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A NOVEL MUTATION IN THE *COL4A5* GENE IN A YAKUT FAMILY WITH ALPORT SYNDROME

Alport syndrome is a hereditary progressive kidney disease associated with sensorineural hearing loss and vision abnormalities, which is caused by mutations in the *COL4A3*, *COL4A4*, and *COL4A5* genes encoding the α3, α4, and α5 type IV collagen chains. This paper presents a case with a novel hemizygous mutation in the *COL4A5* gene in a Yakut family with Alport syndrome. The study involved 228 *GJB2*-negative patients with varying degrees of hearing loss and deafness living in the Republic of Sakha (Yakutia). Brothers were selected from this sample with a history of similar hearing and kidney impairments. For one of the sibs, a complete exome sequencing was performed, which resulted in the discovery of a new hemizygous mutation c.2375delA p.(Asp792fs) in exon 29 of the *COL4A5* gene on the long arm of the X chromosome (Xq22). This mutation was also detected in sibling using PCR-RFLP analysis.

Keywords: Alport syndrome, novel mutation, Yakutia, *COL4A5* gene

Introduction. Alport syndrome (SA) is a hereditary progressive kidney disease associated with sensorineural hearing loss and visual abnormalities [4]. The prevalence of AS is estimated at 1 in 50,000 newborns [16]. In Russia, the frequency of AS, according to epidemiological data, is 17:100,000 of the population [1]. AS is caused by mutations in the *COL4A3*, *COL4A4*, and *COL4A5* genes located on the long arm of the X chromosome (Xq22), which encode the α3, α4, and α5 chains of type IV collagen [10]. Mutations in these genes cause structural anomalies and dysfunctions of the basement membranes of the glomeruli of the kidneys, cochlea, and also cause some visual anomalies with damage to the cornea, lens, and retina [11].

There are three types of AS inheritance: autosomal recessive, autosomal dominant, and X-linked. The most common of these is the X-linked type of inheritance (OMIM #301050), which occurs in 80-85% of AS cases [9, 11]. This type of AS is caused by mutations in the *COL4A5* gene and, in some cases, by mutations in the *COL4A6* gene, which is located adjacent to the 5' end of the *COL4A5* gene [3]. ~14% of patients with AS have an autosomal recessive type of inheritance (OMIM #203780) caused by mutations in the *COL4A3* and *COL4A4* genes in homozygous or compound heterozygous states [9]. And approximately 1% of patients with AS have an autosomal dominant type of inheritance (OMIM #104200) due to mutations in the *COL4A3* and *COL4A4* genes [9].

Typical diagnostic features of AS are persistent hematuria, bilateral sensorineural deafness, family history of kidney disease, and visual anomalies [2]. Among patients with Alport syndrome, most often end-stage renal disease (ESRD) develops in men. Thus, ESRD can develop before the age of 40 in 90% of men and 12% of women with AS [16]. For 50% of men, dialysis or kidney transplantation is required before the age of 30 [16]. Deafness and visual anomalies are observed in 80–90% and 40% of men with X-linked Alport syndrome (XLAS), respectively [12]. However, it is rare to find cases of AS with all of the listed features, due to the age-related manifestation of some of them. For example, sensorineural hearing loss occurs at a later age, and approximately 90% of men and 10-15% of women lose their hearing by the age of 40 [19, 20].

In this article, we describe a case of a new hemizygous mutation in the *COL4A5* gene in a Yakut family with Alport syndrome.

Material and methods. *Patients.* The study involved 228 *GJB2*-negative patients with varying degrees of hearing loss and deafness living in the Republic of Sakha (Yakutia). Among them, 55.7% were women with an average age of 27 years, and 44.3% were men with an average age of 25 years. More than half of the patients (58.4%) were Yakuts, 19.5% were Russians, 9.3% were other nationalities, and 12.8% were metis. From this sample were selected brothers with a history of similar problems with hearing and kidneys (Fig.1, c, II:1, II:2). It was noted that the brothers were prescribed hemodialysis due to end-stage renal failure. During the collection of anamnesis, it was found that the mother (Fig.1, c, I:2) also had similar hearing and kidney disorders. Thus, in the total of the obtained anamnestic data, the diagnosis of Alport syndrome was suggested.

Molecular genetic analysis. For proband II:1 (Fig.1, c), the complete exome sequencing was performed. The analysis was carried out by the method of paired-end reading (2x100 bp) with an average coverage of at least 70-100x. For sample preparation, we used the technique of selective capture of DNA regions belonging to the coding regions of human genes. The sequencing data were processed using an automated algorithm, including alignment of reads to the reference sequence of the human genome (hg19), post-processing of the alignment, identification of variants, and filtering of variants by quality. The search for the c.2375delA p.(Asp792fs) mutation frequency in exon 29 of the *COL4A5* gene (chrX:107850101GA>G, NM_033380.2)

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was carried out using PCR-RFLP analysis. For amplification of fragments in exon 29 of the *COL4A5* gene (281 bp), mismatch primers (F) 5'-CCCCCATGGAAGGAAAAGTA-3' and (R) 5'-AATTCCAGACCTCAGGTGATCC-3' were used. For restriction, the *Hinf I* endonuclease with the G↑ANTC restriction site was used.

3D modeling of the α5 chain structure of type IV collagen. 3D visualization of the experimental spatial structure of the human protein Collagen alpha-5(IV) chain (UniProtKB - P29400 (CO4A5_HUMAN)) was carried out using the AlphaFold program (https://alphafold.ebi.ac.uk/entry/P29400). A PDB file of a protein with c.2375delA p.(Asp792fs) mutation was obtained using Colab notebook. Visualization of normal and truncated α5-chain collagen IV was carried out using the PyMol program (PyMOL Molecular Graphics System).

Ethical control. The study was approved by the local committee on biomedical ethics of the Federal State Budgetary Scientific Institution "Yakutsk Scientific Center for Complex Medical Problems", Yakutsk, Russia (Protocol No. 16, April 16, 2015).

Results and discussion. Whole exome sequencing revealed a novel hemizygous mutation in exon 29 of the *COL4A5* gene (transcript NM_033380.2) c.2375delA p.(Asp792fs). This deletion causes a frameshift, which results in the replacement of aspartic acid with valine at amino acid position 792 (Fig.1, b), which leads to the appearance of a premature stop codon in exon 30 at position 818 of the amino acid sequence.

The identified mutation was also found in the hemizygous state in proband II:2 (Fig.1, c). Affected family members had a history of similar symptoms (hearing problems and kidney disease). These data indicate that the identified new mutation in the *COL4A5* gene may be the cause of Alport's syndrome. In a sample of 226 *GJB2*-negative patients with hearing impairment, this mutation was not found.

The *COL4A5* gene consists of 51 exons and encodes the α5-chain of type IV collagen, consisting of 1685 amino acids. 3D modeling of the structure of the α5-chain of type IV collagen showed that as a result of the c.2375delA p.(Asp792fs) mutation, part of the collagen and the entire NC1 domain (Fig. 2b). It is known that the assembly of heterotrimers is initiated due to interactions of NC1 domains [5, 7, 15]. Deletion of this region of the gene can lead to disruption of the assembly of the necessary heterotrimer (α3α4α5) for

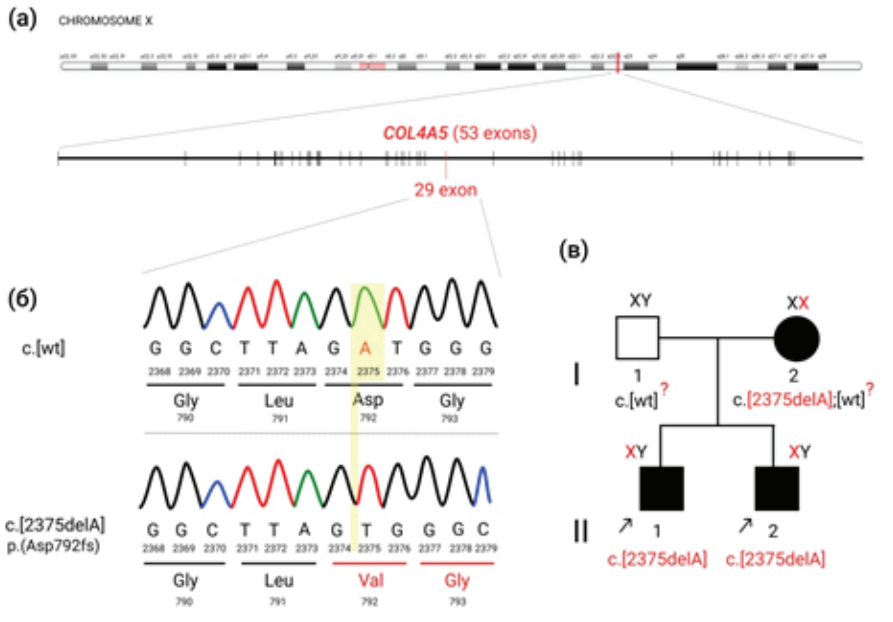

Fig. 1. Mutation with.2375delA p.(Asp792fs) of COL4A5 gene: a) — the localization of COL4A5 gene in the long arm of the X chromosome (q22.3); б) is the sequenogram of c.2375delA mutation with a frameshift, when the deletion of adenine (A) asparaginic acid (Asp - GAT) is replaced by valine (Val - GTG); в) - a pedigree with c.2375delA mutation : squares are men, circles are women; black are patients with mutation c.2375delA; arrows indicate probes; ? - no exact genotype known

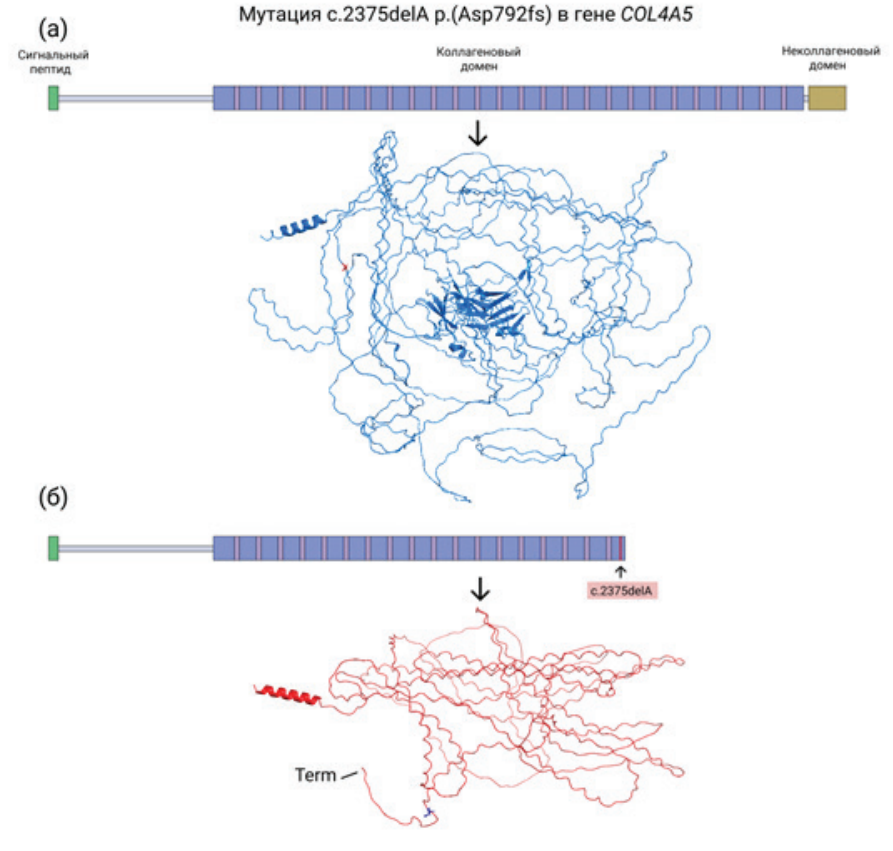

Fig. 2. Normal COL4A5 gene with c.2375delA p.(Asp792fs) mutation and the α5-chain of type IV collagen formed as a result of translation: a - schematic drawing of the COL4A5 gene and the resulting normal α5-chain of type IV collagen; b) shortened (part of the collagen domain and the entire NC domain is missing) α5-chain of type IV collagen as a result of the c.2375delA p.(Asp792fs) mutation in the COL4A5 gene

the proper functioning of the basement membrane in the tissues of the glomeruli of the kidneys, cochlea, and eyes.

It is known that only 10-15% of children with XLAS have *de novo* mutations [12]. In the case of our patients, this mutation was most likely passed from mother to both sons, which excludes the occurrence of this mutation *de novo* in the proband and sibling, but does not exclude the possibility of this variant *de novo* in their mother.

We have not been able to analyze the DNA of the parents of the proband, but it is known that their mother also suffered from kidney disease. Women heterozygous for mutations in the *COL4A5* gene can also have Alport syndrome: some of them may have all the overt signs of AS, as in men (hearing problems, kidney failure, visual abnormalities), others may be only minimally affected, and most can remain healthy throughout life [6, 17]. Thus, in one study, the characteristics of women and girls with proven mutations in the *COL4A5* gene were compared with the characteristics of hemizygous boys and men from 195 families [19]. It was shown that 95% of heterozygous girls and women had hematuria. Proteinuria, hearing loss, and ocular abnormalities developed in 75%, 28%, and 15% of heterozygotes, respectively. The probability of developing end-stage renal disease before the age of 40 was 12%, and deafness 10%. The risk of progression of end-stage renal disease in women increases after 40 years [19].

The reason for such a wide range of pathological phenotypes in women with heterozygous mutations in the *COL4A5* gene is presumably X inactivation, which is used by mammalian cells to equalize the dose of genes between female XX and male XY [8, 13]. At a very early stage of development in females, either the maternal or paternal X chromosome is randomly blocked by a complex cellular mechanism [13, 18]. This choice of inactivation is passed on to all offspring cells, resulting in a woman's body being a mosaic of cells with either an active maternal or paternal X chromosome [8, 13]. It is assumed that in heterozygous women with severe phenotypes, a healthy chromosome may be inactivated, which may explain the preferential expression of the mutant allele [14]. Thus, early, the amount of *COL4A5* mRNA was detected in the kidneys and leukocytes of a woman with two missense mutations in this gene, who suffered from kidney disease. At the same time, a correlation was found between the severe phenotype of Alport's syndrome (the patient underwent kidney transplantation) and the absence of detectable amounts of *COL4A5* mRNA with a normal sequence in the kidneys and leukocytes [14].

Conclusion. In conclusion, it should be noted that the identification of new mutations in AS and associated phenotypes is very important for disease prognosis, clarification of their clinical significance, early DNA diagnostics, and medical genetic counseling for families with AS in Yakutia. The results obtained complement the information available in the literature on the molecular genetic mechanisms of the occurrence of AS.

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