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Case Report

A Novel Variant in Iranian Patient with Cystinuria: A Case Report

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Abstract

Cystinuria is an autosomal recessive disorder in which the renal reabsorption of cystine, arginine, lysine and ornithine are disturbed. The two genes, the pathogenic forms of which are responsible for the disorder, are SLC7A9 and SLC3A1. In this study, we describe a disease that has a new c.916A> T variant (p. K306 *) in exon 5 of the SLC3A1 gene. This variant results in the NMD phenomenon in which the protein product is not produced because of mRNA destruction. In 2020, blood sample of a 41-yr-old man from east Azerbaijan, Iran together with his parents were collected to be studied. PCR and direct sequencing were performed to detect the possible SLC3A1 variant. Whole-gene sequence analysis done by Mutation surveyor Software revealed a novel nonsense homozygous variant in exon 5 of the gene. Parental Sequence Analysis shows that they are heterozygous. According to ACMG guideline, this variant is considered as pathogen. Finding serious mutations can allow rapid screening for cystinuria by analyzing common mutations. It should also be considered as a pathogenic variant in patients' cystinuria.

Keywords: Cystinuria; Kidney stone; Iran

Introduction

In general, 1%-2% of adults and 6%-8% of children suffering from kidney stones have cystinuria (1). Cystinuria is an autosomal recessive disorder in which the renal reabsorption of cystine, arginine, lysine and ornithine are disturbed. Of these amino acids, only cystine is water insoluble which causes formation of stones in kidney (1, 2). The incidence of this disease is 1 in 7,000 (3). The two genes, the pathogenic forms of which are responsible for the disorder, are SLC7A9 and SLC3A1. While the former encodes (b0, + AT) protein, the translated protein product of the latter is rBAT. The aforementioned proteins form a dibasic amino acid transporter located in the apical membrane of proximal renal tubular epithelial cells which facilitate the transport of cysteine from inside the tubules back to the blood. The chromosomal locations of these two genes are at 2p16.3-21 and 19q13.1, respectively (3, 4)

Ever since many mutations in SLC3A1 and SLC7A9 have been reported in patients with cystinuria, most of which are missense mutations (5). From molecular genetics point of view, while mutation in SLC3A1 leads to type A cystinuria, mutation in SLC7A9 type B results in cystinuria type B. The third type of cystinuria, Type AB, has mutations in both genes. Treatment of this disease involves urine alkalization with potassium citrate and limiting sodium intake (6).

In this study, we describe a kind of cystinuria that has a new c.916A> T variant (p. K306 *) in exon 5 of the SLC3A1 gene. This variant results in the NMD phenomenon in which the protein product is not produced because of mRNA destruction.

Case presentation

The patient was a 41-yr-old man who has had nephrolithiasis repeatedly for about 13 years. He was referred to our genetic unit in Ali Asghar Hospital, Tehran, Iran for genetic evaluation of cystinuria in 2020. After obtaining a thorough medical history and drawing a pedigree, the person's parents are third-degree relatives (Fig. 1). Kidney problems, including renal failure and nephrolithiasis, together with blindness due to retinitis pigmentosa are apparently seen in some of his siblings (please refer to the pedigree). For this patients Ethics approval was not required but the consent form was received of patient and his parents.

In the physical examination of the patient there was no abnormal finding. Ultrasound of the patient shows a number of stones in the pelvis and upper and middle calices of both kidneys. Microcytic anemia has been found in blood tests and the presence of cysteine crystals in the patient's urine sample is documented.

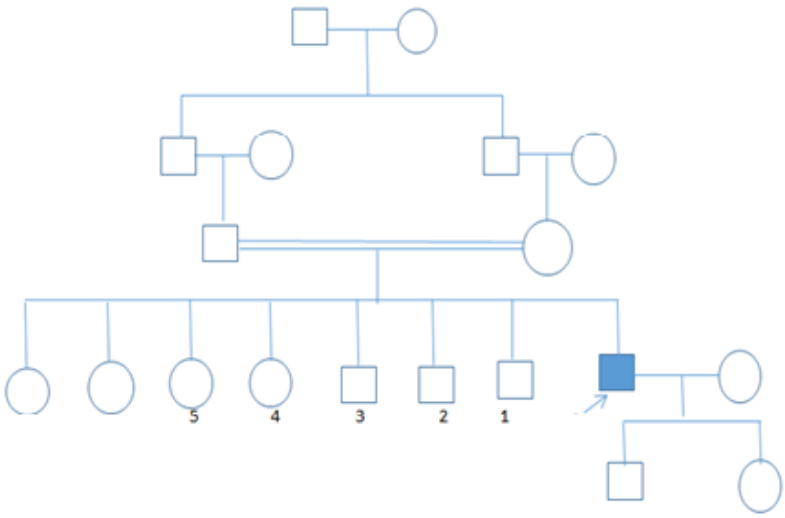

Fig. 1: Pedigree of a Family with Cystinuria. Circles indicate female family members, and squares male family members. Proband with Cystinuria is indicated by solid symbols, and those without JS indicated by open symbols

Differential diagnosis

Other situations in which urinary level of cystine rises are to be considered as differential diagnoses of the disorder. Diagnoses such as renal tubular immaturity in infants, Wilson disease and Fanconi syndrome are those that may increase the level of urinary cytine excretion, a significant sign of cystinuria.

Genomic DNA was extracted from peripheral blood by salting-out method. Primers were designed for all *SLC3A1* gene exons (NM_000341) (Table 1). Then to run PCR, 300 nmol of Forward and Reverse primers were combined with 10 µg of Master Mix (Pishgam Biotech Co., Tehran, Iran), 100 ng DNA and lastly 6 µg of distilled water to reach a final volume of 20 µg. Finally,

PCR was performed according to the following procedure (Sensoquest, Germany).
The initial denaturation temperature was 95 °C for 3 min, then 95 °C for 1 min, 61 °C for 1 min, 72 °C for 1 min and finally 72 °C as the final extension temperature for 10 minutes. All the amplification products were confirmed by setting up them on 2% agarose gel before sequencing them by Sanger method.

Table 1: Primers of *SLC3A1* gene

Exons	***Forward Primer*** ***Reverse Primer***	***Product Size***
EX1	ACCTCTGTACTTTTACCCTTTC	725
	AGGAGCAACTTGGCATGA	
EX2	GTAGAGCTAGATCTTCCTATTTG	774
	AGGCCAGGCACAGTAATG	
EX3	TTAGCCATTACTGTGCCTG	463
	ATTTGCTAGAACACATCATCTC	
EX4	TGAGAACATTGGTTTGCTG	594
	TGCACCAGAGAGCTTAATCC	2229
EX5	TCTTTGAGTTTGCAGTGACAG	767
	TGTCTTCAGAACCCTTGTCAC	3866
EX6	ACTTGAGCATCAAATGAAGC	521
	AGTACGTAGTGTGATCTGCATC	
EX7	AGATAAGCAGCTGTGGAGTG	505
	AGTGTTCTGAGCATAGACAAGC	2196
EX8	AGTCCAGGCTTGCTAGTACC	618
	CAATTTTAGTGTGCCTCTCAAC	3583
EX9	AGTCGTGTAAACTGGCAATAG	568
	TGCAAGGAAGTCTGTGATTG	
EX10	AATTGGAGCAAGTGTTTTGG	792
	TTCTGGTCATCCCTGATTG	

Results

Whole-gene sequence analysis with Mutation Surveyor Software revealed a nonsense homozygous mutation in exon 5. Parental Sequence Analysis shows that they are heterozygous (Fig. 2). According to ACMG guideline and other standard algorithms this variant is considered to be pathogen (Table 2).
Null variant(nonsense) affecting gene *SLC3A1*, which is a known mechanism of disease(PVS1), extremely low frequency in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium(PM2), Multiple lines of computational evidence support a deleterious effect on the gene or gene product(PP3).

Discussion

Cystinuria is the most common inherited form of kidney stone disease (KSD). In fact, 1% of all KSD cases are due to this disorder. Type A, type B, and type AB account for 38%, 47%, and 14% of cystinuria cases, respectively (7, 8). According to Human Gene Mutation Database, 247 different mutations in SLC3A1 gene can be retrieved which lead to the disorder. This amount for SLC7A9 is 160 mutations, all of which responsible for cystinuria (9).

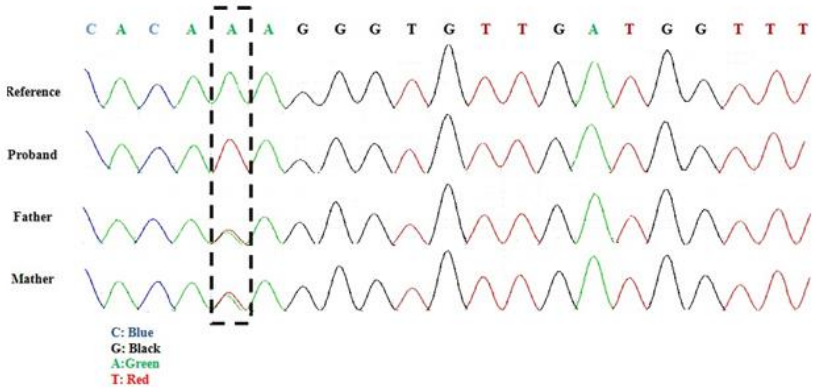

Fig. 2: A nonsense homozygous mutation in exon 5 in proband and parents

Table 2: Predictive algorithms of c.916A> T variant

Predictive Algorithms	*Pathogenicity Scores*
PHRED Score	38
PROVEAN	Deleterious
PolyPhen	Damaging
SIFT	Intolerant
EIGEN	Pathogenic
Mutation Taster	Disease causing

The most common SLC3A1 mutation is the M467T mutation, which accounts for about 26% of cases of cystinuria. While the Y151C mutation is common in northern Europe, dupE5E9 is found more commonly in German population. The absence of both copies of the gene eliminates proximal tubular cystine reabsorption (8, 10). However, in 15% of people with cystinuria, there is no mutation in the two SLC3A1 and SLC7A9 genes (11). SLC3A1 consists of 10 exons (12). The variant we identified in this study is located in exon 5. This kind of genetic alteration is a nonsense variant that creates an early end codon (PTC), which eventually results in the phenomenon of NMD (nonsense-mediated decay). NMD is a phenomenon in which mRNA, with an early end codon, is ultimately destroyed and not translated into any protein product (13). According to the ACMG guide line (14), this variant is pathogenic (privileges found in this variant include: PVS1, PM2, PP3)

Conclusion

New mutations in the disease can be reported by collecting significant data from common mutations in genes associated with cystinuria. The type of mutation can also indicate the severity of clinical symptoms, which paves the way for the control of diseases such as cystinuria. Finding serious mutations can allow rapid screening for cystinuria by analyzing common mutations. It should also be considered as a pathogenic mutation in patients with cystinuria.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that they have no conflict of interest

References

1 Breuning M, Hamdy N (2003). From gene to disease; SLC3A1, SLC7A9 and cystinuria. *Ned Tijdschr Geneesked, 147*(6):245-247.

2 Pereira D, Schoolwerth AC, Pais VM (2015). Cystinuria: current concepts and future directions. *Clin Nephrol* , 83(3):138-46.

3 Fazaeli S, Ashouri S, Kheirolahi M, Mohammadi M, Fazilati M (2017). A novel mutation in SLC7A9 gene in cystinuria. *Iran J Kidney Dis,* 11(2):138-141.

4 Bourderioux M, Nguyen-Khoa T, Chhuon C,et al (2015). A new workflow for proteomic analysis of urinary exosomes and assessment in cystinuria patients. *J Proteome Res,* 14(1):567-577.

5 Adzhubei I, Jordan DM, Sunyaev SR (2013). Predicting functional effect of human missense mutations using PolyPhen-2. *Curr Protoc Hum Genet,* Chapter 7:Unit7.20.

6 Obaid A, Nashabat M, Al Fakeeh K, Al Qahtani AT, Alfadhel M (2017). Delineation of cystinuria in Saudi Arabia: A case series. *BMC Nephrol,* 18:50.

7 Mikhaylenko D, Prosyannikov M, Baranova A, Nemtsova M (2019). Genetic and Biochemical Features of the Monogenic Hereditary Kidney Stone Disease. *Biochemistry (Moscow), Supplement Series B: Biomedical Chemistry,* 13(1):1-12.

8 Randall A (1937). The origin and growth of renal calculi. *Ann Surg,* 105(6):1009-1027.

9 Stenson PD, Mort M, Ball EV,et al (2017). The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Hum Genet,* 136(6):665-677.

10 Mizukami K, Raj K, Osborne C, Giger U. (2016). Cystinuria associated with different SLC7A9 gene variants in the cat. *PLoS One*, 11(7): e0159247.

11 Olschok K, Vester U, Lahme S, Kurth I ,Eggermann T (2018). No evidence for point mutations in the novel renal cystine transporter AGT1/SLC7A13 contributing to the etiology of cystinuria. *BMC Nephrol,* 19(1):278.

12 Endsley JK, Phillips III JA, Hruska KA, et al (1997).Genomic organization of a human cystine transporter gene (SLC3A1) and identification of novel mutations causing cystinuria. *Kidney Int,* 51 (6):1893-1899.

13 Holbrook JA, Neu-Yilik G, Hentze MW, Kulozik AE (2004). Nonsense-mediated decay approaches the clinic. *Nat Genet,* 36(8):801-808.

14 Richards S, Aziz N, Bale S, et al (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics In Medicine,* 17(5):405-423.