Autosomal Dominant Diabetes Arising from a Wolfram Syndrome 1 Mutation

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ABSTRACT

We utilized an unbiased genome wide approach to identify exonic variants segregating with diabetes in a multigenerational Finnish family. At least 8 members of this family presented with diabetes with age-of-diagnosis ranging from 18-51 years and a pattern suggesting autosomal dominant inheritance. We sequenced the exomes of four affected members of this family and performed follow-up genotyping of additional affected and unaffected family members. We uncovered a novel non-synonymous variant (p.Trp314Arg) in the Wolfram syndrome 1 (WFS1) gene that segregates completely with the diabetic phenotype. Multipoint parametric linkage analysis with 13 members of this family identified a single linkage signal with maximum LOD score 3.01 at 4p16.2-p16.1, corresponding to a region harboring the WFS1 locus. Functional studies demonstrate a role for this variant in ER stress, which is consistent with the beta-cell failure phenotype seen in mutation carriers. This represents the first compelling report of a mutation in WFS1 associated with dominantly inherited non-syndromic adult-onset diabetes.

Considerable advances have been made in our understanding of the genetics of monogenic diabetes, which accounts for 1-3% of diabetes cases (1-3). The identification of causative genetic variants responsible for monogenic diabetes has revealed critical elements of the pathways involved in insulin and glucose metabolism, and in some instances has led to important therapeutic interventions (4). But the list of ~20 known causative loci for monogenic diabetes is far from complete, as DNA sequencing of known genes has failed to identify mutations in some families (1). The overlap of genes identified in genome wide association studies (GWAS) and/or candidate gene studies of the more common type 2 diabetes with those of the known monogenic diabetes genes suggests common genetic pathways may be involved (4). Thus, identifying the as-yet-undefined genes involved in monogenic diabetes would also offer new insights into the genetics of type 2 diabetes.
We set out to identify the genetic cause of diabetes in a four-generation family with an apparent autosomal dominant form of adult-onset diabetes (Fig. 1). Previous sequencing of the exons of known Maturity-Onset Diabetes of the Young (MODY) genes in a subset of the affected members of this family failed to identify candidate variants segregating with diabetes. Thus, we sequenced the exomes of four affected members of this family, with follow-up screening of additional affected and unaffected family members and uncovered a novel non-synonymous variant in the Wolfram syndrome 1 (WFS1) gene as the likely causative mutation for diabetes in this family.

RESEARCH DESIGN AND METHODS

Study participants. Informed consent was obtained from study participants, and the respective IRBs for the participating institutions each approved this study. The first participants from this family entered the study through the genetic diagnostic services at the University of Eastern Finland, Kuopio. Blood samples were taken for DNA extraction. Four of the family members underwent an oral glucose tolerance test (a 75 g glucose load). Plasma glucose levels were determined with the glucose oxidase method, plasma insulin and C-peptide levels by time-resolved immunofluorometric assay, and serum glutamic acid decarboxylase (GAD) autoantibodies by ELISA.

Exome sequencing and sequence analysis. Multiplexed, paired-end DNA libraries were prepared for targeted exome capture and sequencing as previously described (5), with minor modifications. Briefly, 1.5 ug of genomic DNA fragmented to an average of 300 bp was end repaired, ligated to adapters containing a 6-base index sequence, and PCR amplified. Targeted capture of exome sequences from libraries was performed with the Roche NimbleGen SeqCap EZ Exome Library v2.0 and sequenced on the Illumina HiSeq 2000 as described by the manufacturer’s protocols for paired 101bp reads.

Reads passing the Illumina "chastity filter" were aligned to the UCSC hg19 genome assembly (PAR region on chrY masked, alternative haplotypes removed) using Novoalign v2.07.18 (http://www.novocraft.com) and duplicate read pairs were removed. Genotypes for SNPs and short indels were called using a Bayesian approach implemented
in MPG (5) and filtered using a score threshold of 10 and a score/depth ratio threshold of 0.5.

Variants were annotated using Annovar (6) and coding transcripts from GencodeV7 (7). Allele frequencies for each variant were estimated based on phase 1 genotypes from the 1000 Genomes project (8), the European Americans among the 6500-exome release of the NHLBI GO Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), and the Finnish whole exome data from the T2D-GENES Consortium.

**Genotyping with HumanOmni2.5-8 BeadChip.** Samples were genotyped with the Illumina HumanOmni2.5-8 BeadChip according to manufacturer’s protocols; genotypes were called using BeadStudio with standard cluster definitions. To obtain the most accurate SNP positions and allele orientations, Illumina probe sequences were mapped to the hg19 genome assembly using bwa v0.5.8c (9), which lead to the exclusion of 32,622 SNPs due to various alignment problems: no alignment, multiple alignments, or presence of known variants near 3' end of probes.

**QC analysis.** Comparison of genotypes called from the exome data with those also called on the HumanOmni2.5-8 BeadChip for each individual showed a mean concordance of 99.60% overall (n=123,376-126,328), 99.83% in coding regions (n=63,929-64,093) and 99.48% at heterozygous sites on the beadchip in coding regions (n=8043-8261).

**Variant validation and screening for variants in additional family members.** Sanger sequencing (ACGT, Wheeling, IL) was used as an alternate platform to validate candidate variants in the 4 exome sequenced individuals. Follow-up genotyping was performed to screen for validated variants in additional family members. The *WFS1* variant was genotyped with the Applied Biosystems TaqMan Allelic Discrimination Assay and the *TNN* variant was genotyped with Sanger sequencing.

**Sanger-sequencing of the *WFS1* promoter, 5'UTR, and nearby regulatory regions.** *WFS1* regions potentially harboring the promoter (upstream of 5'UTR) or regulatory elements such as enhancers/insulators (5'UTR/exon 1 and intron 1) were Sanger-
sequenced (ACGT, Wheeling, IL). The coordinates of the region targeted for sequenced (hg19; chr4: 6,270,603-6,272,808) were defined by chromatin marks (H3K4me3 and H3K27ac) predictive of an “active promoter” state at \( WFS1 \) across 10 cell lines consisting of 9 ENCODE cell types (10) and islets (M. Stitzel, unpublished data). Five amplicons were sequenced to cover this 2.20 kb region, which includes 0.973 kb upstream of the 5’UTR, the 5’UTR (exon 1: 0.165 kb), and 1.067 kb into intron 1.

**Linkage Analysis.** Genotype data from the Illumina Omni2.5-8 BeadChip for 13 family members were available for linkage analysis. Sample-level quality control was carried out with PLINK (11) and included checks for sample contamination and verification of pedigree structure and sex.

SNPs with missing genotypes or Mendelian inconsistencies identified using Pedstats (12) were excluded. A subset of SNPs informative for linkage analysis was selected by first retaining SNPs with at least 7 copies of the minor allele in the 13 genotyped subjects, and then dividing chromosomes into 100kb segments and selecting the first SNP located at least 75kb away from the previous adjacent SNP. This resulted in a map of 25,303 autosomal SNPs with a median intermarker distance of 99kb. Remaining possible genotyping errors were detected by identifying unlikely double recombinants using Merlin (13) and these SNPs were designated as missing for subsequent analysis.

Multipoint parametric linkage analysis was conducted using Merlin assuming autosomal dominant inheritance. To assess robustness of linkage evidence to model parameters, analyses were repeated for a range of penetrance parameters. Allele frequencies were estimated by counting across all individuals and multipoint LOD scores were calculated along a 0.1cM grid. The genetic map was approximated from the physical map by assuming 1cM~1Mb. All positions reported are based on Human genome build hg19 coordinates.

**Luciferase Assay.** For reporter assays, 293T cells were cotransfected with the ERSE (rat promoter GRP78)-luciferase construct (14) and various constructs as indicated. Prior to lysis at 24 hours after transfection, cells were treated with or without 10 nM of thapsigargin for 6h. Firefly luciferase activity was measured using the Dual-Luciferase
Reporter Assay System (Promega, Madison, WI) and normalized to renilla luciferase values of the co-transfected pRL-TK vector (Promega) to control for differences in transfection efficiency.

**Quantitative PCR Assay For Expression of WFS1.** Total RNA was isolated from the cells using RNeasy Mini Kit (Qiagen) and reverse transcribed using 1 µg of total RNA from cells with Oligo-dT primer. For the thermal cycle reaction, the Vii A7 (Applied Biosystems) was used at 95°C for 10 min, then 40 cycles at 95°C for 10 sec and at 55°C for 30 sec.

The relative amount for each transcript was calculated by a standard curve of cycle thresholds for serial dilutions of cDNA samples and normalized to the amount of β-actin. The polymerase chain reaction (PCR) was performed in triplicate for each sample, after which all experiments were repeated twice. The following sets of primers and Power SYBR Green PCR Master Mix (Applied Biosystems) were used for real-time PCR: human actin, ACCATGGATGATGATATCGCC and GCCTTGCACATGCGGG; human WFS1, GAGCCCTGAGGACCTGCC (exon 7) and TCTCCATGATGGCGTGTCA (exon 8).

**Immunoblotting (Western Blots).** Fibroblast cell lysates (30 µg protein equivalent) were electrophoresed at 200 volts for 1 hour using NuPAGE 4-12% Bis-Tris Gel (Life Technologies) and transferred to PVDF membrane (iBlot®, Invitrogen). The membrane was blocked for 1 hour at room temperature (RT) with 5% milk in TBS-Tween, probed overnight at 4°C with primary anti-WFS1 (Cell signaling - rabbit, 1:1000) or anti-tubulin (Sigma: mouse, 1:2000) antibodies, followed by 1 hour incubation at room temperature with secondary HRP-conjugated antibody (Amersham-GE Healthcare: donkey anti-rabbit, 1:10,000) or Santa Cruz: goat anti-mouse, 1:5000). ECL Western blotting and analysis reagents (GE, Healthcare) were used for chemiluminescent detection.

**Immunostaining.** Human skin fibroblasts were fixed in 2% paraformaldehyde for 30 min at room temperature and then permeabilized with 0.1% Triton X-100 for 2 min. The fixed cells were washed with phosphate-buffered saline, blocked with 10% bovine serum
albumin for 30 min, and incubated in primary antibody overnight at 4°C. The cells were washed three times in phosphate-buffered saline/Tween 0.1% and incubated with secondary antibody for 1 h at room temperature. Images were obtained with a FSA 100 microscope (Olympus). Anti-WFS1 antibody and anti-PDI antibody were obtained from Proteintech Group (Chicago, IL) and Stressgen, Victoria, BC, Canada) respectively.

RESULTS

Family participants and clinical phenotypes. Six of the eight affected family members (generations II-IV) were diagnosed with diabetes at the age of 34-51 years, and two of them (IV-2 and IV-4) at the age of 18 and 25 years, respectively (Fig. 1). There is no history of ketoacidosis in any of the family members having diabetes, and seven of them are currently treated with insulin. Body mass index (BMI) values for the eight diabetic family members ranged from 18.1-26.8 kg/m², with six having values < 25 kg/m². Detailed clinical examination of the diabetic family members did not reveal any of the other clinical features of the Wolfram syndrome (hearing impairment in audiograms, optic atrophy or vision impairment in annual ophthalmoscopy examinations or diabetes insipidus).

We performed an oral glucose tolerance test (OGTT) with measured insulin and C-peptide levels for four members of a nuclear family within this multi-generational family who were available for further characterization (Table 1). The diabetic mother and son (III-2 and IV-2) of this family had blunted insulin and C-peptide responses when challenged with a glucose load of 75g. Residual insulin and C-peptide levels, the lack of ketoacidosis, and the absence of glutamic acid decarboxylase autoantibodies exclude a diagnosis of either type 1 diabetes or latent autoimmune diabetes of adults (LADA).

Exome sequencing of 4 family members. To determine the molecular basis of diabetes in this family, we sequenced the exomes of 4 of the 8 diabetic individuals (II-2, III-7, IV-2, and IV-4) representing three generations. An average of 38.8 million reads were obtained for each individual, providing coverage ≥14-fold for 90% and ≥28-fold for 80% of the protein-coding base pairs in GencodeV7 exons. A minimum of 90.5% of these bases was confidently called for each individual. In the 4 sequenced individuals, 153,515
variants (SNPs and small indels) were identified, with an average of 86,989 variants per person. We applied several filters to reduce and prioritize the number of variants for follow-up assessment (Table 2).

Three heterozygous missense variants in three genes fulfilled all criteria; the genes were JMY (junction mediating and regulatory protein, p53 cofactor), TNN (tenascin N), and WFS1 (Wolfram syndrome 1). We used Sanger sequencing to validate these three variants and confirmed the presence of variants in TNN and WFS1 (but not in JMY) in all four of the exome-sequenced diabetics. We then screened additional family members (available to our study), who had not been exome sequenced, for the two validated variants. Based on the screening of additional family members, we excluded the TNN variant as causative as it did not segregate with diabetes status. The remaining WFS1 variant (c.940T>C; p.Trp314Arg) segregated completely with affection status for the 13 affected and unaffected individuals tested (Fig. 1). To our knowledge, this variant is novel and unique to this family as it has not been observed in 1000G, NHLBI exomes, or 962 Finnish samples in T2D-GENE consortium.

**Sanger-sequencing of promoter and potential regulatory regions in 5’UTR/exon 1 and intron 1.** To rule out the contribution of variants in potentially important regulatory sites not captured by exome sequencing, we performed Sanger-sequencing on all 13 members of the family, targeting an approximate 2.20 kb region encompassing 0.973 kb upstream of the 5’UTR, the 5’UTR (exon 1: 0.165 kb), and 1.067 kb into intron 1. Enrichment of histone H3 lysine 4 trimethyl (H3K4me3) and H3 lysine 27 acetylation (H3K72ac) modifications that mark active promoters and enhancers in 9 ENCODE cell types and islets strongly suggest this targeted WFS1 region contains the promoter and potential enhancers. We obtained good quality sequence for 100% of this region and did not identify any variants segregating with diabetes status.

**Linkage analysis with 13 family members across three generations.** Taking advantage of the availability of several DNA samples from this family and to provide additional support for WFS1 as the responsible locus, we performed parametric multipoint linkage analysis with genotype data from the Illumina HumanOmni2.5-8 chip for 13 members of
this family. Assuming complete penetrance and no phenocopies under a dominant inheritance model, we observed evidence for linkage of diabetes to a single region, 4p16.2-p16.1 (maximum LOD score 3.01) (Fig. 2). Repeating this analysis with a range of penetrance parameters and nonzero phenocopy rates did not qualitatively alter these results. The approximately 1.6Mb region (LOD>1.9) of this linkage peak harbors 16 genes, one of which is \textit{WFS1}.

\textbf{\textit{WFS1} variant and ER stress.} \textit{WFS1} mRNA is found in several cell types and is highly expressed in the pancreatic β-cell (15). \textit{WFS1} participates in many important cellular processes including insulin production, processing and secretion, production of cyclic AMP, and regulation of ER calcium levels (as reviewed in (16,17)). \textit{WFS1} also plays a role in the suppression of ER stress-mediated cell death by preventing hyperactivation of the ER stress response, raising the possibility that the p.Trp314Arg variant may cause the dysregulation of the ER stress response. To test this hypothesis, we investigated the effect of both this p.Trp314Arg variant and another rare variant (p.His313Tyr) in an adjacent codon, which was identified as \textit{de novo} mutations in two unrelated Danish individuals with Wolfram syndrome (WS) (18). With no other mutations identified in \textit{WFS1} in these two individuals, it is reasonable to assume that p.His313Tyr is capable of causing WS in the heterozygous state. We cotransfected HEK293T cells with a luciferase reporter construct containing an ER stress response element (ERSE) and an expression vector with either 1) control (pcDNA), 2) wild-type \textit{WFS1} (WT), 3) mutant c.937C>T \textit{WFS1} (p.His313Tyr), or 4) mutant c.940T>C \textit{WFS1} (Trp314Arg). The ERSE reporter reflects activation levels of the ER stress response. In the absence of the ER stress inducer TG, the p.His313Tyr mutant strongly activated the ERSE reporter. After inducing ER stress with TG, both mutants p.His313Tyr and p.Trp314Arg exhibited significantly higher ERSE activity than wild-type WFS1, with the p.Trp314Arg having a milder effect (Fig. 4). This suggests that the Wolfram syndrome-causing mutant p.His313Tyr increases ER stress, whereas the less severely affected p.Trp314Arg mutant may be pathogenic due to a defect in its ability to suppress the ER stress response after the pathway is activated.
**Functional consequences of the WFS1 (c.940T>C; p.Trp314Arg) variant.** To further investigate the effect of the c.940T>C variant on WFS1 transcripts and protein product, we assessed fibroblast cell lines generated from a family quartet of c.940T>C carriers and non-carriers (III-1, III-2, IV-1, and IV-2) for differences in 1) WFS1 transcript levels, 2) WFS1 protein abundance, 3) cellular localization of the WFS1 protein, and 4) cAMP levels, which are regulated by interactions between WFS1 and adenyl cyclase 8 (19). WFS1 mRNA expression levels were comparable in variant and non-variant carriers (Figure 5A). Similarly, WFS1 protein was present in all fibroblast lysates and protein levels did not significantly correlate with variant status (Figure 5B). The variant also did not dramatically alter the subcellular localization of the WFS1 protein; both wildtype and p.Trp314Arg exhibited a diffuse reticular pattern co-localizing with the ER marker protein disulfide isomerase (PDI) (Figure 6). Lastly, cAMP levels did not correlate with carrier status of the c.940T>C; p.Trp314Arg variant (data not shown). Of note, fibroblasts generated from several WS patients carrying known causative WFS1 variants also exhibited no differences in cAMP levels when compared to those from non-mutant WFS1 individuals (F. Urano, unpublished data). Thus, the absence of an effect on WFS1 mRNA, protein, and cAMP levels in fibroblasts suggests a resiliency of this cell type to defective or absent WFS1 and may reflect cell type-specific activities of this protein.

**DISCUSSION**

We sequenced the exomes of 4 members of family with dominantly inherited adult onset diabetes and identified a single novel variant in WFS1 correlating with disease status. Our biological interest in WFS1 was high as coding variants in this gene have been associated with type 1 diabetes in candidate gene studies (20,21) and common non-coding variants nearby this gene have been associated with increased risk for type 2 diabetes in GWAS studies (22,23). Furthermore, coding mutations in WFS1 give rise to two major clinical phenotypes, Wolfram Syndrome (WS or DIDMOAD) and sensorineural hearing impairment (SNHI). WS is a rare progressive neurodegenerative disorder usually characterized by diabetes insipidus, early onset diabetes mellitus, optic atrophy, and deafness (hence the acronym DIDMOAD), with diabetes mellitus and optic atrophy being the most consistent features (17,24). WS is an autosomal recessive disorder
with nearly all cases harboring mutations in both alleles of WFS1 (as reviewed in (17,25)) (Fig. 3). In contrast, SNHI is generally inherited as an autosomal dominant condition attributable to heterozygous missense variants in this same gene (26-29).

The WFS1 gene encodes a multi-span transmembrane protein localized to the endoplasmic reticulum (ER). Partial or complete loss of function of WFS1 protein gives rise to WS, whereas missense changes in the C-terminal domain typically cause SNHI (Fig. 3). The c.940T>C; p.Trp314Arg (exon 8) missense mutation identified in this report is located in the first transmembrane domain and is highly conserved (GERP score of 4.25) and predicted to be deleterious by several algorithms (CDPred, PolyPhen, Condel, SIFT). Other than diabetes mellitus, the p.Trp314Arg carriers in this family had none of the other common signatures of WS such as optic atrophy, diabetes insipidus, or deafness. The native WFS1 protein is a multimer of ~400 kDa and is likely a homooligomer of WFS1 monomers (30). Tetramers composed of purely wild-type monomers in this family would occur infrequently (1/16). Thus, it may be that tetramers composed of p.Trp314Arg mutant and wild-type monomers are not structurally competent or fully functional, leading to diabetes via a dominant negative mechanism. Further investigation of this possibility may provide insight into the molecular effects of heterozygous mutations.

We believe this is the first complete report of dominant inheritance of an adult-onset diabetes phenotype (without other syndromic features of WS) associated with a missense mutation in WFS1. Recently, Johansson et al. identified a single heterozygous WFS1 missense mutation (encoding p.Arg703Cys) in a family with diabetes diagnosed early (14 years) and late (55 and 60 years) in life (31) (Fig. 3b). However, whether p.Arg703Cys is the causative mutation in that family is unclear, as only 3 of the 4 affected family members tested were mutation carriers, and the ages of diabetes diagnoses were vastly different among the carriers.

The report cited above of de novo c.937C>T; p.His313Tyr mutations in two individuals with WS seem to indicate that WS can rarely be caused by heterozygous mutations. Interestingly, a single WFS1 C-terminal missense variant (encoding p.Glu864Lys) has been identified to segregate dominantly in two unrelated families with Wolfram-like (WS-like) disorder (32,33), which is characterized by hearing impairment,
diabetes mellitus, psychiatric illness and variable optic atrophy (34). A small number of WS individuals have been identified to have a single heterozygous variant (EUROWABB open variation database), but it is possible that a second unidentified variant may be present in these individuals.

Taken together, genetic variations in \textit{WFS1} can lead to a spectrum of phenotypes including susceptibility to type 1 diabetes, type 2 diabetes, WS, WS-like disorder and SNHI. Genotype-phenotype correlations emerging from this work imply multiple roles of the WFS1 domains, where variant type and/or location can lead to differential clinical manifestations. In the case of p.Trp314Arg, heterozygous carriers have high penetrance development of adult onset diabetes with relative insulin insufficiency, but the affected individuals lack other features of WS. Functionally, p.Trp314Arg appears to diminish the ability of WFS1 to protect against ER stress, which presumably damages beta-cell function over time, leading to the onset of relative insulin insufficiency and diabetes.

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PH, SP, HH, PN and ML were responsible for the collection of clinical and laboratory data of the family, P.N. designed and performed laboratory measures, and S.P. generated fibroblast cultures from skin biopsies. LLB and AJS performed exome sequencing, variant validation and screening. PSC, NN and RLG developed and ran the analysis pipeline for NextGen sequence data and Illumina Omni2.5 data. JRH performed linkage analyses, and LLB, TH, SL, AJS, MLS and JM contributed to functional analyses. FSC, MB and ML supervised the genetic and linkage studies, and their interpretation. FSC and FU supervised the functional studies and their interpretation. LLB analyzed the sequence
data, and LLB, PSC and JRH contributed to the writing of the manuscript; PH., SP, HH, PN PSC, JRH, AJ, NN, RLG, MLS, JM, MB, FU, FSC and ML reviewed and edited the manuscript. F.S.C. is a guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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| Characteristics                          | III-2 | III-1 | IV-2 | IV-1 |
|----------------------------------------|-------|-------|------|------|
| Glucose tolerance status               | DM    | No DM | DM   | No DM |
| BMI, kg/m2                              | 23.0  | 24.5  | 21.6 | 21.5 |
| Age of onset of diabetes, years         | 51    | -     | 18   | -    |
| History of ketoacidosis                 | No    | -     | No   | -    |
| GAD autoantibodies                      | No    | -     | No   | -    |
| Wolfram syndrome*                       | No    | -     | No   | -    |
| Fasting glucose, mmol/L                 | 7.0   | 6.2   | 14.8 | 6.0  |
| 30 min glucose, mmol/L                 | 9.9   | 8.8   | 19.2 | 9.1  |
| 60 min glucose, mmol/L                 | 10.4  | 6.6   | 22.2 | 7.6  |
| 120 min glucose, mmol/L                | 12.6  | 3.6   | 20.4 | 5.0  |
| Fasting insulin, mU/L                   | 6.0   | 8.0   | 4.0  | 12.0 |
| 30 min insulin, mU/L                   | 19.0  | 62.0  | 12.0 | 61.0 |
| 60 min insulin, mU/L                   | 18.0  | 135.0 | 14.0 | 52.0 |
| 120 min insulin, mU/L                  | 18.0  | 13.0  | 6.0  | 12.0 |
| Fasting C-peptide, nmol/L               | 0.64  | 0.98  | 0.50 | 0.76 |
| 30 min C-peptide, nmol/L               | 1.30  | 3.10  | 0.91 | 2.40 |
| 60 min C-peptide, nmol/L               | 1.50  | 5.40  | 1.20 | 2.80 |
| 120 min C-peptide, nmol/L              | 1.80  | 2.10  | 0.89 | 1.60 |

DM, diabetes mellitus; GAD antibodies, Glutamic Acid Decarboxylase autoantibodies. 
*No visual impairment or optic atrophy, no hearing impairment, no diabetes insipidus.
TABLE 2: Variant Prioritization Strategy

| Filter                                                                 | N Variants |
|------------------------------------------------------------------------|------------|
| Pass primary QC filters                                                | 153,515    |
| Potentially deleterious: Nonsense, frameshift, missense, splice)        | 16,146     |
| Present in 4/4 affected family members*                                | 6,337      |
| Novel or Rare† (Allele freq < 0.02%‡)                                   | 715        |
| Present ≤1X in non-diabetic exomes (n=43) sequenced in laboratory      | 37         |
| Low evidence for being an artifact§                                     | 8          |
| Low frequency in dbSNP 135                                             | 3          |

*Includes variants with no genotype calls in a subset of the 4 samples. †Based on the prevalence of MODY and a dominant genetic model. ‡Data sources: 1000G (n= 1092 samples), NHLBI exomes (n= 6500 samples), and T2D-Gene Consortium Finnish exomes (n=962). §Not in DNA sequence prone to technical sequencing/alignment artifacts including segmentally duplications or variants with genotype calls in <50% of exome samples sequenced in our laboratory with the same capture and sequencing technology.
FIGURE LEGENDS

FIG. 1
Pedigree of 4-generation family with autosomal dominant diabetes and WFS1 p.Trp314Arg carrier status. Black squares and circles = diabetics; white squares and circles = NGT (normal glucose tolerant); M = p.Trp314Arg variant; N = reference allele; ? = unknown; AoD = age of diagnosis of diabetes; asterisks = exome sequenced.

FIG. 2
Parametric multipoint LOD scores based on pruned set of Illumina Omni2.5 SNP chip genotypes for 13 family members (Figure 1: II-2, II-3, II-4, III-1, III-2, III-3, III-5, III-6, III-7, IV-1, IV-2, IV-4 and IV-5). A maximum LOD score of 3.01 was identified on chromosome 4p16.2-p16.1.

FIG. 3
Variants in WFS1 identified in individuals with Wolfram Syndrome (WS), Maturity Onset Diabetes of the Young (MODY), or sensorineural hearing impairment (SNHI): Variants as listed in the EURO-WABB open variation database (https://lov.d.euro-wabb.org/home.php?select_db=WFS1). A: Nonsense or frameshift variants. B: Missense variants. Gray boxes are exons, tick marks extending up from exons represent variants identified in a homozygous or compound heterozygous state; extending below exon represent variants identified as a single heterozygous variant, although some of the cases of WS may be those in which the second allele has not yet been identified. Tick marks: Blue = variants in WS individuals, Red = variants in SNHI individuals, Green = variant p.Arg703Cys identified in MODY individual, Purple with asterisks = p.Trp314Arg identified in current study showing autosomal dominant inheritance of diabetes.

FIG. 4
Luciferase reporter assays in HEK293T cells transfected with the ERSE reporter together with control (pcDNA), wild-type WFS1 (WT), mutant c.937C>T WFS1 (p.His313Tyr), or mutant c.940T>C WFS1 (p.Trp314Arg) expression plasmid. Cells were untreated (UT) or treated with thapsigargin (TG, 10 nM) for 6 hours. Relative intensity of luciferase (Promega Dual-Luciferase Reporter Assay System) was then measured (n=3; values are mean ± S.D.). Transfections were normalized with the pRL-TK vector (Promega) as an internal control. Welch's t-test on log-transformed data was used for determining the significance between two treatments. *** p<0.05

FIG. 5
Expression of WFS1 in fibroblasts from c.940T>C (p.Trp314Arg) carriers and non-carriers. A: Expression of WFS1 mRNA in skin fibroblasts. B: Western blot analysis of WFS1 protein abundance with tubulin as loading control. *Relative densitometry measurements (WFS1/tubulin) made with ImageJ64 (http://imagej.nih.gov/ij).

FIG. 6
Cellular localization of WFS1. Skin fibroblasts obtained were double immunostained for WFS1 and PDI and representative single channel fluorescence images are shown.
individually and merged. III-1 (A), III-2: p.Trp314Arg (B), IV-1 (C), and IV-2: p.Trp314Arg (D).
A

Relative WFS1 expression

- III-1
- IV-1
- III-2
- IV-2

p.Trp314Arg carriers

B

| Lane | Sample               | *Relative Units |
|------|----------------------|-----------------|
| 1    | IV-1                 | 0.67            |
| 2    | III-1                | 0.45            |
| 3    | III-2 (p.Trp314Arg)  | 0.85            |
| 4    | IV-2 (p.Trp314Arg)   | 0.68            |

WFS1 Tubulin
