The mutation spectrum of hereditary diseases genes in populations of Russia

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I. INTRODUCTION

The main feature of modern medical genetics, analyzing hereditary diseases, is active using methods of molecular biology, which allow to detect structural disturbances in human genome, i.e., in fact, to discover the first cause in pathogenesis of the diseases, and to develop high-informative methods of diagnostics and effective methods of medical treatment on its base. Many of hereditary diseases are characterized by clinic polymorphism and genetic heterogeneity, that’s why the decision of the most important problem of medical genetics is determination of population singularity and structural features of genes, determining development of these diseases, that is necessary for optimal methods of DNA-diagnostics development for each particular region on the one hand, and, on the other hand, broaden our knowledge about structure of human genome and its functioning and make contribution to gene pool analysis in different populations. Here we report the main results of molecular analysis of genes, responsible for phenylketonuria, Wilson disease and Hereditary motor and sensory neuropathies. This region is at the boundary between Europe and Asia; its populations are each characterized by complex ethnogenesis, specific gene pool, and specific population structure, and combine both Caucasian and Mongoloid components in varying proportion. Linguistically, populations of the region belong to the Turkic (Tatars, Bashkirs, Chuvash), Finno-Ugric (Mari, Mordvinias, Udmurts, Komi), and Slavonic (Russians, Ukrainians) stocks (Khusnutdinova, 1999). Genomic analysis of monogenic hereditary disorders was initially carried out in Bashkortostan Republic. The spectrum and frequency of mutations in these genes, typical for the main ethnic groups of Bashkortostan Republic (Bashkirs, Tatars, and Russians) were shown. These findings became a base for optimal scheme of DNA-diagnostics development of these hereditary diseases in the Volga-Ural region and ascertainment of the mutations origin, leading to the diseases.

II. PHENYLKETONURIA

Phenylketonuria is the most common inborn error of amino acid metabolism in Europeans. It is caused by an autosomal recessive deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH). Failure to convert phenylalanine to tyrosine leads to an increase of phenylalanine in the body fluids and severe mental retardation unless phenylalanine intake is restricted. The average frequency of phenylketonuria in Europe is approximately 1:100000 newborns (Woo et al., 1993). The disease is caused by mutations in phenylalanine hydroxilase gene (PAH), located on 12q22-24 chromosome (Lidsky et al., 1984). Gene PAH consists of 13 exons; its length is about 90000 bp. More than 450 mutations in PAH gene are described, a spectrum and frequency of mutations are characterized by considerable population differences (http:www.mcgill.ca/pahdb). The most frequent mutation in Europe is R408W (p.Arg408Trp).

We analyzed structural features of PAH gene in 59 patients with phenylketonuria and 126 members of their families from Bashkortostan (Akhmetova et al., 2003). The frequency of p.Arg408Trp mutation in common sample of patients was 53% that corresponds to the frequency of the mutation in European populations. p.Arg408Trp mutations appeared to be the most frequent among patients of Russian (60%) and Tatar (23%) ethnic origins.

In PAH gene we have identified six mutations in patients from Bashkortostan: p.Arg408Trp (53%), p.Arg261Gln (10%), p.Arg252Trp (3%), p.Pro281Leu (3%),
**III. WILSON DISEASE**

Wilson disease (WD) is a heavy autosomal recessive disorder, characterized by copper and protein metabolism disturbances, resulting in combined liver and brain affection owing to toxic effect of copper, accumulating in human organs. The frequency of Wilson disease in world populations is approximately 1:30-40000 (Scheinberg, Sternlieb, 1984). The gene of Wilson disease (ATP7B), coding copper-transporting ATPase, is located on 13q14.3 chromosome and consists of 80000 bp. and 21 exons (Frydman et al., 1985; Bull et al., 1993). More than 370 mutations are identified and described in ATP7B gene. The mutation spectrum is heterogeneous in various populations (Wilson Disease Mutation Database [http://www.wildsondiseases.med.ualberta.ca]; Human Gene Mutation Database [http://www.hgmd.cf.ac.uk/ac/index.php]). The *p.His1069Gln* mutation is the most spread in European populations (10-70%) (Stapelbroek et al., 2004).

Molecular genetic analysis of Wilson disease was performed in 44 WD patients and 104 members of their families (33 families: 6 families of Russian ethnic origin, 9 –Tatar, 4 - Bashkir, 2 – Chuvash, probands in 12 families were from mixed marriages) (Karunas et al., 2009). We have screened the 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20th exons of *ATP7B* gene. Using SSCP analysis followed by direct sequencing, we have characterized the molecular defect in 81.82% of WD chromosomes and identified 9 different mutations (c.2152 G>C (p.Ala718Pro) and c.2304dupC (p.Met769HisfsX26) – in 8th exon; c.3170T>C (p.Leu1057Pro), c.3190G>A (p.Glu1064Lys) and c.3207C>A (p.Leu1057Pro)- in 14th exon; c.3402delC (p.Ala1135GfsX13) – in 15th exon; c.3556+1G>T (p.IVS16+1G>T) – in 16th intron; c.3914T>C (p.Leu1305Pro) and 3942-3943delCA; 3947delG (p.Lys1315_Arg1316delinsGlu) – in 19th exon) and 3 polymorphic variants (c.1366G>C, p.Val456Leu) and 3947delG (p.Lys1315_Arg1316delinsGlu) and c.3903+6T>C). Four mutations revealed - p.Ala718Pro, p.Leu1057Pro, p.IVS16+1G>T and p.Lys1315_Arg1316delinsGlu had not been described before.

The common *p.His1069Gln* mutation was represented in 43.94% of WD chromosomes; its frequency rate in Bashkirs was 44.44%, in Russians – 47.62% and in Tatars – 36.36%. Mutation *p.Lys1315_Arg1316delinsGlu* was detected in 18.18% of Tatar chromosomes and 22.22% of Bashkir chromosomes.

Alleles and haplotypes, statistically significantly associated with mutant and normal chromosomes were detected, using polymorphism of microsatellite loci D13S316, D13S133 and D13S228 analysis in families with Wilson disease and control DNA samples (Karunas et al., 2000). The most widespread haplotype (40%) on mutant chromosomes was 6-18-3, which was not revealed on normal chromosomes. Haplotypes 7-18-2 (19%) and 7-18-4 (9%) were the most on normal chromosomes; their frequency on mutant chromosomes was 7%. Haplotypes association analysis of polymorphic markers with mutations has shown that 90% of chromosomes with *p.His1069Gln* mutation had most widespread haplotype 6-18-3,
revealed also on chromosomes with these mutations in other investigated populations. Taking this fact into consideration, we assumed that \( p.\text{His1069Gln} \) mutation appeared long ago and had a common source of origin. Three chromosomes, bearing \( 3402\text{delC} \) mutation, had identical haplotype 4-7-4, and one chromosome with \( p.\text{Glu1064Lys} \) mutation had 6-7-2 haplotype, revealed neither on other mutant chromosomes in families with Wilson disease nor on normal chromosomes. The mutation \( p.IVS16+1\text{G}>T \), revealed for the first time, had the same haplotype 7-18-4, which had not met on other mutant chromosomes and was detected in three WD patients of Tatar ethnic origin that confirms its Turkish origin.

IV. HEREDITARY MOTOR AND SENSORY NEUROPATHY

Hereditary motor and sensory neuropathies (HMSN) (Charcot–Marie–Tooth disease, CMT) represent a genetically heterogeneous group of diseases affecting peripheral nervous system. Typical symptoms of most of HMSN cases are progressive weakness and atrophy of the distal leg muscles and the hand muscles on latter stages, depression of tendon reflex, foot deformities of the pes cavus type, and sensory disturbance of polyneuritic type (Harding et al., 1980). The disease mechanics include degenerative changes of myelin sheath, or of the axons of motor and sensitive fibers of either peripheral nerves, or spinal roots. Accordingly, two main forms of the disease are distinguished, including demyelinating form, HMSN I, and axonal form, HMSN II. Clinical differentiation between these two types is possible only using electroneuromyographic (ENMG) investigation, which provides evaluation of the median nerve conduction velocity (MNCV). Within two HMSN types, the autosomal dominant, autosomal recessive, as well as X-linked forms were described (Dyck et al., 1993). Up to now, more than 25 HMSN loci were mapped and 22 genes were identified (http://www.neuro.wustl.edu/neuromuscular/time/hmsn.html). Prevalence of different HMSN forms in world populations is different, ranging from 10 to 40 in 100000 individuals (Skre, 1974; Emery, 1991; Ionasescu, 1995). In the European countries and United States the most prevalent form is HMSN type 1A (HMSN 1A), which is caused by duplication of the gene for peripheral myelin protein (PMP22) (Timmerman et al., 1990; Patel et al., 1990). The second most frequent form is HMSN type 1X, a dominant X-linked form, which is found in 10 to 20% of all HMSN cases (Nelis et al., 1996). The genetic cause of this disease form are the mutations in the \( \text{GJB1} \) gene (gap junction B1 type), mapped to the long arm of X chromosome region Xq131 (Bergoffen et al., 1993). At present, more than 300 different mutations, involving all regions of the protein molecule, have been described (http://www.molgen.ua.ac.be/CMTMutations). The functional importance of the mutations is different, depending on their type and localization. Consequently, clinical characteristics of HMSN type 1X are variable. Furthermore, the HMSN 1X phenotype is not always clearly differentiated from HMSN type II, demonstrating the features of both myelinopathy and axonal degeneration (Ionasescu et al., 1996).

Mutations of \( \text{MPZ} \) (major peripheral myelin protein zero) gene are also frequent cause of HMSN. Gene \( \text{MPZ} \) is mapped to the region 1q21.3 – q23, consists of 6 exons, and more than 120 point mutations are known to result in HMSN (www.molgen.ua.ac.be/CMTMutations). Most of them refer to HMSN type I – HMSN IB (34 mutations) and other variants of HMSN I (41 mutations). The analysis of the \( \text{MPZ} \) gene in Dejerine-Sottas syndrome (DSS) showed sixteen mutations; either sporadic or recessive variants of the disease are known (Hayasaka et al., 1993; Warner et al., 1996). Some \( \text{MPZ} \) gene mutations result in congenital dominant hypomyelinating neuropathy (CHN, CMT4E) (Warner et al., 1996; Kochanski et al., 2004). Thirteen various mutations in the \( \text{MPZ} \) gene were found in HMSN type II (CMT2I, CMT2J) (Misu et al., 2000; Senderek et al., 2000). The frequency of the \( \text{MPZ} \) gene mutations among HMSN patients of different ethnic origin varies. The high frequency of mutations is demonstrated in HMSN patients from Spain – 10.6% (Bort et al., 1997), low – in Finland – 4.8% (Silander et al., 1998). The frequency of \( \text{MPZ} \) gene mutations in Russia is estimated as 4.6% (Mersiyanova et al., 2000).

In addition, the mutations in the early growth response 2 (EGR2) gene (10q21.1-q22.1) segregating as either dominant or recessive alleles have been demonstrated in HMSN I and congenital
hypomyelination (Warner et al., 1998). The coding part of the gene consists of 4300 bp and is represented by 2 exons. Although only 9 mutations and 1 polymorphism are known for today in this gene, it is intensively investigated in molecular-genetic analysis of HMSN in populations.

In recent years, a survey on the prevalence and molecular bases of HMSN has been carried out in Bashkortostan. The HMSN frequency in Bashkortostan Republic is 10.3:100000. HMSN type I is prevalent in this region. We examined CMT1A duplication of 17p11.2-p12, point mutations of PMP22, MPZ (P0), GJB1 (Cx32) and EGR2 genes in 173 patients from 131 unrelated families (42 Russian, 37 Tatar, 27 Bashkir, 3 Ukrainian families, and by one patient of Chuvash, Mari, and Azerbaijani origin; 19 probands originated from interethnic marriages).

The frequency of the PMP22 gene duplication, which is the cause of HMSNIA was determined. It constituted 26.72% of all HMSN types, which was somewhat lower compared to other regions of Russia. PMP22 gene duplication was found in Tatars more often (37.14%) compared with Russians (31.43%) and Bashkirs (14.78%). Deletion of the PMP22 gene, resulting in Hereditary Neuropathy with Liability to Pressure Palsies development, was found in 2 patients (1.53%).

SSCP-analysis, followed by direct sequencing, allowed to determine only one nucleotide change – c.309-29 G>A in PMP22 gene, which hadn’t been described previously. In MPZ gene in one patient (0.8%) was detected mutation p.Ser88Leu (c.263C>G), have written earlier Fabrizi et al. (2000) and marked them us p.Ser49Leu, and in two patients – the nucleotide change c.714C>T (p.Ser238Ser). In EGR2 gene in HMSN patients from Bashkortostan Republic any one changes was not revealed.

In GJB1 gene 4 missence - mutations were detected (Khidiyatova et al., 2008): p.Pro87Ala (c.259C>G), with frequency 10% among HMSN patients, p.Arg22Gln (c.65G>A) (2.98%); p.Arg15Gln (c.44G>A) (1.5%) and p.Thr86Ile (c.257C>T) (0.8%), the last one was not described previously. It was demonstrated that the frequency of the p.Pro87Ala mutation in the HMSN patients from Bashkortostan was high, but the distribution of this mutation in different ethnic groups was heterogeneous. The highest mutation frequency was observed among Bashkirs, where it was the reason for HMSN in 33% of the cases. The mutation was rare among Russians, and was almost not detected among Tatars. According to the literature data, the mutation p.Pro87Ala was earlier described in the patients Belgium (Nelis et al.,1997). Taken together, the GJB1 mutations were detected in 18 out of 131 unrelated HMSN patients from Bashkortostan, which constituted 13.7% for this sample. These data are consistent with those published elsewhere and according to which in European populations, HMSN 1X makes 10 to 20% of all HMSN cases (Nelis et al., 1996).

At present we continue to study HMSN in Bashkortostan Republic, analyzing genes responsible for other rare disease forms.

V. CONCLUSION

So, taking into consideration all the results received, it is possible to make a conclusion that according to structure of the genes, responsible for hereditary diseases development, the Volga-Ural region is heterogeneous; and it is most similar to European populations. At the same time, in patients of different ethnic origin and various hereditary disorders we identified mutations and polymorphic variants, which had not been described in the world before that determine genetic singularity of the Volga-Ural region populations. On the basis of the received data, DNA-diagnostics of analyzed hereditary diseases was developed and introduced to genetic consulting.

References
11. http://www.megill.ca/PAHDB


