Mutation analysis in the *COL1A1* gene in osteogenesis imperfecta patients from the Republic of Sakha (Yakutia)

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Summary

DNA sequencing of 51 exons and exon-intron junctions in *COL1A1* gene in patients with osteogenesis imperfecta from Yakutia was performed. Two different mutations: mutations shifting the reading frame c.3540_3541insC (p.Gly1181AlafsX38) was identified in a patient Yakut ethnicity and splicing site mutation c.4005 +1 G> T in patient Russian ethnic origin. Mutation c.3540_3541insC (p.Gly1181AlafsX38) hasn't been described early. Mutations lead to a clinical 1 type of osteogenesis imperfecta, identified in heterozygous state and are unique to each family.

Keywords: osteogenesis imperfecta, collagen type 1, mutations.

Introduction

Osteogenesis imperfecta (OI) is a systemic disease of connective tissue, characterized by a wide spectrum of clinical manifestations, the main of which is the high bone fragility. Nowadays there are eight genes responsible for the development of 11 types of OI. The genetic defect which cause the development of OI type 5, hasn't been identified yet. OI type 1 is the most common form of the disease, which occurs in 60-80% of patients and is characterized phenotypically by blue

sclerae and minimal bone abnormality [5, 18, 20]. This form of the disease is characterized, as a rule, by the decrease of collagen type I due to frameshift mutations, stop codons or splicing mutations in the genes of collagen type 1 - the main structural protein of bone tissue. The molecule of collagen type I is constructed of 3 polypeptide α-chains: two α1 (I) and one α2 (I) chain. Each strand contains approximately 1,000 amino acids [3, 23]. The *COL1A1* gene encoding the alpha 1 chain is located on chromosome 17q21.31-q22, and contains 51 exons. Its length is 18 kb [6, 19]. At present time more than 200 mutations in the *COL1A1* gene are revealed in OI patients: insertions, deletions, nucleotide substitutions, splicing and nonsense mutations [23]. Mutations are different in their types and location and result in insufficient amount of protein by reducing of normal collagen secretion in quantitative mutations or in formation of abnormal collagen in qualitative mutations [4]. The majority of identified mutations are missence mutations, which account for more than 70% of all the changes; frameshift mutations are less common; deletions, duplications, and insertions are rare. [4, 13]. The spectrum of mutations in the collagen type 1 gene is described by many researchers in American and European populations [4, 14, 16, 21]. There are several studies of OI patients from Asia [7, 9, 10]. It is shown that the expression profile and the spectrum of mutations for each population depend on the ethnic origin [9].

The high genetic homogeneity of indigenous population of Yakutia due to high levels of isolation (low frequency of interethnic marriages) let refer the population to "ideal" in point of the possibility to identify specific genes and mutations of hereditary diseases and to map genes of complex diseases [1, 2]. In such populations with a traditional way of life existing for a long time in relative isolation, the new allele comes from outside by the founder of population («founder effect»), or arises de novo. Taking into consideration these facts, we studied nucleotide sequence changes in the *COL1A1* gene in osteogenesis imperfecta patients from the Republic of Sakha (Yakutia) to determine «founder effect» mutations, and analyzed correlations of the identified mutations with the form and type of inheritance of OI.

Material and methods

We used DNA samples of 15 patients with clinical diagnosis "osteogenesis imperfecta" from 12 families from the Republic of Sakha (Yakutia), registered in the medical and genetic counseling of "Republic hospital № 1 - National Medical Center." Genomic DNA was isolated from peripheral blood lymphocytes by phenol-chloroform extraction, Mathew, 1984 [11]. The search of changes in the nucleotide sequence of the *COL1A1* gene was performed by single-strand DNA conformational polymorphism analysis (SSCP) using the method proposed by Orita M., et al. with alkaline and thermal denaturation [12]. We used primer pairs flanking the exons and adjacent intron region

which were previously described [8]. After denaturation, the samples were applied on 8% polyacrylamide gel (PAAG). Gel electrophoresis of 20 cm long, 1 mm thick was held at room temperature at a voltage of 100V for 20 - 40 hours. The gel was stained in 0.09% silver nitrate (AgNO3) within 25 minutes. Analysis of samples was carried out by the presence or absence of additional bands in comparison with the control DNA. Determination of the nucleotide sequence in samples with altered mobility of single-stranded DNA was performed using an automatic sequenator ABI PRISM model 310 («Applied Biosystems») with the use of fluorescent labeling kit DYEnamicTM ET, according to the protocol of the manufacturer («Amersham Pharmacia Biotech» DYEnamicTM ET Terminator Cycle Sequencing Kit .) For the nucleotide sequence analyses MegAlign software package from DNAStar Inc (1993-2002) and BioEdit v.5.0.9. (1997 - 2001) were used.

The impact of newly identified substitutions on the probability of occurrence / loss of splice sites was performed using the program «Splice Prediction using Consensus Sequences» (WebGene): http://www.itba.mi.cnr.it/webgene.

Results and discussion

The molecular-genetic study was performed in 15 patients from 12 families, 10 of them were of Yakut ethnic origin, one family – of Russian- and one – of Even ethnic origin. The main method we used was SSCP, the main advantages of which are simplicity and high sensitivity [12]. However, there are also some disadvantages of SSCP method – low sensitivity, if the analyzed fragment exceed 400 bp and a long duration of the electrophoretic separation of the amplification products. However, the method is widely used by many researchers to search for polymorphic DNA sites. Exons with the length of more than 400 bp were directly sequenced. DNA samples with altered mobility of single-stranded DNA were followed by sequencing.

The *COL1A1* gene encoding the alpha 1 chain of type I collagen contains 51 exons and consists of 38 kb. Currently, about 200 mutations are found in OI patients in different populations of the world [https://oi.gene.le.ac.uk/]. Despite the large number of detected mutations in the *COL1A1*gene, each population is characterized by a spectrum consisting of a small number of mutations, ranging from 6 in patients from Brazil to 14 in patients of Jews origin from Israel [4, 8, 15, 16, 17]. In patients from Lithuania 11 types of mutations, in Americans – 10, in Japanese – 9, in Chinese – 8 were revealed [4, 7, 10, 22]. In addition, each researcher finds previously undescribed mutations, as well as known mutations. Most of the mutations resulting in OI development are unique and reflect the high variability of the *COL1A1* gene.

We analyzed 51 exons and adjacent intronic regions of *COL1A1* gene in OI patients and

found two types of altered single-strand DNA mobility in exons 49 and 50 of the *COL1A1* gene in two unrelated patients from Sakha Republic.

Subsequent sequencing of the sample with an altered mobility of single-stranded DNA allowed to detect previously unreported mutation c.3540_3541insC (p.Gly1181AlafsX38) in exon 49 of *COL1A1* gene in OI patient of Yakut ethnic origin (Fig. 1). The proband has deafness and blue sclera and such associated diseases, as residual encephalopathy, cerebroasthenic syndrome, neuropathy, and myotonic syndrome. This mutation hasn't been revealed in parents, suggesting that the mutation is de novo.

Analysis of samples with altered mobility of single-stranded DNA in the 50th exon of *COL1A1* gene allowed to identify the splicing mutation c.4005 +1 G> T in a family of Russian ethnic origin (Fig. 2). The patient has blue sclera, multiple fractures of arms and legs, ribs, vertebral compression fracture and dentinogenesis imperfecta. The patient also had concomitant diseases: uranostaphyloschisis, residual encephalopathy and pollinosis. Unfortunately, the proband's parents were not available for DNA analysis, however, the pedigree analysis suggests an autosomal dominant inheritance of the disease from his father. This mutation was previously described only by Italian researchers [https://oi.gene.le.ac.uk].

According to the literature data, frameshift and splicing mutations often lead to OI type 1 with mild disease form [24], which is consistent with our data. Both patients from Yakutia with mutations s.4005 +1 G> T and c.3540_3541insC (p.Gly1181AlafsX38) in the *COL1A1* gene has OI type 1 with concomitant diseases.

We haven't found *COL1A1* gene mutations caused by «founder effect» in OI patients from the Republic of Sakha (Yakutia). Our results are in consistent with those of scientists from Israel, who also conducted the analysis of mutations in the *COL1A1* gene in 65 OI patients of Jewish origin to identify «founder effect». They identified 14 different mutations (missense, nonsense, frameshift and splicing mutations), but all the detected mutations were unique for each family and were scattered throughout the gene [16]. It is possible that OI is not characterized by «founder effect» mutations, even in relatively isolated populations.

Conclusions

Thus, two types of mutations were identified in 16.7% of OI families from Yakutia: frameshift and splicing mutations, which are quantitative and lead to the decrease of normal collagen level due to the instability of abnormal RNA resulting in mild clinical manifestations of the disease. These mutations were identified in the heterozygous state and were unique for each family. Mutation c.3540_3541insC (p.Gly1181AlafsX38) in the *COL1A1* gene hasn't been described

previously. Families with the mutations detected are informative for prenatal DNA diagnostics. Our research has contributed significantly to the understanding of molecular-genetic features of the disease and to gene-phenotype correlations in Osteogenesis Imperfecta. The results of this work can also be a theoretical and methodological basis for the development and optimization of molecular-genetic diagnostics of such complicated diseases as Osteogenesis Imperfecta.

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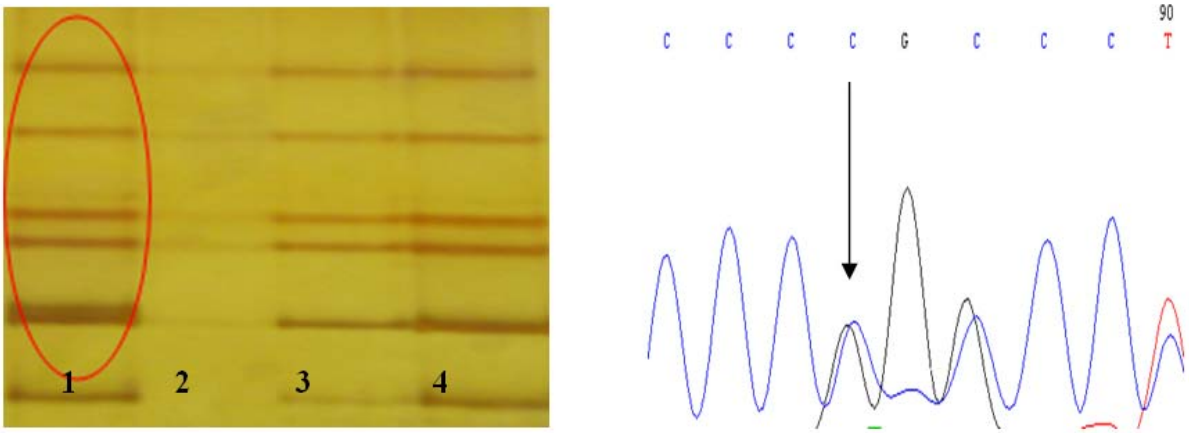

Fig. 1. Identification of c.3540_3541insC (p.Gly1181AlafsX38) mutation in *COL1A1* gene in OI patient of Yakut ethnic origin.

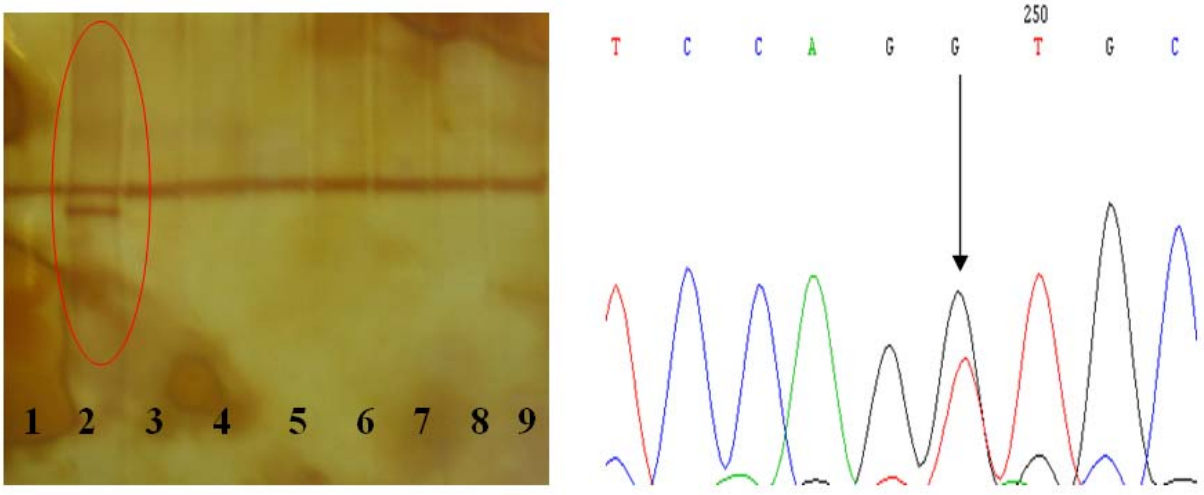

Fig 2. Identification of c.4005+1G>T mutation in *COL1A1* gene in OI patient of Russian ethnic

origin.