A Novel Compound Heterozygous Mutation in the WFS1 Gene (C.1997 G>A and C.2113_2114 ins T) Causes wolfram Syndrome: A Case Report

Reyida Aishajiang
  Jilin University First Hospital   https://orcid.org/0000-0001-9496-3905

Cheng Li
  Jilin University First Hospital

Bo-Tao Shen
  Jilin University First Hospital

Jian Sun
  Jilin University First Hospital

Wei Zhao (✉ w_zhao@jlu.edu.cn)
  Jilin University First Hospital

Case Report

Abstract

Background:

Wolfram syndrome (WS) is a rare autosomal recessive disorder associated with early-onset diabetes mellitus (DM), diabetes insipidus (DI), optic atrophy (OA) and hearing impairment. Most patients with WS have mutations in the *WFS1* gene, which encodes wolframin. This case report describes a patient with a novel heterozygous mutation of *WFS1*.

Case Presentation:

The proband was a 27-year-old Chinese male with WS who had developed DM at the age of 2 years, DI in the first decade, OA, neurogenic bladder and urinary tract infections in the second decade, and neurological abnormalities in later life. Magnetic resonance imaging suggested superior sagittal sinus enlargement and atrophy of the medulla and pons. Sequencing showed that the proband's asymptomatic parents were both carriers: the father carried a heterozygous c.1997G>A mutation that creates a premature stop at codon 666 (W666X) and that the proband's asymptomatic mother carried a heterozygous c.2113_2114insT mutation that generates a frameshift downstream to codon 705 (K705Ifs*7) and leads to a stop at codon 711. The proband had both the above C-terminal mutations, resulting in the substitution of N-glycosylation sitesthat are associated with the stability of wolframin.

Conclusion:

We have identified a novel compound heterozygous mutation of *WFS1* that is associated with WS. Our findings may facilitate future screening of WS carriers.

Background

Wolfram syndrome (WS) is an autosomal recessive disorder with varied manifestations, and this genetic disorder is also known as DIDMOAD because its typical clinical features include diabetes insipidus (DI), diabetes mellitus (DM), optic atrophy (OA) and deafness. WS was first reported in 1938 by Wolfram and Wagener, who described eight siblings with juvenile DM and OA. WS is considered a rare disease and has an estimated prevalence of 1/770,000 in the UK and a carrier frequency of 1 in 354. (1) Most cases of WS are caused by mutations in the *WFS1* gene, and the first case was reported in 1998. *WFS1* spans approximately 33.4 kb of genomic DNA on chromosome 4p16.1 and encodes wolframin, a protein with 890 amino acids.(2) Wolframin is expressed in a wide variety of tissues, particularly pancreatic beta cells and the brain. Biochemical studies have revealed that wolframin is an endoglycosidase H-sensitive membrane glycoprotein localized predominantly to the endoplasmic reticulum (ER). (3) Although the exact function of wolframin remains to be elucidated, it is speculated that mutations in *WFS1* lead to absent or dysfunctional wolframin that in turn causes ER dysfunction.

This case report describes a patient with WS associated with a novel compound heterozygous mutation of *WFS1* consisting of a previously reported c.1997G > A mutation (inherited from the father) and a new c.2113_2114insT mutation (inherited from the mother). Both C-terminal frameshift mutations in wolframin caused the substitution of putative N-glycosylation sites, presumably resulting in loss of function.

Case Presentation

A 27-year-old Chinese male (the proband) presented to our hospital with a history of type 1 DM (T1DM), DI, visual impairment and neurogenic bladder. The patient was diagnosed with T1DM (non-autoimmune) at the age of 2 years, since when he had received insulin therapy. DI was diagnosed at the age of 13 years following the development of polyuria and polydipsia, and the patient was receiving desmopressin as a treatment. He developed an impairment of vision 2 years later, and the ocular history revealed a gradually decreasing visual acuity and complete loss of color vision. During the previous decade, the proband had experienced symptoms of neurogenic bladder and repeated urinary tract infections, for which he had received intermittent catheterization. The parents were non-consanguineous and had no signs or symptoms of WS. There was a family history of essential hypertension on the father's side (including the proband's father and the brother and parents of the proband's father) and a family history of type 2 diabetes DM (T2DM) on the mother's side (including the proband's mother and the sister of the proband's mother, who were both diagnosed with T2DM at around the age of 45 years). During hospitalization, the patient complained of high blood pressure (ranging from 140−210/85−90 mmHg) for 1 month, but there were no signs of renal vascular abnormalities.

Fundoscopy revealed bilateral OA without diabetic retinopathy (Fig. 1). Magnetic resonance imaging (MRI) of the brain showed a high signal in both lateral ventricles in T2-weighted turbo inversion recovery magnitude (TIRM)-dart-fluid sequences (Fig. 2). Contrast-enhanced scanning suggested a slightly irregular signal along the superior sagittal sinus, which was considered to represent enlargement of parts of the venous sinus, as well as moderate atrophy of the medulla oblongata and pons (Fig. 2). A diagnosis of WS was made based on the clinical and imaging findings.

Blood samples were obtained from the proband and his parents to investigate whether WS was associated with a mutation in *WFS1*. The study protocols were approved by the hospital ethics committee, and informed consent was obtained from the proband and his parents. In brief, the methods used were as follows. Genomic DNA was extracted from peripheral blood using a commercially available kit (MyGenostics, Beijing, China). The enrichment libraries were sequenced (HiSeq 2000 sequencer, Illumina, San Diego, CA, USA) for a final size of 350−450 bp. Polymerase chain reaction (PCR) was performed for all 8 exons of *WFS1*. The PCR mix (final volume, 100 μL) contained 20 μL of Phusion HF buffer, 2 μL of 10 mM dNTP, 30 μL of template DNA, 1 μL each of Illumina PE primer #1 and #2, 1 μL of Hotstart Phusion, 5 μL of dimethyl sulfoxide and 40 μL of water. The eluted DNA was amplified using a denaturation step at 98ºC for 30 s, 15 cycles at 98ºC for 25 s, 65ºC for 30 s and 72ºC for 30 s, followed by final extension at 72ºC for 5 min. Purified products were obtained using SPRI beads (Beckman Coulter,

Brea, CA, USA). The enrichment libraries were sequenced (HiSeq 2000 sequencer) using 100 bp paired-end reads. The potential functional impact of any identified *WFS1* mutations were evaluated using five widely used algorithms: SIFT (Sorting Intolerant From Tolerant) ,(4) PolyPhen-2 (Polymorphism phenotyping-2) ,(5) MutationTaster ,(6) GERP++ (Genomic Evolutionary Rate Profiling) (7) and REVEL (Rare Exome Variant Ensemble Learner) .(8)

Sequencing revealed that the proband's father carried a G-to-A heterozygous mutation at nucleotide position (np) 1997 (c.1997G > A) that creates a premature stop at codon 666 (W666X) and that the mother carried a heterozygous T-insertion at np 2113 (c.2113_2114insT) that generates a frameshift downstream to codon 705 (K705Ifs*7) and leads to a stop at codon 711 (Figure. 3). The proband was identified as having both the above mutations (Figure. 3). The effects of the W666X mutation were considered deleterious by MutationTaster and GERP + + but unknown by SIFT, PolyPhen-2 and REVEL. The effects of the K705Ifs*7 mutation were considered unknown by all the above algorithms.

Discussion And Conclusions

The clinical features of WS are varied and include T1DM, OA, progressive sensorineural deafness, DI, autonomic nervous system dysfunction and, ultimately, brainstem atrophy and premature death. Although the medical and family histories are vital for the diagnosis of WS, genetic testing is becoming increasingly important. The majority of WS cases are caused by *WFS1* mutations. Wolframin, the protein encoded by *WFS1*, is predicted to have a hydrophobic central domain comprising 9 membrane-spanning segments connected to a hydrophilic N-terminal domain and a hydrophilic C-terminal tail .(2) Wolframin is a component of the ER, which carries out post-translational modification, folding and assembly of newly synthesized proteins such as insulin. *WFS1* may play a role in the negative regulation of ER stress signaling, thereby inhibiting the apoptosis of cells (including pancreatic beta cells) in response to ER stress.(9)

Wolframin is abundantly expressed in the pancreas, brain, heart and muscle. N-glycosylation of wolframin is thought to affect its biogenesis and stability, and there are five predicted Asn-glycosylation sites at amino acid positions 28, 335, 500, 661 and 746 .(3) Hofmann et al. reported that the R629W mutation causes instability and rapid degradation of wolframin .(2) The proband in the present case report carried a compound heterozygous W666X and K705Ifs*7 mutation (Fig. 4), the latter of which is a frameshift mutation that completely changes the N-glycosylation site. Mutation analysis has indicated that individual amino acids in specific regions are critical for correct protein folding, with substitutions at these loci inducing major functional changes .(10) Thus, the amino acid mutations identified in the proband in this study may be critical for correct folding of wolframin.

Most mutations underlying WS are located in exon 8 of *WFS1*, which encodes the transmembrane region and C-terminal tail of wolframin. However, the same mutations in exon 8 can have heterogeneous clinical presentations. The patient in our study had a compound heterozygous mutation (c.2113_2114insT and c.1997G > A) and presented with three of the four components of DIDMOAD (DI, DM and OA) but not

hearing impairment, which was reported previously for a patient with the c.1997G > A missense mutation.(11) Phenotype variation has also been described for another mutation (c.1346C > T, p.T449I): one affected patient showed urethral involvement and severe anorexia while her younger sister exhibited microalbuminuria without neurological or urinary tract involvement.(12) The c.1997G > A mutation identified in the proband described here causes premature termination at codon 666 and an incomplete hydrophilic C-terminal tail. Most mutations associated with deafness occur in exon 8, which contains the conserved C-terminal domain that seems vital to cochlea function.(13) The C-terminal may interact with other proteins, and its deletion may disrupt these interactions and protein function.(14) The patient in our study also had an nucleobase insertion at codon 705 that resulted in frameshift and rearrangement. This insertion mutation may be in a region that impacts on the interaction with other proteins, causing the phenotypic difference with regard to hearing impairment. However, it should be noted that WS in our patient could also have been due to an allelic variant in the promoter regulatory region in the intronic sequences of *WFS1* or a mutation in a different gene that was not detected with the current technology.

The proband in this case report developed T1DM at the age of 2 years, DI in the first decade, OA, neurogenic bladder and urinary tract infections in the second decade, and neurological abnormalities later in life. Although clear genotype-phenotype correlations have yet to be characterized, it has been suggested that missense mutations have a relatively mild phenotype while inactivating mutations (deletions, insertions, nonsense mutations and splice site mutations) cause more severe disease .(15) This is supported by another study reporting that patients with WS caused by missense mutations in *WFS1* had a mild phenotype.(16) Consistent with the above hypothesis, the patient in our study had a nonsense mutation and insertion in *WFS1* and exhibited a severe phenotype, including the onset of DM at only 2 years of age. Inactivation of both *WFS1* alleles is known to be associated with early-onset DM.(17) Interestingly, a study of juvenile-onset DM in Lebanon identified a particular *WFS1* mutation encoding a protein with an extended C-terminal domain that resulted in a delayed onset or absence of extrapancreatic features.(18) *In vitro* and *in vivo* animal experiments have found that wolframin depletion causes a reduction in insulin content, impairment of glucose-stimulated insulin secretion and activation of apoptosis.(19) Interestingly, single nucleotide polymorphisms in *WFS1* are strongly associated with DM risk in the general population.(20) Since the proband's mother had a family history of T2DM, it will be interesting to determine whether the K705Ifs*7 mutation is associated with an increased risk of T2DM. Further research is needed to establish the association between *WFS1* genotype and phenotype.

In summary, we have described a patient with WS likely caused by a novel compound heterozygous mutation in *WFS1* (c.2113_2114insT and c.1997G > A). The patient developed DM at a very young age followed by DI and OA in the next decade and neurological abnormalities later in life. His severe phenotype may be related to the presence of inactivating (nonsense and frameshift) mutations in *WFS1*. Since genetic mutations tend to be population-specific, the identification of this novel mutation may help in future screening for carriers of WS.

Abbreviations

WS

Wolfram syndrome

DM

Diabetes melitus

DI

Diabetes insipidus

OA

Optic atrophy

ER

Endoplasmic reticulum

PCR

Polymerase chain reaction

Declarations

Ethics approval and consent to participate

Consent for publication

Obtained

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests

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Authors' Contributions

Jian Sun, Wei Zhao and ReyidaAishajiang cared for the patient, Wei Zhao and Bo-Tao Shen designed the study. Cheng Li analysis for interpretation of data, ReyidaAishajiang drafting and revising the manuscript. All authors have approved the final version and agree to be accountable for all aspects of the work. None of the authors has conflicts of interest to declare. All of the authors have seen and approved the submission and take full responsibility for the manuscript.

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Footnotes

ReyidaAishajiangand Cheng Liare co-primary authors

ReyidaAishajiang and Cheng Li are joint first authors.

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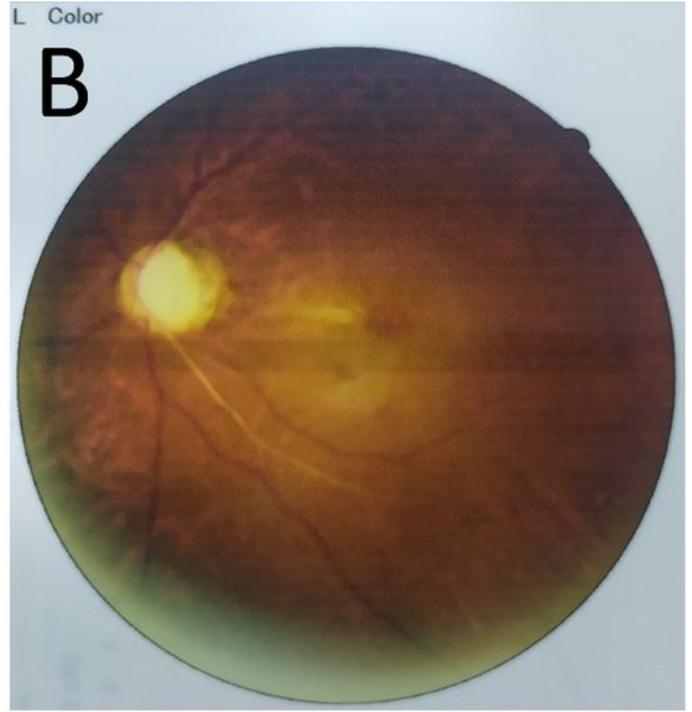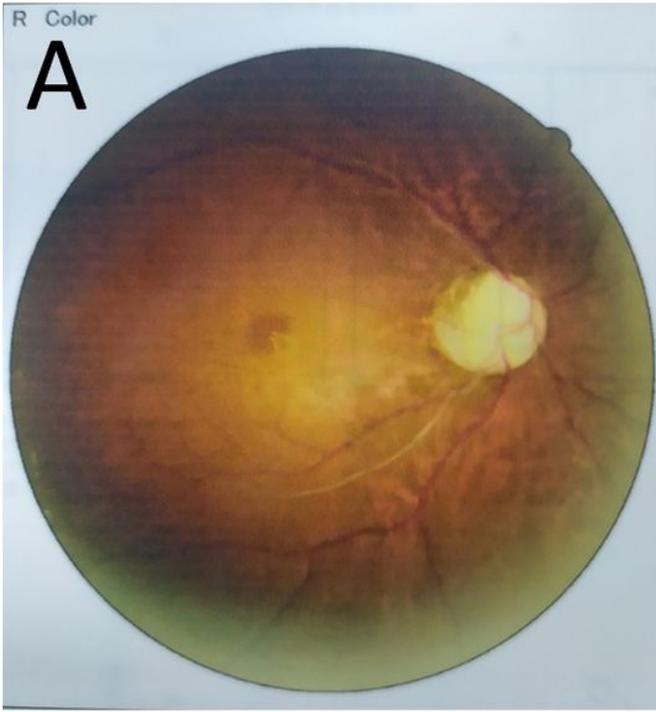
Figures

Figure 1

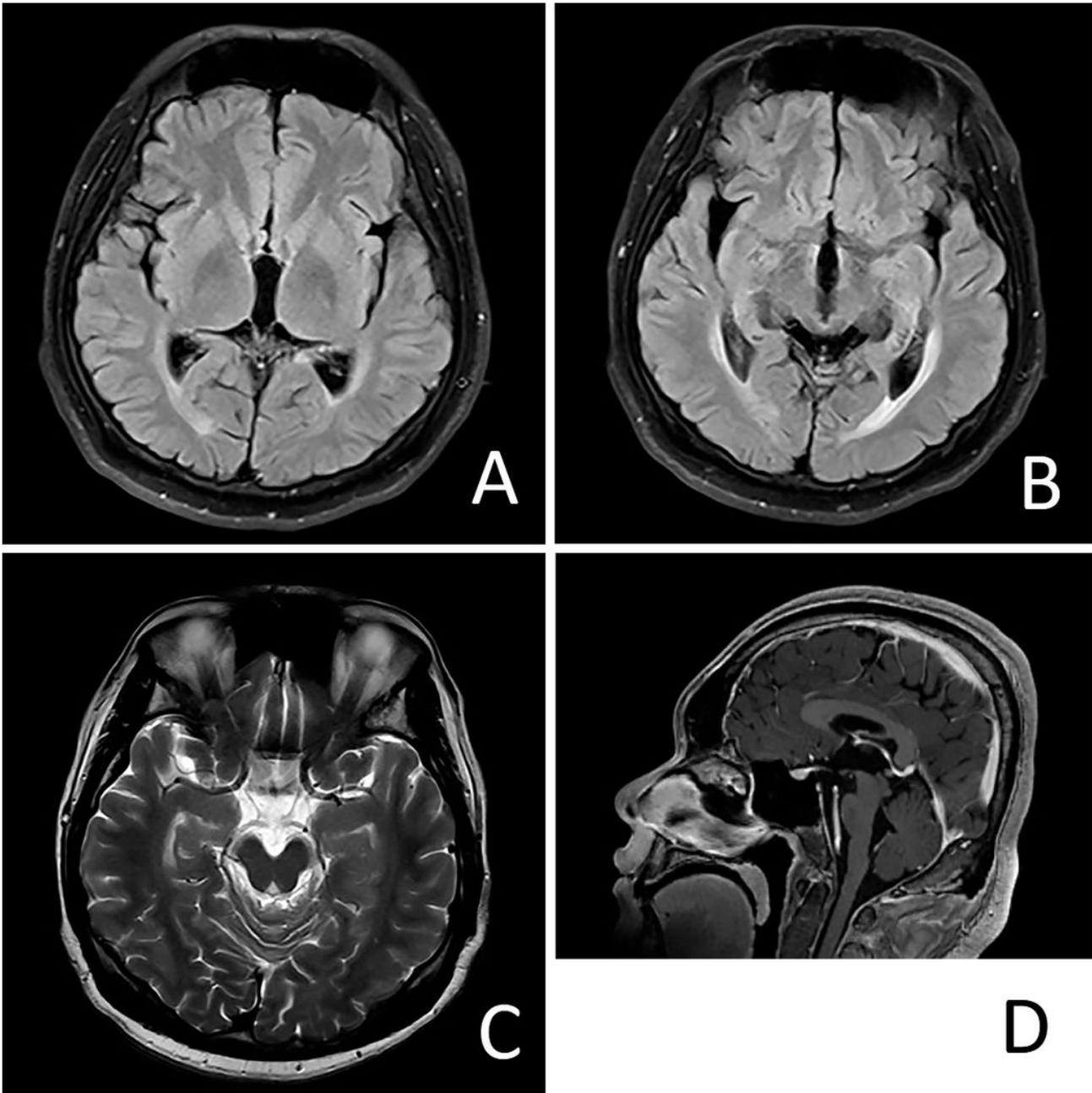Optic atrophy.Fundoscopy revealed bilateral optic atrophy(A,B) with no signs of diabetic retinopathy.

Figure 2

Brain MR images.Magnetic resonance imaging of the brain showed signal-intensity abnormalities in both lateral ventricles (A, B), moderate shrinkage of the medulla oblongata and pons (C, D) and an irregular signal along the superior sagittal sinus (D).
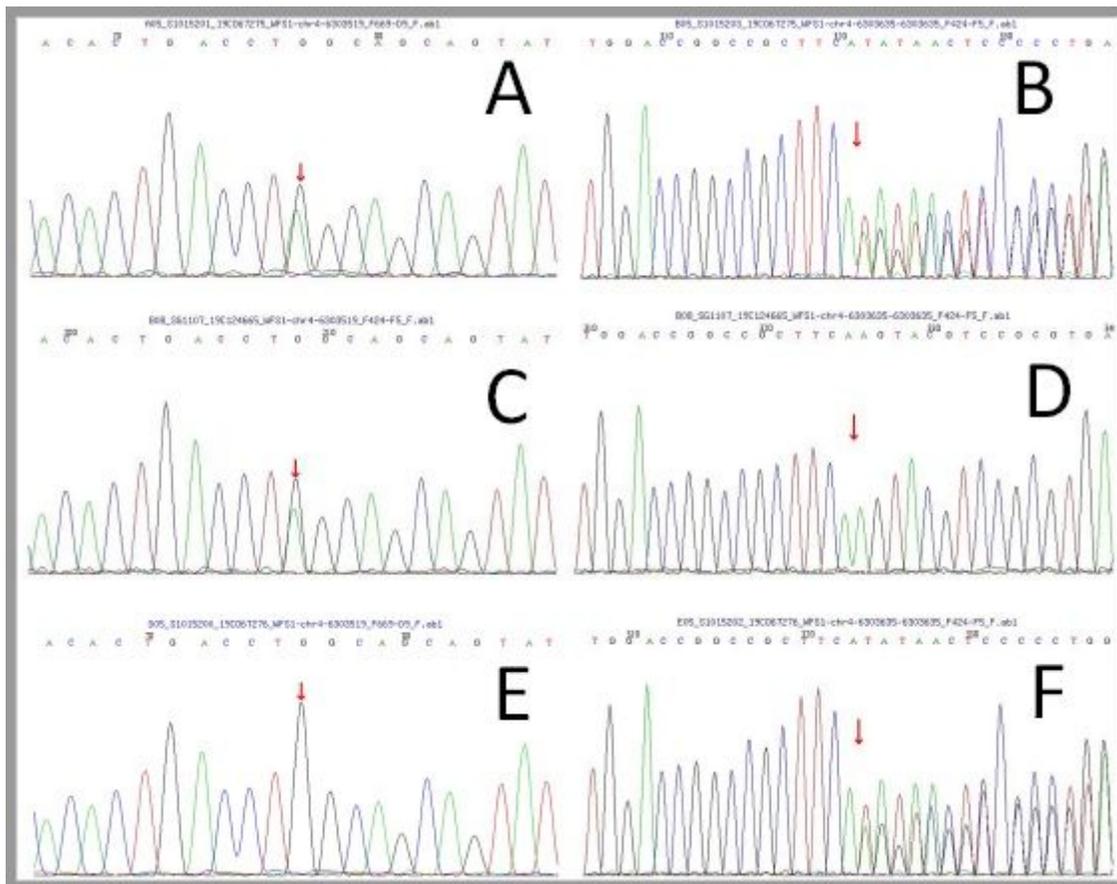
Figure 3

DNA sequencing.Identification of WFS1 gene mutations using next-generation sequencing of WFS1. Chromatograms are shown for the proband (A, B), proband's father (C, D) and proband's mother (E, F). The proband had a heterozygous c.1997 G>A mutation (A) also present in his father (C) as well as a heterozygous (c.2113_2114insT) mutation (B) also present in his mother (F).
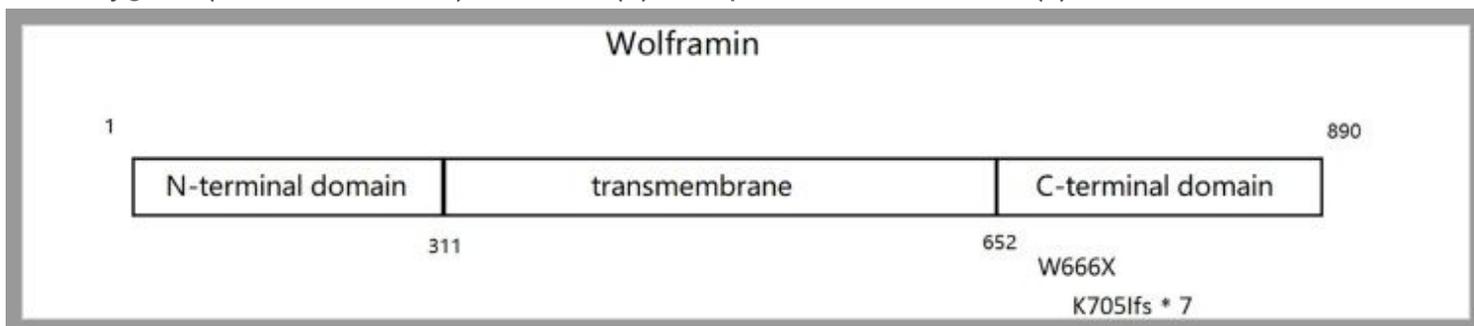

Figure 4

Schematic of wolframin.The proband had both the W666X and K705lfs*7 mutation in C-terminal, the K705lfs*7 mutation resulting in the substitution of N-glycosylation sitesthat is associated with the stability of wolframin.

Supplementary Files

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