**Supplemental Methods**

**1) DNA amplification and sequence analysis**

The patient’s parents provided written informed consent. Genomic DNA was extracted from peripheral blood leukocytes. The ethical committee of Hokkaido University approved this study. Theexon of *WFS1* was amplified by polymerase chain reaction (PCR) using primers reported previously (1). After amplification, PCR products were purified and sequenced directly using anApplied Biosystems 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA).

**2) Cell culture**

Human embryonic kidney (HEK)-293 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen Carlsbad, CA) with 10% heat-inactivated fetal bovine serum and 0.3% penicillin/streptomycin.

**3) Site-directed mutagenesis and plasmid construction**

Human *WFS1* cDNA was inserted into a pcDNA3.1 vector (gift from F. Urano, Washington University and K. Tanabe, Yamaguchi University). Themutant p.N325\_I328del (designated as Del) was created by site-directed mutagenesis using the KOD-plus-Mutagenesis kit (Toyobo, Tokyo, Japan). We also created p.Q194X, and p.L543R, which were previously reported (1, 2). FLAG tagged mutant WFS1 expression plasmids (Del, p.Q194X, and p.L543R) were also created from a FLAG tagged wild type (WT) *WFS1* expression plasmid (gift from F. Urano, Washington University) by site direct mutagenesis. The *WFS1* cDNA fragments were inserted into a pGFP vector (Clontech, CA) with *Hin*d III and *Xba*I cloning sites and used to create mutant GFP tagged *WFS1* expression plasmids (*WFS1*-GFP). The plasmid constructs were confirmed by DNA sequencing before transfection. The human full-length ATF6α expression plasmid was a gift from S. Oyadomari (Tokushima University). The rat GRP78 reporter plasmid (ERSE luciferase plasmid) was provided by F. Urano (Washington University). An ATF6 binding site luciferase reporter plasmid (pGL4.39) and nuclear factor of activated T-cell (NFAT) luciferase reporter plasmid (pGL4.30) were purchased from Promega (Madison, WI).

**4) Luciferase assay**

For each ERSE, NFAT, ATF6α luciferase assay, we seeded HEK-293 cells into 12-well plates to 70-80% confluency. TransIT-LT1 Transfection Reagent (Mirus, Madison, WI) was used according to the manufacturer’s protocol. All transfections were performed in triplicate and the experiment was repeated three times. Values arerepresented as the mean ± SEM. Statistical significance was tested by one-way ANOVA followed by Tukey’s test.

**ATF6α luciferase assay**

HEK-293 cells were transfected with each of 1.5 µg WT, Del, p.Q194X, and p.L543R *WFS1* construct plasmids along with 0.5 µg pGL4.39 plasmid, 0.5 µg ATF6α expression plasmid and 10 ng *Renilla reniformis* luciferase expression plasmid. Cells were treated with or without 4 mM chemical chaperone 4-phenylbutyrate (4-PBA). The assays were performed as described above.

**5) Western blotting**

HEK-293 cells were plated on a 6-well plate, and cultured to 70-80% confluency. Cells were transfected with 3.0 µg of WT or Del FLAG-tagged *WFS1* expression plasmids with 3.0 µg HA-tagged ATF6α expression plasmids (Genecopoeia, Rockville, MD) for cells in 6-well plates. Twenty-four hours after transfection, cells were lysed on with ice-cold Mammalian Protein Extraction Buffer (GE Healthcare, Buckinghamshire, UK) containing a protease inhibitor cocktail (Sigma Aldrich, St. Louis, MO) for 10 minutes on ice. Then the lysates were centrifuged at 15,000 rpm for 30 minutes at 4 °C. We used NuPAGE LDS Sample Buffer (Thermo Fisher Scientific, Waltham, MA) for denaturing. Lysates were normalized for to total protein (25 µg per lane) and separated by NuPAGE Novex 4-12% Bis-Tris Protein Gels (Thermo) followed by electroblotting using an iBlot Dry Blotting System (Thermo). Anti-HA antibody was purchased from WAKO Pure Chemical (Osaka, Japan). Anti-FLAG antibody and anti-β actin antibody were purchased from Sigma Aldrich, respectively. The membrane was blocked with 5% nonfat milk for 1 hour and then added 1:3000 anti-FLAG antibody or 1:3000 anti-HA antibody was added overnight at 4°C or 1:3000 anti-β actin antibody for 1 hour at room temperature. Anti-mouse HRP labeled IgG (GE Healthcare) was used as secondary antibody and detected with ECL Western blotting reagents (GE Healthcare).

**6) Primers used for quantitative PCR (qPCR)**

For qPCR, the following sets of primers were used: human CHOP (forward) 5’-AGAACCAGGAAACGGAAACAGA-3’, (reverse) 5’-TCTCCTTCATGCGCTGCTTT-3’; human sarcoendoplasmic reticulum Ca2+ ATPase (SERCA)2b (forward) 5’-CGCTACCTCATCTCGTCCA-3’, (reverse) 5’-TCGGGTATGGGGATTCAA-3; human β-actin (forward) 5’-CATGTACGTTGCTATCCAGGC-3’, and (reverse) 5’-CTCCTTAATGTCACGCACGAT-3’.

**Supplemental information**

**Table**

**Laboratory data of the patient**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| WBC | 9,500 | /µl |  | BUN | 4.9 | mmol/l |  | Blood glucose | 17.3 | | mmol/l | |
| Seg. | 22.0 | % |  | Cr | 18.5 | µmol/l |  | HbA1c (NGSP) | 11.6 | | % | |
| Lym. | 73.0 | % |  | UA | 214 | µmol/l |  | Glycoalbumin | 36.4 | | % | |
| Mono. | 3.0 | % |  | Na | 133 | mmol/l |  | Anti-GAD antibody | <0.4 | | U/ml | |
| Eos. | 2.0 | % |  | K | 4.9 | mmol/l |  | Anti-IA2 antibody | <0.4 | | U/ml | |
| RBC | 466 | /µl |  | Cl | 102 | mmol/l |  | IRI | 10.2 | | pmol/l | |
| Hgb | 11.8 | g/dl |  | Ca | 2.61 | mmol/l |  | CPR serum | | 0.33 | | nmol/l |
| Ht | 36.1 | % |  | P | 1.17 | mmol/l |  | urine\* | 7.6 | | µg/m2/day | |
| Plt | 33.6 | ×104/µl |  | Total-chol | 5.30 | mmol/l |  | Total ketone body | 102 | | µmol/l | |
| AST | 27 | U/l |  | HDL-chol | 1.62 | mmol/l |  | 3-hydroxybutyric acid | 47 | | µmol/l | |
| ALT | 13 | U/l |  | TG | 1.42 | mmol/l |  | Acetoacetic acid | 55 | | µmol/l | |
| γ-GTP | 8 | U/l |  | BGA (vein) |  |  |  |  |  | |  | |
| CK | 82 | U/l |  | pH | 7.491 |  |  |  |  | |  | |
| Amy | 40 | U/l |  | pCO2 | 3.47 | kPa |  |  |  | |  | |
| LDH | 260 | U/l |  | HCO3 | 19.7 | mmol/l |  |  |  | |  | |
|  |  |  |  | BE | -2.0 | mmol/l |  |  |  | |  | |

WBC, white blood cell; Seg., segmented cell; Lym., lymphocyte; Mono., monocyte; Eos., eosinocyte; RBC, red blood cell; Hgb, hemoglobin; Ht, hematocrit; Plt, platelet: AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyltranspeptidase; CK, creatine phosphokinase; Amy, amylase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; Cr, creatinine; UA, uric acid; Total-chol, total cholesterol; HDL-chol, high density lipoprotein cholesterol; TG, triacylglycerol; BGA, blood gas analysis; BE, base excess; Anti-GAD antibody, Anti-glutamic acid decarboxylase antibody; Anti-IA2 antibody, anti-insulinoma-associated 2 antibody; IRI, immunoreactive insulin; CPR, C-peptide immunoreactivity

\*Average of 3 days (normal range of 3-year old child; 40 µg/m2/day)

**Fig. S1**

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**ATF6-luciferase reporter assays in HEK-293 cells.**

HEK-293 cells were transfected with the ATF6-luciferase reporter (pGL4.39, Promega) and ATF6α expression plasmid together with WT *WFS1* or mutant (p.N325\_I328del, p.Q194X, p.L543R) expression plasmids (*n* = 9 for each). Statistical significance was tested by one-way ANOVA followed by Tukey’s test. \*, *P* < 0.001; \*\* *P* = 0.0003.

ATF6 promoter activity was elevated in p.N325\_I328del. The chemical chaperone 4-phenylbutyrate removes the ATF6 promoter activity.

WT, wild type; Del, p.N325\_I328del; 194, p.Q194X; 543, p.L543R; 4-PBA; 4-phenylbutyrate.

**Fig. S2**



**Western blotting with protein extracted from HEK-293 cells.**

HEK-293 cells were transfected with WT or p.N325\_I328del *WFS1*-FLAG expression plasmids together with the ATF6α-HA expression plasmid. Protein was extracted and the protein expression levels were analyzed by western blotting using anti-FLAG, anti-HA and anti-β actin antibodies.

Protein expression levels of p.N325\_I328del WFS1 were extremely low compared with WT. This result corresponds to Fig. 4. The inactive form of ATF6α protein (90 kDa) is highly expressed in the lane transfected with WT *WFS1.* In contrast, the active form of ATF6α protein (50 kDa) is higher in the p.N325\_I328del transfected cells.

WT, wild type; Del, p.N325\_I328del**References**

1. Hansen L, Eiberg H, Barrett T, Bek T, Kjaersgaard P, Tranebjaerg L, et al. Mutation analysis of the WFS1 gene in seven Danish Wolfram syndrome families; four new mutations identified. Eur J Hum Genet. 2005; 13:1275-84.

2. Colosimo A, Guida V, Rigoli L, Di Bella C, De Luca A, Briuglia S, et al. Molecular detection of novel WFS1 mutations in patients with Wolfram syndrome by a DHPLC-based assay. Hum Mutat. 2003; 21:622-9.