ORIGINAL RESEARCH

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ANALYSIS OF THE FREQUENCY OF HETEROZYGOUS CARRIAGE OF SIX MUTATIONS OF AUTOSOMAL RECESSIVE DISEASES AMONG RUSSIAN OLD-SETTLERS OF YAKUTIA

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Among the populations of Eastern Siberia, the Russian old-settlers of the village of Russkoe Ustye (Allaikhovsky district of Yakutia) occupy a special place: their origin is relations with people from the old possessions of Novgorod the Great (presumably around 1570). The investigation of the hypotheses of the origin of the people of Russkoe Ustye, are of considerable scientific interest for the study of the colonization process of Northern Eurasia. In this study analysis of the carrier frequency of the six mutations responsible for autosomal-recessive diseases in 30 unrelated individuals from the village of Russkoye Ustye was performed. In the population of the Russkoe Ustye we did not find any heterozygous mutations, which are the cause of phenylketonuria (0/30), Wilson disease (0/30), congenital cataract (0/30), progressive deafness (0/30), and methemoglobinemia (0/30). In this Siberian population with a carrier frequency of 6.7%, the c.35delG mutation of the GJB2 gene (2/30) responsible for autosomal recessive deafness 1 A was detected. The absence of local East-Siberian variants of founder mutations associated with congenital cataract, progressive deafness, and methemoglobinemia, which are prevalent among Turkic-speaking Yakuts, indicates that this Turkic component in population of Russkoe Ustye is absent or represented to a small extent. Increasingly, than the Turkic component in the in population of Russkoe Ustye is represented a common West-Eurasian component, as indicated by the presence of the c.35delG mutation of the GJB2 gene, which is common in Europe.

Keywords: Russkoe Ustye, carrier frequency, Wilson disease, phenylketonuria, congenital deafness, congenital cataract, juvenile deafness, methemoglobinemia.

Introduction. Among the populations of Eastern Siberia, the Russian old-settlers of the village of Russkoe Ustye (Allaikhovsky district of Yakutia) occupy a special place: their origin is relations with people from the old possessions of Novgorod the Great (presumably around 1570), who fled from the persecution of the guardsmen of Ivan the Terrible. However, in chronicles the first mention of the village dates back to 1638, it was in this year that a Cossack detachment led by the Tobolsk Cossack Ivan Rebrov opened the sea route to Indigirka [6]. To date, there are several versions of the origin of the residents of Russkoe Ustye their ancestors could be either Novgorodians who fled from the oprichnina of Ivan the Terrible, or descendants of Cossacks and industrial people (in particular, from the village of Pokhodsk, it was from this village that Cossack explorers began their campaigns to meet with non-peaceful Chukchi), or settlers from the city of Zashiversk, liquidated by a royal decree in 1805 [1, 3]. Hypotheses of the origin of the people of Russkoe Ustye, are of considerable scientific interest for ethnography, history, anthropology and the study of the processes of settlement of Northern Eurasia.

In addition to the generally accepted archaeological and ethnographic approaches, of molecular genetics methods have become widely used in recent years to resolve the issues of the origin of individual ethnic groups and restore the evolutionary history of various regions. The study of the gene pool of populations can include both classical markers based on typing of paternal (Y-chromosome) and maternal lines (mtDNA) and analysis of autosomal markers. From the autosomal markers, one of the most interesting markers is mutations of autosomal recessive diseases, which are characterized by the founder effect. An analysis of the carrier frequency of founder mutation to some extent allows us to characterize the relationship with certain migration processes of the past, and can be used in the study of the population genetic cluster of autosomal markers. On the other hand, the analysis of the carrier frequency of hereditary diseases is important for assessing the burden of hereditary diseases, and can be applied in clinical genetics and the epidemiology of hereditary diseases.

In this regard, the aim of this work was to analysis of the carrier frequency of six major founder mutations responsible for the occurrence of autosomal recessive diseases in the population of Russkoye Ustye.

Material and methods. Genomic DNA of 30 unrelated individuals from the village of Russkoye Ustye (Allaikhovsky District of Yakutia) was isolated using phenol-chloroform extraction with informed written consent of all participants of this study. Amplification of the desired fragments was carried out by polymerase chain reaction (PCR) on a programmable thermal cycler BioRad T100 Thermal Cycler (Bio-Rad Laboratories, Inc., USA) using the original sequences of oligonucleotide primers. Methods for detecting major mutations that are the main cause of six autosomal recessive diseases are presented in Table 1.

Brief description of investigated autosomal recessive diseases.

Phenylketonuria (OMIM 261600) is an autosomal recessive disease associated with impaired activity of the enzyme phenylalanine-4-hydroxylase [12], which normally catalyzes the conversion of phenylalanine to tyrosine. If left untreated (excludes phenylalanine from the diet), it leads to the accumulation of phe-

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Table 1

Methods for detecting major mutations, which are the main cause of six autosomal recessive diseases

Disease (OMIM)	Gene	Mutation	Primers sequencing	Size of PCR-product (bp)	Restriction ferment	Size after restriction
Congenital cataract (610019)	*FYCO1*	c.1621C>T p.(Gln541*)	F: TTGGCCTGCCGGAGCTCTT R: AGTGACCTGGAGGAGCAGAAGAAGCAGC**C**CATT	266	*Pct* I GAATGCN↑ / CTTAC↓GN	wt/wt 239/27 wt/mut 266/239/27 Mut/mut 266
Methemoglobinemia (613213)	*CYB5R3*	c.806C>T p.(Pro269Leu)	F: CCTCAGTTGCCACATCTGTAA R: TCATGGGTGGGGGGCCACACATCAGCACC**C**G	448	*Sfr*303 I CCGC↑GG / GG↓CGCC Режет при норме	wt/wt 30/418 wt/mut 448/418/30 mut/mut 448
Progressive deafness (607293)	*CLIC5*	c.1121G>A (p.Trp374*)	F: CGTCATCTCAGCCGGGATATCATAGTTGCG R: TGCTGGTATCATGGGAACTCCA	293	*Bsc4* I CCNNNNN↑NNGG GG↓NNNNNCC	wt/wt 258 wt/mut 293/258 mut/mut 293
Phenylketonuria (261600)	*PAH*	c.1222C>T p.(Arg408Trp)	F: TCCAAATGGTGCCCTTCAC R: GGACACAAGGGTAATTCTGACCT	351	*Mnl* I CCTC(N)7↑ / GGAG(N)6↓ Режет при норме	wt/wt 59/73/217 wt/mut 59/73/217/297 Mut/mut 297/59
Autosomal-recessive deafness 1 A (220290)	*GJB2*	c.35delG p.(Gly12fs)	F: ACTCAGGTGAACAAGCTACT R: TCTTTCCAATGCTGGTGGAGTGTTTGTTC**C**CA	337	*Bsc4* I CCNNNNN↑NNGG / GGN-N↓NNNNNCC	wt/wt 303/34 wt/mut 334/303/34 Mut/mut 334
Wilson disease (27900)	*ATP7B*	c.3207C>A p.(His1069Gln)	F: ACCCTGAGATTGAACGACAGA R: CTTTACAGTATTTGGTGACTGCCACGCCCAC**C**G	337	*Dra* III CACNNN↑GTG/ GTG↓N-NNCAC Режет при норме	wt/wt 306/31 wt/mut 337/306/31 Mut/mut 337

Note: A mismatch substitution in the primer sequence is indicated by an underscore; wt/wt – norma, wt/mut – heterozygote, mut/mut – homozygote.

nylalanine and its toxic products, which affect, among other things, the central nervous system (phenylpyruvic oligophrenia). A decrease in the enzymatic activity of phenylalanine-4-hydroxylase is associated with homozygous and compound-heterozygous mutations in the *PAH* gene mapped at the locus 12q23.2 **(NC_000012.12)** causing this disease, which has spread widely in Europe as a result of the founder effect. Kalaydjieva et al (1991) found this mutation at high frequency in Bulgaria, Lithuania and Germany, where it was found in a common haplotype (haplotype-2). The c.1222C>T p.(Arg408Trp) mutation frequency exhibited a downward gradient from East to West, suggesting that its distribution is associated with the Balto-Slavic population of Eastern Europe [17]. The main reasons for this east-west gradient may reflect the migrations of early Slavic or Germanic peoples in the middle of the first millennium AD [9]. Another haplotype with this mutation was found in Northern Europe (with accumulation in Ireland) [10]. However, subsequent analysis of VNTR polymorphisms suggest of common founder haplotype in chromosomes with c.1222C>T p.(Arg408Trp), and its divergence is probably associated with early recombination and subsequent migrations in Europe [10].

Wilson disease (OMIM 277900) is an autosomal recessive disease characterized by accumulation of intracellular copper in the liver with subsequent hepatic and neurological disorders caused by homozygous or compound heterozygous mutations in the *ATP7B* gene mapped at the 13q14.3 locus (NC_000013.11). The most common cause of Wilson's disease in Europe was the c.3207C>A p.(His1069Gln) variant of the *ATP7B* gene. Presumably, this pathogenic variant arose as a result of a very ancient single mutational event on the territory of modern Europe [2,16]. The subsequent distribution of the variant c.3207C>A p.(His1069Gln) in the vector from northeast to southwest across Europe from the center of settlement in the territory between the Vistula and Elbe rivers in the 5th-6th centuries associated with West Slavic tribes [13].

Autosomal recessive deafness type 1A (OMIM 220290) is the most common form of hearing loss in most populations of the world. This form of the disease is caused by biallelic mutations in the homozygous or compound-heterozygous state in the *GJB2* gene **(NC_000013.11)** encoding the gap junction protein connexin 26 (Cx26). The c.35delG mutation accounts for up to 70% of all pathological *GJB2* alleles in Caucasians from

northern and southern Europe and North America, while the carrier frequency ranges from 1.3% to 2.8% [11]. It has been shown that the high frequency of c.35delG in the *GJB2* gene in populations of European origin is the result of the founder effect [7].

Methemoglobinemia (OMIM 250800) is a rare autosomal recessive disease caused by a deficiency in the methemoglobin repair system, which is caused by homozygous or compound heterozygous mutations in the *CYB5R3* gene mapped on chromosome 22q13.2 (**NC_000022.11).** Clinically, methemoglobinemia is manifested by headaches, dizziness, and shortness of breath, tachycardia, fatigue, and drowsiness, possibly a lag in physical and mental development as a result of constant cerebral hypoxia. In Yakutia, a missense substitution c.806C>T p.(Pro269Leu) of the *CYB5R3* gene, unique for this region, was identified [14]. Based on the analysis of haplotypes, the assumption of the existence of a founder effect for this disease was confirmed; the estimated age of distribution of the detected mutation was 285+135 years, which explains the high prevalence of this disease (1:1250) in this region of Siberia [14].

Congenital cataract (OMIM 607182) is the leading cause of vision loss in children worldwide, as it leads to clouding of the lens, which can therefore lead to various visual impairments, including complete loss of vision [15]. In Yakutia, a nonsense mutation c.1621C>T p.(Gln541*) specific to Eastern Siberia was found in exon 8 of the *FYCO1* gene (3p21.31, **NC_000003)** (involving in controlling of autophagy process), which leads to a premature stop codon and in the homozygous state is the main cause of congenital cataract (86% all cases of congenital cataract) [8]. Reconstruction of mutant STR-haplotypes with c.1621C>T indicates that the expansion of c.1621C>T (p.Gln541*) carriers in Eastern Siberia occurred as a result of the founder effect about 260±65 years ago [8].

Progressive deafness (OMIM 607293) is the second most common cause of hearing loss in Yakutia, after autosomal recessive deafness type 1 A, and is caused by the specific homozygous mutation c.1121G>A (p.Trp374*) of the *CLIC5* gene (6p21.1**, NC_000006.12)**. This transition leads to the formation of a premature stop codon at the 374th amino acid position (p.Trp374*), which terminates the synthesis of the polypeptide chain of the CLIC5 protein. Most patients with this form of the disease noted a late onset of hearing loss that occurred in the postlingual period (after acquiring speech skills) [4]. Haplotype analysis indicates that the c.1121G>A (p.Trp374*) mutation most likely spread as a result of the founder effect, mainly in the Arctic regions, among the Paleo-Asiatic, Tungusic and Turkic peoples of Yakutia, approximately in the middle of the 18th century [5].

Results and discussion. Among 30 unrelated individuals from the Russkoy Ustye, the analyses of carrier frequency of six major mutations causing of Mendelian diseases with autosomal-recessive pattern of inheritance was performed (Table 2). Three diseases (congenital cataract, progressive deafness, and enzipopenic methemoglobinemia) are associated with local variants of mutations that are common only in Eastern Siberia, mainly among Turkic-speaking peoples, and to a lesser extent among Tungus-speaking peoples, which is due to the founder effect [5,8,14]. The other three diseases (phenylketonuria, Wilson disease, and autosomal recessive deafness type 1A) are associated with common West-Eurasian variants that spread as a result of the founder effect, mainly during in the Neolithic period [2, 7, 9, 10, 13, 17].

As a result, of the six tested diseases, heterozygous c.35delG mutation in the *GJB2* gene was detected in two individuals (Table 2). The carrier frequency of the c.35delG mutation of the *GJB2* gene in the population of Russkoy Ustye was 6.7% (2/30). The c.35delG mutation leads to stop codon in the coding exon 2 of the *GJB2* gene, which leads to termination of the translation of the polypeptide chain of the connexin 26 protein. The expansion of this mutation was causing by common founder effect, and its approximate age was estimated at 10,000 years [7]. The initial center of distribution of this mutation probably is the territory of the Middle East, from where, during the Neolithic period, it was brought to Europe through

Table 2

The carrier frequency of major mutations of six autosomal recessive diseases in residents of the village of Russkoye Ustye

№	Disease (OMIM)	Gene	Mutation	Common origin / «age» of mutation	Carrier frequency
Eastern-Siberian variants of mutations					
1	Congenital cataract (610019)	*FYCO1*	c.1621C>T p.(Gln541*)	Founder effect in Yakut population / 250 years [8]	0/30
2	Progressive deafness (607293)	*CLIC5*	c.1121G>A p.(Trp374*)	Founder effect in Yakut, Even and Evenk populations / 350 years [5]	0/30
3	Methemoglobinemia (613213)	*CYB5R3*	c.806C>T p.(Pro269Leu)	Эффект основателя в популяции якутов / 285 лет [14]	0/30
West-Eurasian variants of mutations					
4	Phenylketonuria (261600)	*PAH*	c.1222C>T p.(Arg408Trp)	West-Eurasian common founder effect [17]/ the first millennium AD [9]	0/30
5	Wilson disease (277900)	*ATPB7*	c.3207C>A p.(His1069Gln)	West-Eurasian common founder effect [13] / 12 000 - 13 000 years [16] and 1600 years [13]	0/30
6	Autosomal-recessive deafness 1 A (220290)	*GJB2*	c.35delG p.(Gly12fs)	West-Eurasian common founder effect / 10 000 years [7]	2/30 (6.7%)

the Mediterranean Sea, and then, together with subsequent waves of migrations, spread to the territory of North and South America and Australia [7]. This study shows that this mutation is also found on the Arctic coast of Eastern Siberia among Russian old-settlers.

Conclusions

1) In the population of Russkoe Ustye we did not find major mutations that cause phenylketonuria (0/30), Wilson disease (0/30), congenital cataract (0/30), progressive deafness (0/30) and methemoglobinemia (0/30). With a carrier frequency of 6.7% the c.35delG mutation of the *GJB2* gene (2/30), causing of congenital autosomal recessive deafness type 1 A was found.

2) The absence of local East-Siberian variants of founder mutations associated with autosomal recessive diseases, which are prevalent among Turkic-speaking Yakuts, indicates that this Turkic component in population of Russkoe Ustye is absent or represented to a small extent. Increasingly, than the Turkic component in the in population of Russkoe Ustye is represented a common West-Eurasian component, as indicated by the presence of the c.35delG mutation of the *GJB2* gene, which is common in Europe.

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