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Evaluation of pathogenic variant in *WFS1* in a patient with Wolfram syndrome

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Abstract

Objective Wolfram syndrome (WS) is a genetically disorder that affect on many organs, and neurodegenerative disorder. Although various clinical dysfunctions may have different onset times, they can collectively contribute to delays in the diagnosis of the disorder. To date, more than 200 pathogenic and likely pathogenic variant have been identified. In the present investigation, we evaluated three families with WS and reported a mutation in the *WFS1*.

Methods This study, we have evaluated mutation in the *WFS* gene in three consanguineous families including three patients with a history of young-onset DM, progressive hearing loss and optic atrophy further neurological abnormalities.

Results Sequencing results showed a novel homozygous stop-gain variant, c.1444A > T (p.K482X), and two previously reported mutations (c.2006A > G and c.2105G > A) in exon 8 of *WFS1* gene. The variant interpretation was done according to the genetic guidelines. Finally, p.K482X was determined as a novel pathogen variant. Also, analysis showed that variants in parents were heterozygous.

Conclusions The present survey, revealed a novel nonsense mutation in the wolframin protein, creates a frameshift which causes a premature stop codon truncating the protein in amino acid 482 residues. This mutation occurs in transmembrane domain and causes elimination of 46% of wolframin protein.

Keywords Wolfram syndrome (WS), *WFS1* gene, Wolframin

Introduction

Wolfram syndrome (WS) is a genetically disorder that affect on many organs, and neurodegenerative disorder. Although various clinical dysfunctions may have different onset times, they can collectively contribute to delays in the diagnosis of the disorder. "Early-onset diabetes mellitus (DM) and optic atrophy (OA) are the primary clinical symptoms that should be considered for WS evaluation". The most common time of death is the fourth decade of life due to brain stem atrophy [1, 2]. The prevalence of WS dependent to conditions, estimated from 1 in 68,000 in Lebanon to 1 in 770,000 in the UK [3–5].

The location of *WFS1* gene is on chromosome 4p16 which contain 8 exons. Exon 8, the largest exon encode, is the most pathogenic variant reported. This gene encodes an 890 residue glycoprotein (wolframin), which is expressed in the majority of organs. Wolframin consists

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of three fragments, including central nine-transmembrane domains, cytoplasmic N-terminal domain, and luminal C-terminus. Wolframin is responsible for the regulation of endoplasmic reticulum (ER) stress, intracellular homeostasis, integrity, and survival of the cell [6]. *WSF1* mutations by influencing the proline-rich, extensin-like receptor kinase-1 (PERK1), Inositol-requiring enzyme type 1 (IRE1), and activating transcription factor 6 (ATF6) pathways, activate the ER stress and cell apoptosis such as neural system, and pancreases [7].

To date, more than 200 likely pathogenic mutations have been identified. The majority of them are null variant that is either nonsense mutations or frameshift mutations suggesting that the loss of function of wolframin protein is responsible for the disease [28]. Studying rare autosomal recessive disorders in regions with consanguineous marriage, such as Iran and Northern Africa gives us more insight about the management and prevention of disease. In this regard, in the present investigation, we evaluated three families with WS and reported a novel mutation in *the WFS1* gene.

Materials and methods

Population study

Three affected patients from three Iranian Family 1, 2, and 3, aged 14–29, with a history of early-onset DM, OA, and hearing impairments, belonging to consanguineous parents were enrolled for this study. Clinical patient information was obtained from clinical file records, and both a questionnaire and physical examination were performed by a group of physicians in the diabetes clinic. The minimum diagnostic criteria for WS are MD and OA, which are usually presented in the first decade and second decade, respectively.

Variant analysis

Four mL of peripheral blood from each patient was collected in EDTA tubes. Genomic DNA was extracted by the Cinna Teb Gene Azma (CTGA) PersPure Ultra Rapid DNA Extraction Kit, according to manufacture protocol. The quantity of extracted DNA was done using 1.2% agarose gel and spectrophotometry (Thermo Scientific, USA). We obtained the coding sequence data of the *WFS1* gene (NM-006005) from GenBank. The *WFS1* gene primers were designed using Primer3 v.0.4.0 online software to amplify exons and exon–intron boundaries of all coding regions of this gene. Exon 8 was amplified in 7 overlapping fragments termed 8–1 to 8–7 (Table 1).

PCR was conducted under standard circumstances: 1 µL isolated DNA (70 mg), 1 µL of each of the designed primers, 9.5 µL ddH2O, 12.5 µL of 2X Master Mix (Ampliqon®, Denmark), and standard thermal cycling circumstances for each exon according to its product size. After analyzing PCR reactions on 1.2% of the agarose gel, direct sequencing of amplified sequences was performed, at first for fragments of exon 8 (because the majority of mutations occurred in this region), bi-directionally on an automated sequencer ABI 3130 (Applied Biosystems 3130). Co-segregation analysis was carried out by PCR-Sanger sequencing in members of family 1, which included the healthy father and mother of the patient.

Table 1 The sequencing of primers used for WFS1 variant

Exon	Sequence	PCR product (bp)
Exon 1	CGGAGATGTGGAGTGATTGG ATCAGCTCGGAAGGGGAAAC	401
Exon 2	CCATGGGGACTGTACTGAGTG CAATGCTGAACTGCAGAGGAC	321
Exon 3	GAAGACCCTCATGCCTTGTC ATCTCAGGCACCGACACTTC	272
Exon 4	GCCTAGCCTAGTGGACATGC GAAACTGAAATTTCCCAACAGC	222
Exon 5	AGAGTTGGCAGGGTCAGAGTG GGAAGGTCCTGGCTCCTGTG	375
Exon 6	CCAGCTACTGGAGGTACAGAGG CTACTCCCAGCGTCCAGAAC	486
Exon 7	AGCCCATTGCTCTGTGTGAGG GGCACGGCTGTAAGACACTCT	354
Exon 8–1	GTCAGAGGGAGGCGTGAGAT GCCTGCTCCACATCCAGGTT	392
Exon 8–2	GGTGTTCCAGGACAGCAAGG CCGACAGGCACGGTGATGAA	393
Exon 8–3	GCTATCGCTGCTGCCCTCCA AGCTCCAGAGACGTGAACCAC	381
Exon 8–4	GTGAGCTCTCCGTGGTCATC CCCTCTGAGCGGTACACATAG	346
Exon 8–5	CTCCATGGTCAAGCTCATCCT TGTAGCGGTCGAACTTCTTGAT	431
Exon 8–6	GAGTCTGCCATCAACATGCTC TGGGAAGAAAAAGAAGTCGAAG	507
Exon 8–7	CTTCGAGCTCAAGGCCATCAG AGAGCTACACAGCAGCCTTCC	342

Table 2 Clinical features and age of onset of the main clinical characteristics in 5 patients with Wolfram syndrome

Patients	Age	Sex	DI	DM	OA	D	Other disorders
I (Patient1)	26	F	–	9	12	23	–
II (Patient2)	29	F	25	7	14	15	Psychiatric disease
III (Patient3)	14	F	–	5	9	12	–

Abbreviations: M: Male, F: Female, DM: diabetes mellitus, OA: optic atrophy, DI: Diabetes Insipidus, D: deafness

Bioinformatic studies

The sequence data were analyzed using SeqMan v5.00 (DNASTAR) and aligned to the reference sequence GenBank, for variant calling. Identification of the Consequence of variants was performed using by investigating The Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/), LOVD (www.lovd.nl), and Iranome (http://www.iranome.ir/) databases. In silico prediction tools including PROVEAN, SIFT (http://sift.jcvi.org), Polyphen2 (http://genetics.bwh.harvard.edu/pph2), Mutation Assessor (http://mutationassessor.org), MutationTaster (http://www.mutationtaster.org/), FATHMM, PANTHER and CLUSTALW online software were applied to evaluation of pathogenicity and conservation degree of identified variants. The detected mutation classification and nomenclature were made according to the American College of Medical Genetics and Genomics (ACMG) (www.acmg.net) and Human Gene Mutation Database (HGMD) (www.hgmd.org) rules, respectively. In silico pathogenicity prediction tools were utilized to assess the c.1444A > T (p.K482X) variant, revealing a consensus on its deleterious effects. PROVEAN, SIFT, PolyPhen2, Mutation Assessor, MutationTaster, and FATHMM all indicated a damaging or disease-causing impact, while PANTHER highlighted significant functional consequences. Additionally, CLUSTALW showed high conservation of the mutation site across species, further supporting its potential pathogenicity.

Protein structure prediction

The p.K482X mutation affected on stop codon that causes disruption in protein formation. Structural

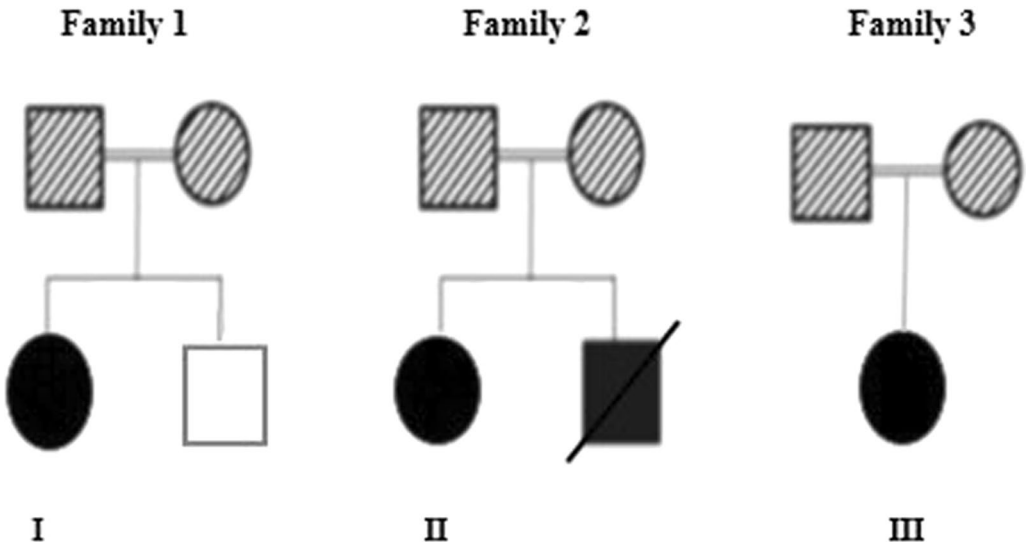

Fig. 1 Family pedigrees of Wolfram syndrome patients. Black-filled boxes: were homozygous affected members, while black shaded boxes: were carrier members, and white boxes: were mutation-negative unaffected members

Table 3 Mutations detected in the *WFS1* gene in families

Family	Exon	Nucleotide change	Amino acid change	Type of mutation	References
I	8	c.1444 A > T	p.K482X	Nonsense	Novel
II	8	c.2006 A > G	p.Tyr669Cys	Missense	[8]
III	8	c.2105 G > A	p.Gly702Asp	Missense	[9]

predictions suggest significant alterations in the protein's three-dimensional conformation, which could disrupt normal function [29, 30].

Results

Clinical findings and sequencing results

In the present survey, three families with suspicion of WS were evaluated. Early-onset diabetes was the primary clinical manifestation in all patients (at the age of 5–9 years). The parents of all three families had

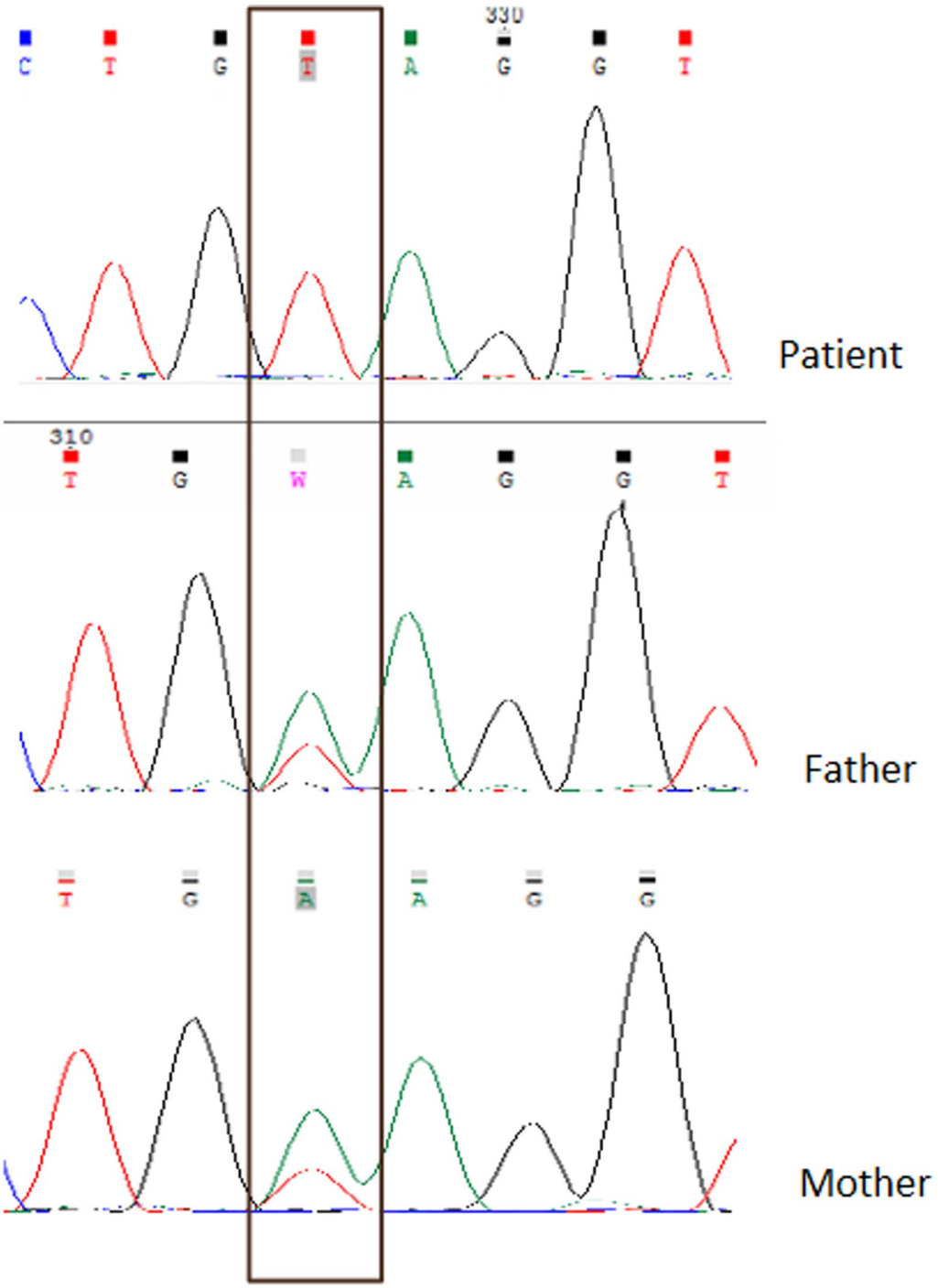

Fig. 2 The electropherograms of this family 1 reveal the homozygous state of the patient and the heterozygous state of the father and mother for the p. K482X variant

consanguineous marriages. The age of the patients showed in Table 2.

Our case 1 is a 26-year-old female with diagnosed insulin-dependent diabetes (Type 1) and OA at the ages of 9 and 12, respectively. Also, hearing impairment at 8000 Hz was diagnosed in this patient at the age of 23 (Fig. 1).

Our mutational screening revealed 3 variants, including 2 missense and 1 nonsense in exon 8. Two missense mutations, including c.2006A > G and c.2105G > A (family 2 and 3) were previously reported. The variant of c.1444A > T, p.K482X was detected in Family 1 (Table 3) (Fig. 2). The reported pathogenic variants of *WFS1 gene* that create WS phenotype were presented in Table 4.

The results of the WFS1 gene sequence showed A to T homozygous at position 31,391 (g.31391A > T), which caused the conversion of the amino acid Lysine to a stop codon at position 482 (p.K482X) (Fig. 2). Co-segregation analysis revealed that both parents carry the p. K482X novel variant in a heterozygous status.

Bioinformatics results

Based on the HMMTOP and PRED-TMR servers, wolfram protein has nine-transmembrane helixes. The arrangement of amino acid fragments of transmembrane helixes is as follows; 314–333, 342–361, 405–423, 432–451, 464–481, 496–515, 528–549, 564–583, and 635–652, respectively. The p.K482X occurred in the fifth transmembrane domain (464–481). Our analysis revealed that the novel nonsense mutation, c.1444A > T (p.K482X) creates a frameshift which causes a premature stop codon truncating the protein in amino acid 482 residue. The substitution of Lysine to a stop codon results in a truncated protein with 482 amino acids long and producing only 54% of wolframin protein (Fig. 3).

In silico prediction tools predicted the deleterious effect of this variant on protein function, stability, and structure (Table 5). The detected variant was absent from ClinVar databases, 1000 genomes project phase 3, gnomAD, HGMD, Iranome, and literature. Multiple sequence alignment across species signified that the amino acid at position 482 is a conserved residue from humans to other mammalians (Fig. 4).

Table 4 The pathogenic gene variant in *WFS1* in ClinVar database

HGVS coding	HGVS protein	Coding impact	Location
c.76C > T	p.Arg26Ter	Nonsense	Exon 2
c.173C > T	p.Ala58Val	Missense	Exon 2
c.265G > T	p.Glu89Ter	Nonsense	Exon 3
c.307C > T	p.Gln103Ter	Nonsense	Exon 3
c.319G > C	p.Gly107Arg	Missense	Exon 4
c.320G > A	p.Gly107Glu	Missense	Exon 4
c.328 T > A	p.Tyr110Asn	Missense	Exon 4
c.330C > A	p.Tyr110Ter	Nonsense	Exon 4
c.334C > T	p.Gln112Ter	Nonsense	Exon 4
c.406C > T	p.Gln136Ter	Nonsense	Exon 4
c.472G > A	p.Glu158Lys	Nonsense	Exon 5
c.643C > T	p.Gln215Ter	Nonsense	Exon 6
c.676C > T	p.Gln226Ter	Nonsense	Exon 6
c.743 T > G	p.Val248Gly	Missense	Exon 7
c.757A > T	p.Lys253Ter	Nonsense	Exon 7
c.873C > G	p.Tyr291Ter	Nonsense	Exon 8
c.937C > T	p.His313Tyr	Missense	Exon 8
c.1048 T > A	p.Phe350Ile	Missense	Exon 8
c.1309G > C	p.Gly437Arg	Missense	Exon 8
c.1381A > C	p.Thr461Pro	Missense	Exon 8
c.1433G > A	p.Trp478Ter	Nonsense	Exon 8
c.1456C > T	p.Gln486Ter	Nonsense	Exon 8
c.2084G > T	p.Gly695Val	Missense	Exon 8
c.2104G > A	p.Gly702Ser	Missense	Exon 8

Differences in patient phenotypes

Our analysis identified three variants, including one novel nonsense mutation (c.1444A > T, p.K482X) and two previously reported missense mutations (c.2006A > G and c.2105G > A). Patients with the novel nonsense mutation (Family 1) presented with earlier onset of diabetes mellitus (DM) at age 9 and optic atrophy (OA) at age 12, compared to the other two patients who showed slightly later onsets of these symptoms. Additionally, the patient with the novel variant exhibited hearing impairment at age 23, whereas the others had more pronounced psychiatric and neurological manifestations. This phenotypic variability may be linked to the functional impact of the mutations,

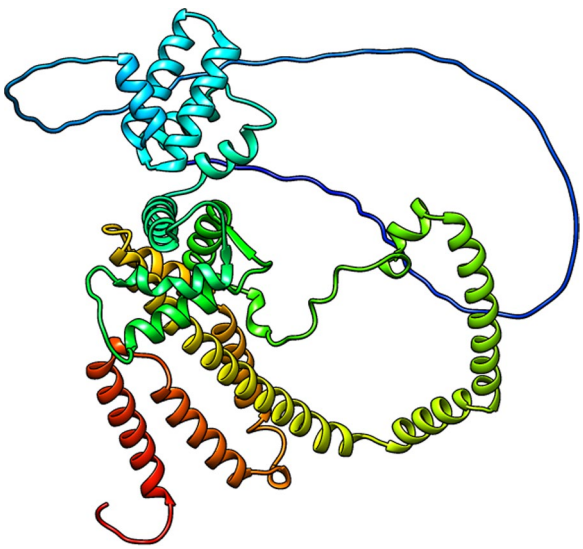

Fig. 3 The novel nonsense mutation, c.1444A > T in transmembrane domains causes elimination of 46% of wolfram protein

Table 5 Results of In silico analysis for c.1444A > T p.K482X variant

Tool	Information	Link	Prediction	Value/range
MutationTaster2	Disease-causing potential	https://www.mutationtaster.org	Pathogenesis	NA*
MuPro	Energy and stability biomarker	http://mupro.proteomics.ics.uci.edu/	Instability	G = −0.9968398
Franklin	Pathogenicity classification	https://franklin.genoox.com/clinical-db/home	Likely Pathogenic	–
ACMG	Pathogenicity classification	https://www.acmg.net/	Pathogenic	–

* Not available

as the novel mutation leads to significant truncation of the wolframin protein.

Discussion

WSF1 contains 8 exons, which encode Wolframin protein with 890 amino acids. Wolframin is mainly placed in the ER and plays a role in ca^{+} hemostasis. The brain and pancreas have the highest level of wolframin, while the kidney and spleen have the lowest level of wolframin [10]. Most mutations of *WSF1* have been identified in exon 8. Mutation in *WSF1* causes loss of function of the Wolframin protein, which is engaged in the cell apoptosis. Loss of function mutations of *WSF1* such as nonsenses and stop causes instability of nonsense transcript [11]. The novel nonsense mutation detected in our study, c.1444 A > T, influences the wolfram localization in ER. Generally, the mutations before exon 8, in amino acids 1–670 and 701–890 cause complete degradation, while mutations after exon 8 cause disrupt protein information [12–14].

Na^{+}/K^{+} ATPase in pancreas β cells has a critical role in insulin secretion regulation and ca^{+} hemostasis [15]. Two subunits of Na^{+}/K^{+} ATPase, including α_1 and β_1 are vital for catalytic and regulatory roles, respectively [16]. Any disruption in Na^{+}/K^{+} ATPase function is accompanied by cell apoptosis [17]. To maintain the proper Na^{+}/K^{+} ATPase function, the interaction between the β_1 subunit and wolframin protein is essential [18, 19]. There are two domains in wolframin for interaction in transmembrane [19]. The novel mutation detected in the present study, c.1444 A > T, causes the premature stop codon at p.K482x amino acid and eliminates the wolframin protein after the fifth part of the transmembrane. This mutation reduces the connection between wolframin protein and Na^{+}/K^{+} ATPase, which results in instability of Na^{+}/K^{+} ATPase localization and subsequently cell apoptosis. Also, we diagnosed a missense mutation, c.2106 A > G after the substitution of glycine by aspartic acid at codon 702 in the C-terminal domain of wolframin (Patient 3). In addition to the importance of amino acid 652–890 in wolframin and Na^{+}/K^{+} ATPase interaction, it was demonstrated that any gene defect in the C-terminal domain leads to the severe phenotype of WS [16]. Additionally, Na^{+}/K^{+} ATPase involved in K^{+} hemostasis and osmolarity of the inner ear [20, 21]. Hence, any Na^{+}/K^{+} ATPase dysfunction is accompanied by hearing loss [22], which was observed in our patient with c.1444 A > T mutation. Steady-state level analysis indicated that wolframin in insulinoma cells approximately is 50 folds higher than the

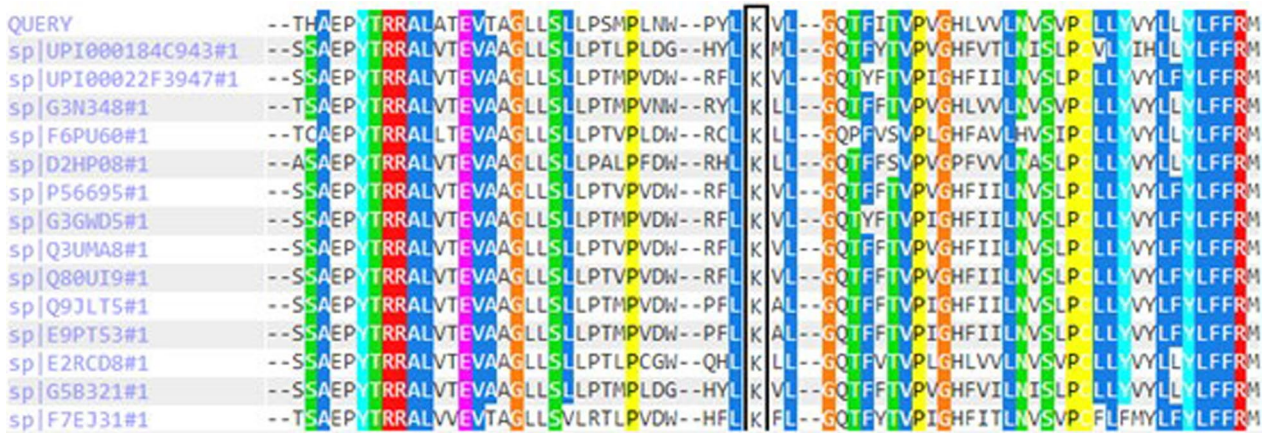

Fig. 4 Illustrates the conservation of the K482X residue across multiple species, including Homo sapiens, Mus musculus (mouse), Rattus norvegicus (rat), Pan troglodytes (chimpanzee), and Canis lupus familiaris (dog). The alignment highlights the high evolutionary conservation of this residue, supporting its critical role in protein function. This finding aligns with prior studies that suggest conserved residues are likely to be functionally significant

other cells [11]. This explained the early-onset DM in our patients.

As mentioned earlier exon 8 of the *WSF1* gene is a hot spot region for the majority of mutations. Nevertheless, there are five hot spots, including Tyr669, Gly674, Gly780, Asp797, and Lys836 which correspond to phenotype-genotype inconsistency in WS [23]. The second diagnosed mutation in our study was with missense mutation c.2006 A > G due to substitution of p.Tyr669Cys and created DIDMOAD phenotype (characterized by Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and Deafness) in a 29-year-old female (Patient 2). In a study from Italy, it was demonstrated that among patients with WS, 46.7% showed the DIDMOAD phenotype [24].

WS is recognized as a rare neurodegenerative disorder, although different onset of more clinical dysfunctions can delay the disorder diagnosis, especially in patients with autoantibodies [25]. In addition, the genetic abnormalities create various clinical manifestations; for example, in patients with biallelic null variants, the mean onset age of DM significantly is younger than in patients with biallelic missense variants [26]. In contrast, it was demonstrated that some dominant forms do not create WS due to reduced penetrance [27]. Heredia et al. investigated the genotype–phenotype correlation of 412 patients with WS; their results indicated that despite the relevant literature, DM and OA might not be the first two clinical symptoms in all patients, mutations are nonuniformly distributed in WFS1, the onset age of DM, hearing loss, diabetes insipidus, and disease progression are highly depended on the genotype [14]. Hence, the genetic analysis should be conducted for these patients.

Conclusion

The present survey revealed a novel nonsense mutation in wolframin protein; c.1444A > T (p.K482X) creates a frameshift which causes disrupt protein formation in 482 residues. This mutation occurs in transmembrane domain and causes elimination of 46% of wolframin protein. Our data demonstrated the importance of genetic analysis in patients with early onset of DM.

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Author contributions
M.At has conceived the manuscript and revised it. M.H wrote the manuscript. M.H and M.J, provided clinical data, information and performed the technical tests. S.N conducted statistical analysis. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declaration

Ethics approval and consent to participate
The current study is based on the ethical committee of Isfahan University of Medical Sciences (IR.ARI.MUI.REC.1400.011).

Consent for publication
Informed consent was obtained from all individual participants included in this study.

Competing interests
No potential conflict of interest relevant to this article was reported.

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