonadism, or thyroid dysfunction. The association between mean $HbA_{1c}$ during childhood and adolescence and the number of Wolfram syndrome symptoms at ages 10, 11–16, and 17–21 years was assessed using the Spearman rank correlation test.

WSD patients were divided into two groups according to their mean $HbA_{1c}$ during childhood and adolescence: one group consisting of probands with a good control of diabetes, as evidenced by a mean $HbA_{1c}$ ≤7.5% and the other group with a mean $HbA_{1c}$ >7.5%, indicative of a state of chronic hyperglycemia. Age at onset of additional symptoms of Wolfram syndrome and their prevalence were compared in the two groups. Cox proportional hazard regression analysis, adjusted for the covariate "age at onset of WSD," was used to calculate the probability of any additional Wolfram syndrome symptom to appear in the years after the diagnosis of diabetes, and the results were plotted as estimated symptom-free survival function curves.

### Genotype–phenotype analysis

Patients were subdivided into the three categories according to the predicted effect of their *WFS1* genotype on wolframin function: *1*) mutations with a predicted complete loss of function, *2*) mutations with a predicted partial loss of function, and *3*) mutations with a putative minor loss of function. Age at onset of diabetes was compared among these groups using ANOVA.

## RESULTS

### Characterization of WSD and comparison with type 1 diabetes

The phenotypic characteristics of the WSD cohort compared with the data of 24,164 patients with type 1 diabetes from the DPV database are summarized in Table 1.

The initial manifestation of Wolfram syndrome was diabetes in 43 patients (86%), hearing loss in 4 (8%), and optic atrophy in 3 (6%). All but four patients were treated with insulin from the time of diagnosis of diabetes.

WSD was diagnosed earlier than type 1 diabetes (5.4 ± 3.8 vs. 7.9 ± 4.2 years; $P < 0.001$), with a lower prevalence of ketoacidosis (7 vs. 20.3%; $P = 0.049$). Autoantibodies were less often positive (10 vs. 86%; $P < 0.001$). Mean duration of remission was longer (2.3 ± 2.4 vs. 1.6 ± 2.1 years; $P = 0.064$ NS). Severe hypoglycemia occurred at a higher frequency in WSD (37 vs. 7.9%; $P < 0.001$).

As calculated from the German dataset, the prevalence of Wolfram syndrome in children and adolescents aged <21 years in Europe is 33/17,000,000 (~1:500,000). The prevalence of WSD among children and adolescents aged <21 years with diabetes is 33/24,000 (~1:730).

### Neurodegenerative disease in WSD and effect of diabetes control on disease progression

Age at onset and the prevalence of additional Wolfram syndrome symptoms in our patient cohort are reported in Table 2. In 74% of the patients, optic atrophy was the first neurodegenerative symptom after diabetes onset. Patients with atonic bladder had urinary tract dilation and/or recurrent renal tract infections. Neurologic symptoms included learning difficulties (24%), coordination deficits (16%), ataxia (12%), dysarthria or aphasia (8%), electroencephalograph abnormalities (6%), mental or motor retardation (6%), and dysphagia (4%). Magnetic resonance imaging showed cortical atrophy in 18%. Symptoms of hypo- or hypergonadotropic hypogonadism occurred in 34%, including late puberty or pubertal arrest, abnormalities in menstrual bleeding, testicular atrophy with testosterone deficiency, and erectile dysfunction.

Sudden and complete loss of visual acuity was observed in one patient after prolonged severe hypoglycemia. Acute neurologic deterioration occurred in one patient after a severe febrile infection and in one patient after general anesthesia during the second decade of life. In all patients, this acute disease progression was partially reversible over time.

A correlation was found between the number of Wolfram syndrome symptoms and the patient's mean $HbA_{1c}$ during childhood and adolescence. In the patient group, observed until the age of 17 to 21 years, this correlation was statistically significant (Spearman rank correlation coefficient, 0.485; $P = 0.012$).

The prevalence of diabetes insipidus, deafness, and neurologic and psychologic symptoms was lower, and the time of symptom-free survival for each of these additional Wolfram syndrome symptoms after diagnosis of WSD was higher, in the well-controlled WSD group with a mean $HbA_{1c}$ ≤7.5 vs. >7.5%. This is

**Table 1—*Diabetes phenotypes in patients with WSD and type 1 diabetes***

| Variable | *n* | Frequency occurrence (%) | Mean (SD) | Median (range) | Median (lower/upper quartile) values | *P* |
|---|---|---|---|---|---|---|
| Age at onset of diabetes (years) | | | | | | |
| WSD | 50 | | 5.4 (3.8) | 4.3 (1–17) | | <0.001* |
| Type 1 diabetes | 24164 | | 7.9 (4.2) | | 7.7 (4–11) | |
| Ketoacidosis pH <7.3 at diabetes manifestation | | | | | | |
| WSD | 41 | 7 | | | | 0.049† |
| Type 1 diabetes | 13,644 | 20.3 | | | | |
| Autoantibodies against β-cell elements | | | | | | |
| WSD | 31 | 10 | | | | <0.001† |
| Type 1 diabetes | 11,144 | 86 | | | | |
| $HbA_{1c}$ (%) at diagnosis of diabetes | | | | | | |
| WSD | 29 | | 10.6 (2.9) | 10.0 (5.8–16) | | 0.436* |
| Type 1 diabetes | 23,850 | | 11.0 (2.4) | | 10.8 (9.3–12.5) | |
| Remission (years)‡ | | | | | | |
| WSD | 33 | | 2.3 (2.4) | 2.0 (0–8.5) | | 0.064* |
| Type 1 diabetes | 10,195 | | 1.6 (2.1) | | 0.8 (0.4–1.7) | |
| Insulin requirements (IU/kg/day) | | | | | | |
| WSD | 46 | | | (0.46–0.9) | | |
| Type 1 diabetes | 24,018 | | 0.81 (0.3) | | 0.79 (0.6–1.0) | |
| $HbA_{1c}$ (%) | | | | | | |
| WSD | 47 | | 7.9 (1.4) | 7.8 (5–12) | | 0.064* |
| Type 1 diabetes | 23,850 | | 8.0 (1.7) | | 7.6 (6.9–8.7) | |
| Diabetic ketoacidosis after insulin substitution | | | | | | |
| WSD | 41 | 7 | | | | 0.427† |
| Type 1 diabetes | 23,504 | 4.4 | | | | |
| ≥1 severe hypoglycemia during childhood/adolescence | | | | | | |
| WSD | 38 | 37 | | | | <0.001† |
| Type 1 diabetes | 22,324 | 7.9 | | | | |
| Persistent microalbuminuria | | | | | | |
| WSD | 50 | 0 | | | | 0.005† |
| Type 1 diabetes | 15,547 | 11.1 | | | | |
| Diabetic retinopathy | | | | | | |
| WSD | 50 | 0 | | | | <0.001† |
| Type 1 diabetes | 12,096 | 0.6 | | | | |

Mean age (years) at the time of data acquisition was 18.1 for WSD and 11.9 for type 1 diabetic patients. The mean time (years) of observation from the time of diagnosis of diabetes was 12.6 for WSD and 4.0 for type 1 diabetes. *n*, number of patients examined. *One sample *t* test: $P > 0.05$ is NS. †Fisher exact: $P > 0.05$ is NS. ‡ Insulin <0.5 IE/kg/day, $HbA_{1c}$ <7.5%.

summarized in Table 2 and illustrated in Supplementary Fig. A1. Owing to the relatively small numbers of patients in each symptom-specific category, statistical significance was reached only in one category (psychologic symptoms) and in an analysis with all symptoms pooled ($P = 0.031$). No correlation was found concerning insulin usage (IU/kg/body weight) and $HbA_{1c}$ (Supplementary Fig. A2).

## Genotype–phenotype analysis

Among 39 patients screened for *WFS1* mutations, 34 mutations were detected, 13 of which were novel (Table 3). The mutational spectrum included missense, nonsense, and splice site mutations as well as smaller deletions and insertions, which were commonly associated with a shift of the *WFS1* reading frame. Most mutations were located within exon 8 of the *WFS1* gene, which encodes the COOH-terminal extracellular part of wolframin at the luminal site of the ER membrane.

Mutations were divided into three categories according to the predicted functional consequences on wolframin function (Fig. 1). Assuming that Wolfram syndrome results from a loss of *WFS1* function, patients were grouped as follows:

- Group 1: individuals carrying mutations with a predicted complete loss of function, including N-terminal nonsense and frameshift mutations;
- Group 2: individuals carrying mutations with a predicted partial loss of function, including COOH-terminal nonsense and frameshift mutations or small in-frame deletions and individuals compound heterozygous for a predicted complete and partial loss of function mutation; and
- Group 3: individuals carrying mutations with a putative minor loss of function, including missense mutations and individuals compound heterozygous for a predicted partial and minor loss of function mutation (Fig. 1).

Comparison of age at onset of WSD among these groups revealed significant differences (ANOVA, $P = 0.028$). Thus, mean age at WSD onset was 3.7 ± 1.7 years in individuals carrying mutations with a predicted complete loss of function, 5.8 ± 2.6 years in those carrying mutations with a predicted partial loss of function, and 7.5 ± 6.0 years in

**Table 2—*Symptoms of Wolfram syndrome: age at onset, prevalence, and symptom free survival with regard to glucose control in WSD***

| Variable | Optic atrophy Mean (range) | Diabetes insipidus Mean (range) | Deafness Mean (range) | Bladder dysfunction Mean (range) | Neurologic problems Mean (range) | Psychologic problems Mean (range) |
|---|---|---|---|---|---|---|
| Age in years at symptom onset | | | | | | |
| All patients | 9 (2–18) | 13 (5–22) | 10 (1–20) | 15 (1–22) | 11 (1–22) | 14 (8–20) |
| Patients with a mean $HbA_{1c}$ ≤7.5% | 9 (4–18) | 13.5 (5–18) | 6 (1–18) | 16 (1–22) | 10 (1–22) | 14.5 (14–15) |
| Patients with a mean $HbA_{1c}$ >7.5% | 9 (2–17) | 11 (5.5–22) | 11 (1–18) | 13.5 (7–18) | 11 (4–19) | 14 (8–20) |
| | % | % | % | % | % | % |
| Prevalence of symptoms | | | | | | |
| All patients | 92 | 52 | 68 | 48 | 62 | 34 |
| Patients with a mean $HbA_{1c}$ ≤7.5% | 94.7 | 31.6 | 58 | 47.4 | 42.1 | 10.5 |
| Patients with a mean $HbA_{1c}$ >7.5% | 89.2 | 64.3 | 75 | 50 | 75 | 53.6 |
| | Median (95% CI) | Median (95% CI) | Median (95% CI) | Median (95% CI) | Median (95% CI) | Median (95% CI) |
| Symptom-free survival in years after WSD diagnosis* | | | | | | |
| All patients | 4.7 (3.2–6.2) | 13 (9.7–16.3) | 9.8 (7.4–12.2) | 14.0 (12.1–15.9) | 9.9 (6.4–13.4) | 14 (10.2–17.8) |
| Patients with a mean $HbA_{1c}$ ≤7.5% | 2.2 (0.8–3.6) | 16.7 (8.21–25.2) | 9.8 (0.8–18.8) | 11.9 (9.4–14.4) | 20.7 (–) | — |
| Patients with a mean $HbA_{1c}$ >7.5% | 5.2 (4.6–5.8) | 13 (6.6–20.4) | 8.9 (6.3–11.5) | 14 (11.8–16.2) | 8 (4.7–11.8) | 12.3 (10.7–13.8) |
| | *P* | *P* | *P* | *P* | *P* | *P* |
| Significance of differences | | | | | | |
| Each individual symptom | 0.349 | 0.115 | 0.865 | 0.841 | 0.089 | 0.033 |
| All symptoms† | 0.031 | 0.031 | 0.031 | 0.031 | 0.031 | 0.031 |

*Cox regression. †The symptom-free survival of all neurodegenerative symptoms pooled in Wolfram Syndrome is significantly different ($P = 0.031$) in patients with a mean $HbA_{1c}$ higher than 7.5%, compared with patients with a mean $HbA_{1c}$ lower than or equal to 7.5%.

individuals carrying mutations with a putative minor loss of function (Fig. 1).

**CONCLUSIONS**—This is the first study characterizing WSD in a large European cohort of patients in comparison with a nationwide cohort of patients with type 1 diabetes. We demonstrated that WSD occurs earlier in life than type 1 diabetes. This may be because the process of ER stress–mediated β-cell decline in WSD already starts at the beginning of life, whereas autoimmune-mediated β-cell destruction in type 1 diabetes is triggered later. In addition to β-cell loss, insulin secretion has been shown to be impaired in *wfs1* knockout mice (14). Thus, defective insulin secretion may contribute to the early diabetes onset in WSD. In line with previous reports, WSD was autoantibody-negative and not commonly complicated by ketoacidosis in our cohort (6,15). Although the differences in remission period and insulin requirement between the WSD and type 1 diabetes groups did not reach statistical significance, a high degree of variability was noted in these features among patients with WSD. Some WSD patients showed a remarkably long remission period of >8 years or an insulin requirement of <0.5 IU/kg/day. Similarly, Fishman et al. (16) described Wolfram syndrome patients with measurable C-peptide 8 years after diabetes onset, and Cano et al. (10) reported that the daily insulin requirement and mean $HbA_{1c}$ were lower in WSD than in type 1 diabetes. These findings suggest that progression of diabetes toward total insulin deficiency may be slower in WSD than in type 1 diabetes. Thus, WSD might be considered if diabetes occurs without diabetic ketoacidosis in a preschool child with negative autoantibodies and a strikingly long remission period.

In addition, we noted that severe hypoglycemia occurred at a higher frequency in patients with WSD than with type 1 diabetes. This was not caused by cortisol deficiency and may be explained by impairment of hypoglycemia awareness as a result of neurologic dysfunction.

We also observed that transient severe glucose deprivation in patients with WSD was accompanied by an acute, partially reversible, neurologic or visual deterioration. Hypoglycemia is known to perturb ER function (5). This should urge professional and family caretakers to carefully adjust insulin therapy in strict avoidance of hypoglycemic events. Our data also confirm a decreased prevalence of microvascular complications in WSD, as reported previously (10,15). This is an intriguing finding because hyperglycemia occurs on top of tissue ER stress in Wolfram syndrome. Although the underlying molecular mechanisms remain to be investigated, this may suggest a protective effect of wolframin deficiency on the development of classical diabetes complications.

The ER is the cell organelle where secretory proteins are folded. Accumulation of unfolded or misfolded proteins causes ER stress and induces the unfolded protein response (UPR), a signaling network that aims at restoring ER homeostasis by halting protein translation and activating molecular chaperones involved

**Table 3—*WFS1 mutations in patients with WSD***

| Mutation group | Age at WSD onset (years) | Exon | Nucleotide change | Amino acid change | Type of mutation | First description |
|---|---|---|---|---|---|---|
| — | 0.6 | 8 + intron 3 | c.1367G>A+c.316–37C>T | p.R456H+3′splice* | Missense + unknown | Awata 2000† + novel |
| 3 | 1 | 8 | c.937C>T | p.H313Y‡ | Missense | Hansen 2005 |
| 1 | 1 | 8 | c.1362_1377del16 | p.Y454fs | Frameshift | Colosimo 2003 |
| 3 | 1.1 | 8 | c.1885C>T | p.R629W | Missense | Kadayifci 2002 |
| 1 | 1.3 | 8 | c.1619G>A | **p.W540X** | Nonsense | **Novel** |
| 1 | 1.5 | 7 | c.740_741delTT | **p.F247fs** | Frameshift | **Novel** |
| 1 | 1.7 | 8 | c.1096C>T | p.Q366X | Nonsense | Strom 1998 |
| 1 | 1.8 | 4 + 8 | c.409_424dup+c.1193_1243dup | **p.V142X+p.V415fs** | Nonsense + frameshift | **Novel + novel** |
| 1 | 2 | 7 | c.740_741delTT | **p.F247fs** | Frameshift | **Novel** |
| 2 | 2.6 | 7 + 8 | c.740_741delTT+c.2048T>G | **p.F247fs+p.M683R** | Frameshift + missense | **Novel + novel** |
| 3 | 3.1 | 8 | c.1243_1245delGTC | p.V415del | In-frame deletion | Hardy 1999 |
| 1 | 3.3 | 8 | c.1919_1929del | **p.L640fs** | Deletion | **Novel** |
| 1 | 3.4 | 8 | c.1362_1377del16 | p.Y454fs | Frameshift | Colosimo 2003 |
| 2 | 3.7 | 5 + 8 | c.599delT+c.2006A>G | p.L200fs+p.Y669C | Frameshift + missense | Strom T 1998 |
| 3 | 3.8 | 8 | c.1943G>A+c.2336T>G | p.W648X+**p.V779G** | Nonsense + missense | Inoue 1998 + **novel** |
| 1 | 3.8 | 8 | c.1362_1377del16 | p.Y454fs | Frameshift | Colosimo 2003 |
| 2 | 4 | 8 | c.1251_1252delinsG+c.1973delA | **p.F417fs+p.K658fs** | Frameshift | **Novel + novel** |
| 1 | 4.1 | 8 | c.1362_1377del16 | p.Y454fs | Frameshift | Colosimo 2003 |
| 1 | 4.2 | 8 | c.1775_1776insGGAT | **p.E593fs** | Frameshift | **Novel** |
| 1 | 4.3 | 8 | c.1919_1929del | **p.L640fs** | Deletion | **Novel** |
| 1 | 4.3 | 5 | c.599delT | **p.L200fs** | Frameshift | **Novel** |
| 2 | 4.3 | 8 | c.1380_1388del 9 | p.T461_463del | In-frame deletion | Strom 1998 |
| 1 | 4.8 | 8 | c.1362_1377del16 | p.Y454fs | Frameshift | Colosimo 2003 |
| 2 | 4.9 | 8 | c.1997G>A | p.W666X | Nonsense | Hong 2009 |
| 1 | 5.1 | 8 | c.1838G>A | p.W613X | Nonsense | Smith 2003 |
| 1 | 5.3 | 8 | c.1362_1377del16 | p.Y454fs | Frameshift | Colosimo 2003 |
| 2 | 5.5 | 8 | c.1380_1388del 9 | p.T461_463del | In-frame deletion | Strom 1998 |
| — | 6 | 8 + intron 4 | c.1228delCTCT+**c.461–8T>C** | p.L410fs+**3′splice*** | Frameshift + unknown | Colosimo 2003 + **novel** |
| 1 | 6 | 8 | c.1362_1377del16 | p.Y454fs | Frameshift | Colosimo 2003 |
| 1 | 6 | 8 | c.1096C>T | p.Q366X | Nonsense | Strom 1998 |
| 1 | 6.5 | Intron 4 | c.460+1G>A | 5′splice | Splice | Strom 1998 |
| 3 | 7.5 | 8 | c.1973delA+c.1289C>T+c.1367G>A | **p.K658fs+p.S430 L**+p.R456 H | Frameshift + missense + missense | **Novel + novel** + Awata 2000† |
| 3 | 7.8 | 8 | c.2206G>A+c.1380_1388del9 | p.G736S+p.T461_463del | Missense + in-frame deletion | Hardy 1999; Strom 1998 |
| 2 | 8.5 | 8 | c.1943G>A | p.W648X | Nonsense | Inoue 1998 |
| 2 | 8.9 | 8 | c.2164_2165ins24 | p.M722fs | Frameshift | Strom 1998 |
| 2 | 9.8 | 8 + 4 | c.2648_2651delTCTT+c.409_424dup16 | p.F883fs+p.V142fs | Frameshift + frameshift | Hardy 1999; Gomez 2001 |
| 3 | 10 | 8 | c 2051C>T | p.A684V‡ | Missense | Tessa 2001 |
| 3 | 16 | 8 | c.1367G>A+c.1885C>T+c.2648_2651delTCTT | p.R456H+p.R629W + p.F883fs | Missense + missense + frameshift | Awata 2000†; Kadayifci 2000; Hardy 1999 |
| 3 | 17.3 | 8 | c.2385 G>C+c.2390 A>T | **p.E795D+p.D797V** | Missense + missense | **Novel + novel** |

Novel mutations are indicated in boldface. *Putative splice mutation, not experimentally examined. †Variant associated with type 1 diabetes and type 2 diabetes. ‡Individual with only one detectable *WFS1* mutation.

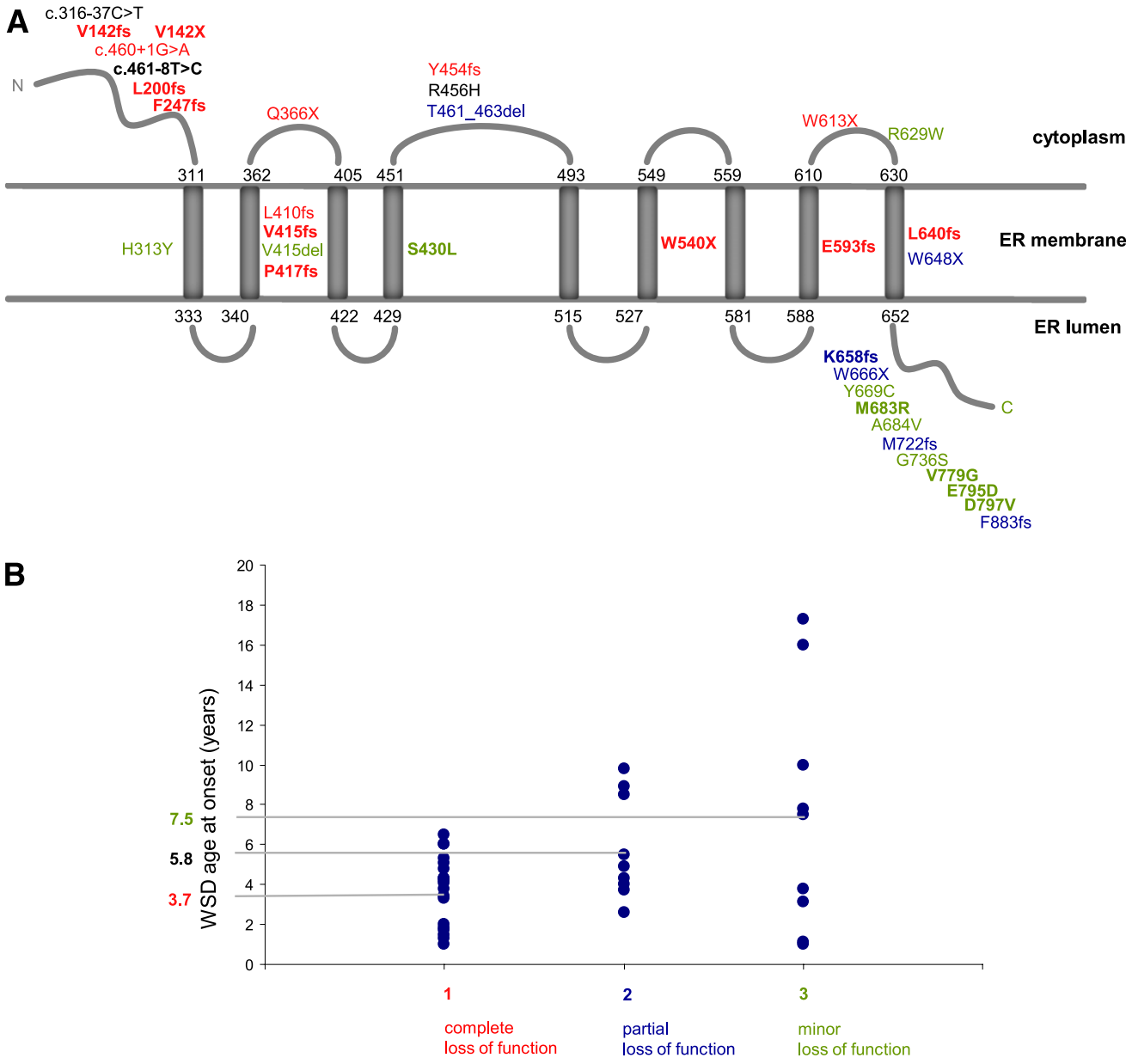


**Figure 1**—A: *A schematic presentation of the wolframin protein and analysis of genotype-genotype correlation shows the relative positions of* WFS1 *mutations within the wolframin polypeptide chain with the five transmembrane domains indicated (adapted from Gene Reviews, University of Washington, Seattle, WA). Mutations are color-coded according to their mutation categories: group 1 (red), group 2 (blue), and group 3 (green). Unclassifiable variants are indicated in black; novel mutations are indicated in bold.* B: *Genotype–phenotype correlation; the differences in mean age at WSD onset between group 1 (3.7 ± 1.7 years), 2 (5.8 ± 2.6 years), and 3 (7.5 ± 6.0 years) are significant (ANOVA,* P = *0.028).*

in protein folding (17). However, if ER stress persists, the UPR shifts from a survival-promoting to a proapoptotic pathway, thereby ensuring proper disposal of irreversibly damaged cells.

Wolframin is a UPR component and promotes cell survival by mitigating ER stress signaling. Deficiency of wolframin caused by mutations in the *WFS1* gene leads to an inadequate activation of the UPR in response to accumulation of unfolded proteins within the ER lumen, especially in tissues with high secretory demands (18), which results in apoptotic cell death (5). In patients with Wolfram syndrome, these mechanisms underlie progressive neurodegeneration and endocrine dysfunction. In fact, ER stress–induced β-cell loss and atrophy of the islets of Langerhans have been shown to cause infancy-onset WSD (18,19).

Consistent with previous reports, diabetes was the first manifestation of Wolfram syndrome in most patients in this study. An intriguing observation was a positive correlation between a high mean $HbA_{1c}$ and the number of neurodegenerative and endocrine symptoms that occurred after the onset of WSD. This indicates that ER stress due to wolframin deficiency may only partially account for disease progression after the onset of WSD. Because additional Wolfram syndrome symptoms are more likely to develop in patients with poor glycemic control, hyperglycemia may be involved

as well. The deleterious effects of glucose on tissues, collectively described as glucose toxicity, involve oxidative stress by reactive oxygen species (20,21). Antioxidant enzyme activities in Langerhans islets are relatively low compared with other tissues (3,20). In patients with type 2 diabetes, high glucose concentrations impair expression and secretion of insulin in β-cells and accelerate apoptosis of islet cells (22,23). These observations may explain why the duration of residual β-cell function after onset of WSD showed extreme variations. Our study provides evidence that wolframin-deficient subjects with infancy-onset and poorly controlled diabetes experience a faster progression of neurodegenerative disease than those with good diabetes control. From these findings, we hypothesize that ER stress in patients with WSD is enhanced by additional chronic oxidative stress resulting from chronic hyperglycemia. Hence, tissues with high wolframin expression would be put in double jeopardy, and the process of cell death would be accelerated. This underscores the importance of good diabetes control in Wolfram syndrome to retard the neurologic and endocrine degenerative course of the disease.

We also observed in individual patients that febrile infections and general anesthesia may induce acute neurologic deterioration in Wolfram syndrome, indicating that these conditions may constitute an additional source of ER stress enhancement. Oxidative stress is also inducible by cytokine storm (3). High-fat diet, amino acid starvation, environmental toxins, hypoxia, or radiation are other oxidative stressors (3,5,24). Knowledge of these ER stress-enhancing factors may be important in the clinical management of patients with Wolfram syndrome.

In our cohort, we identified 34 *WFS1* mutations, including 13 distinct previously unreported mutations, expanding the spectrum of known mutations in Wolfram syndrome. We aimed at determining the relationship between genotype and phenotype by dividing patients into three groups according to the predicted consequences of their mutations on wolframin function and assuming translation of mutated *WSF1* transcripts, as previously shown (25). Using this approach, we showed genotype–phenotype correlation suggesting a role for *WFS1* in determining the age at onset of WSD. This is in line with the data from a meta-analysis by Cano et al. (13) showing that the presence of two inactivating mutations predisposes to an earlier onset of diabetes and optic atrophy. However, validation of any genotype–phenotype correlation must await detailed functional analysis of mutations on a cellular and molecular level.

Taken together, we demonstrated that ER stress–mediated decline of β-cells in WSD occurs earlier in life than autoimmune-mediated β-cell destruction in type 1 diabetes, that poor glycemic control is associated with a faster progression of neurodegeneration, and that *WFS1*-mutations influence the age at onset of WSD. Because ER stress has been recognized as contributing to insulin resistance in type 2 diabetes (5,22), the dissection of pathogenic mechanisms in monogenic ER stress–mediated β-cell loss may also contribute to the understanding of type 2 diabetes pathogenesis.

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