A Novel Nonsense Mutation in The *WFS1* Gene Causes The Wolfram Syndrome

Abstract: Wolfram syndrome is a rare autosomal neurodegenerative disorder usually caused by mutations in the *WFS1* gene. The *WFS1* gene is active in cells of body, with highly expression in the, brain, lungs, heart, inner ear, and pancreas. Within cells, *WFS1* gene encodes wolframin protein that is located in a structure of endoplasmic reticulum. Endoplasmic reticulum has critical role in protein folding and material transportation within the cell or to the surface of cell. Although the actual function of wolframin protein is unknown, but based on location, defect of this protein may cause the problem in protein folding or cellular transportation. In this study DNA sequence of *WFS1* gene was analyzed in a 9 years old boy, to confirm wolfram syndrome. We found the novel pathogenic nonsense mutation in exon 4 of *WFS1* gene (c.330 C>A). The heterozygosity for parents also confirmed by Sanger sequencing.

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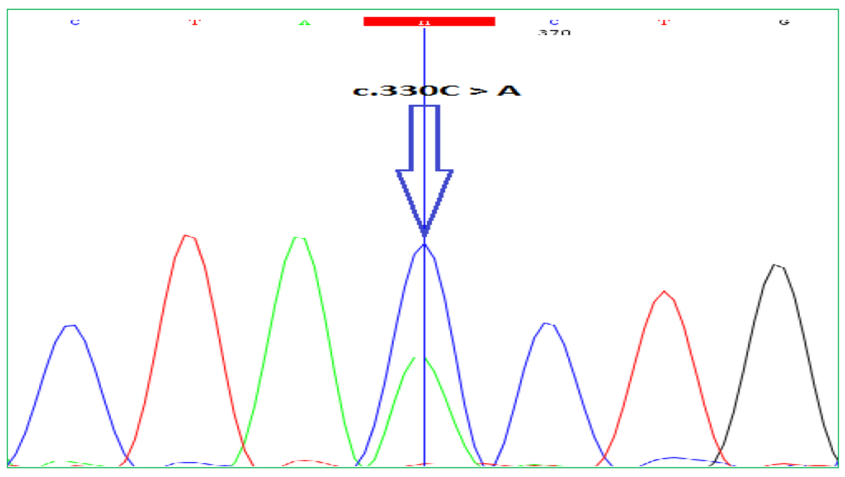
CASE PRESENTATION

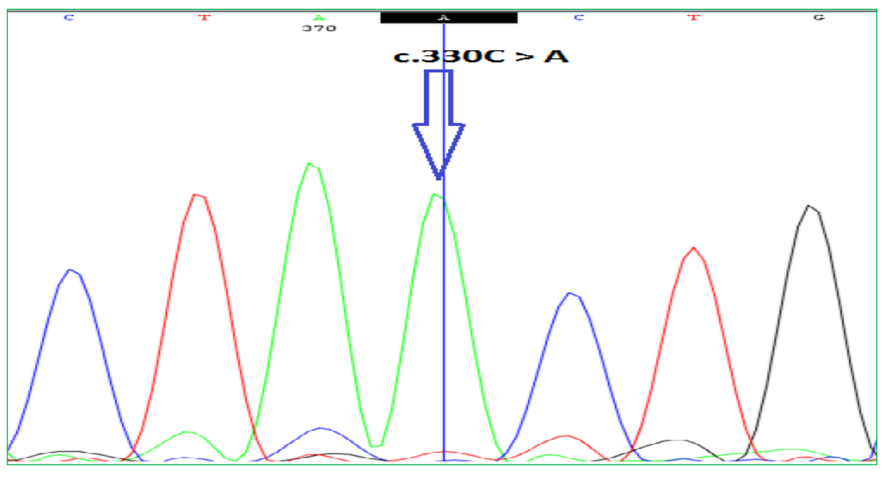
We report a 9 years old boy who was born from consanguineous parents. Due to his learning disabilities he was not educable in primary school. He was diagnosed with insulin depended diabetes mellitus when he was at age of 3 years. He had progressive visual deterioration without retinopathy for 6 years because of bilateral optic atrophy. Auditory evaluation detected mild bilateral sensory neural hearing loss. His medical history revealed frequent urination and symptoms of progressive nighttime enuresis from one year ago. Direct Radionuclide voiding Cystourethrography (DRNC) showed no vesicoureteral reflux, but after urodynamic tests, neurogenic bladder diagnosis was established. Fluid restriction test were performed and enabled us to diagnose central DI. In physical examination, height and weight were 128cm (25 percentile) and 30 kg (50 percentile) respectively, testis development, axillary and pubic hair was tanner stages one. In laboratory step, fasting blood sugar (FBS), HbA1c, and urine density were 140 mg/dl (normal range 70 to 99), 8% (normal< 5.7%) and 1.004 (normal range 1.005 to 1.030) respectively. The patient was clinically diagnosed as Wolfram syndrome with these findings.

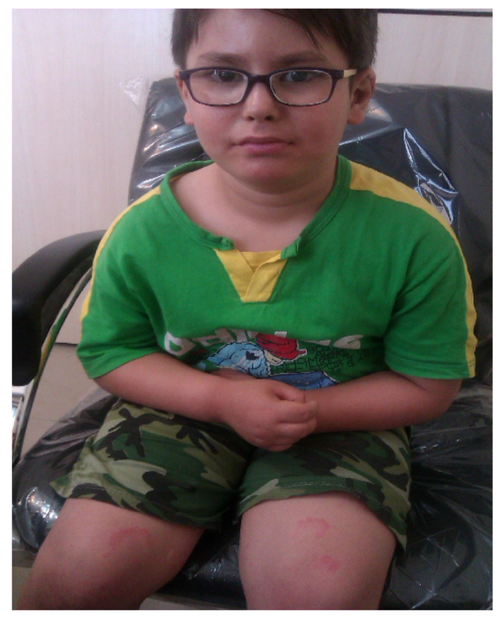
METHOD

Blood sample was collected from the patient after written consent from his parents. Genomic DNA using salting out method was extracted from blood. Genetic testing for confirmation of Wolfram syndrome was performed. Not only all exons but also exon-intron boundaries of *WFS1* was amplified and sequenced by Illumina Genome Analyzer (BGI-Clinical laboratories, China). Mutant homozygote mutation in patient, also heterozygosity of parents was confirmed by Sanger sequencing of polymerase chain reaction (PCR) product. PCR was performed including 100 ng extracted DNA, 1.5um mgcl2, four units of dNTPs, 1 unit TaqDNA polymerase, and specific primers for exon 4 of WFS1 gene (ABI Veriti Thermal cycler). The sequences of forward and backward primers are TGGTTCTGATTTCCATGCATTGA and ATTTCCCAACAGCATCACCAG, respectively. The sequences of PCR products were carried out using the ABI Prism 3130 Genetic Analyzer (ABI, USA).

Homozygote mutation in exon 4 of *WFS1* gene (c.330 C>A) in patient, also heterozygote mutation of parents were confirmed with Sanger sequencing technique (Pars Genome laboratory, Karaj, IRAN). (Figure 1)







DISCUSSION

The nucleotide substitution (c.330C > A) was found in the patient, lead to Y110X, It means nonsense mutation. Transition mutation, C to A in exon 4 of *WFS1* gene in patient leads to a Tyrosine at position 110 (UAC) replaced by stop codon (UAA). To the best of our knowledge, this mutation has been reported for first time. We used Mutation Taster (www.mutationtaster.org) for prediction of damaging effects of this variant which was a nonsense mutation. This software checks if the resulting protein will be elongated, truncated, or whether nonsense-mediated mRNA decay (NMD) is likely to occur. Truncated is referred to as either slightly truncated (if less than 10% of the wild-type protein length are missing) or strongly truncated (if more than 10% of original protein length are missing). Mutation Taster determines the NMD border as last intron/exon junction minus 50 bp and analyses if a given premature termination codon occurs 5' to this border thus leading to NMD. The original wolframin protein length concluded 890 aminoacids, which in our case this length had been reduced to 109 aminoacids, strongly truncated protein. If these aberrant mRNAs were translated, results in gain-of-function or dominant-negative activity of the proteins. Mutation Taster concludes that an alteration causes NMD, this alteration is automatically regarded as a disease mutation. This premature stop codon in heterozygous parents tolerate because of NMD action, and normal allele produce normal protein. In patient, Homozygote mutation, activation of NMD in both transcribed mRNA from both mutant allele results in no wolframin protein production. This novel nonesense mutation causes classic form of WS which can be used for prenatal diagnosis (PND). As mutations of genetic disorder may be population specific, finding new mutations in a population helps to design screening tests for carrier detection and PND.

REFERENCES

1. Eiberg H, Hansen L, Kjer B, Hansen T, Pedersen O, Bille M, Rosenberg T, Tranebjaerg L. Autosomal dominant optic atrophy associated with hearing impairment and impaired glucose regulation caused by a missense mutation in the WFS1 gene. J. Med. Genet. 2006;43:435-440.
2. Strom TM, Hortnagel K, Hofmann S, Gekeler F, Scharfe C, et al. Diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) caused by mutations in a novel gene (Wolframin) coding for a predicted transmembrane protein. Hum Mol Genet 1998; 7: 2021–2028.
3. Ghirardello S, et al.The Diagnosis of Children with Central Diabetes Insipidus Pathology. Journal of Pediatric Endocrinology and Metabolism. 2007 ; 20:359–376
4. Najjar SS, Saikaly MG, Zaytoun GM, Abdelnoor A. Association of diabetes insipidus, diabetes mellitus, optic atrophy, and deafness. The Wolfram or DIDMOAD syndrome. Arch Dis Child.1985 Sep;60(9):823-8.
5. Sonya G. Fonseca, Mark Burcin, and etc .Endoplasmic Reticulum Stress in Beta Cells and Development of Diabetes**.** Curr Opin Pharmacol. Dec 2009; 9(6): 763–770.
6. Barrett TG, Bundey SE, Macleod AF. Neurodegeneration and diabetes: UK nationwide study of Wolfram (DIDMOAD) syndrome. Lancet 1995; 346(8988): 1458-63.
7. Norooziasl S, Javadinia SA. A Case report of Wolfram Syndrome. J Birjand Univ Med Sci 2013; 20(1): 102-7. [In Persian].

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