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GENETICS OF VILYUI ENCEPHALOMYELITIS

ABSTRACT

Viliuisk encephalomyelitis (VE) is a neurodegenerative disease with unknown etiology in development of which genetic factors have significant role. Study of HLA markers and other genes of immunity and analysis of exom sequencing in VE patients revealed features of the Yakut genome, predisposing to immunity dysfunction and functional insufficiency of proteolitic and phosphatase activity. In extreme environmental conditions these features may lead to dystrophic process development in the brain and distinguish type of encephalopathy as basis of VE pathogenesis.

Keywords: Viliuisk encephalomyelitis, genes of immunity, human exom, DNA sequencing.

INTRODUCTION

Viliuisk encephalomyelitis (VE) is the regional pathology of Republic Sakha (Yakutia). Study of etiology and pathogenesis of this disease since difficult nature remains subject of fundamental medicine.

At present time VE consider as primary chronic form of disease, in which a patient experience physical weakness of nervous system due to the gradual degradation of neural circuits and the death of neurons. Disease may have form of encephalopathy with stable, steady flow and minimal degrees of motor and cognitive impairments. However, in some of these patients undergone extreme physical stress (undercooling, trauma, childbirth), external factors can provoke acute inflammation of the brain (encephalitis) with a local immune response and development of a clinical picture of an acute or subacute form of the VE with possible fatal outcome [4]. Patients who survive acute inflammation develop a chronic process typical for all patients with VE. The nature of the primary neurodegenerative process in VE remains unresolved. Currently, the working hypothesis is a violation of the control function of intracellular autophagy of the neuron, inducing atrophy of tissue and spongios of the brain. The reason of which may be a violation of the molecular mechanisms that control this function. The study of the genetic component in the etiology and pathogenesis of the disease is an important aspect of the disclosure of the molecular nature of VE pathology. In recent years, modern methods of molecular genetics are being used to solve this problem.

1. Population-genetic studies of VE

The disease is known to be endemic (the focus of the disease is limited by the Vilyui river region) and afflicts only Sakha people. The disease is sporadic, group cases of the disease are not observed, but generic connections are traced. The involvement of heredity in the development of the disease was confirmed by population-genetic studies on the material of a mass neurological survey of the population of the districts of the Vilyui basin for 1969-1977 in the works of Goldfarb LG, et.al. [6]. Segregation analysis (the ratio of patients among siblings) showed the absence of a monogenic type of autosomal recessive inheritance, but does not exclude the inheritance of more than one gene, the mutual action of which predisposes to the disease. To analyze the contribution of the genetic component and environmental factors to the pathology of the VE, the Falconer-Edwards mathematical model was used to calculate the coefficient of heritability (H). The coefficient of heritability of the phenotype of the disease for relatives of the 1st degree of kinship was 22.14% in the Vilyui district and 28.94% in the other districts of the Vilyui basin [6]. This means that the disease of VE among the Yakut population is due to the genetic component by 22-29% and due to the action of external environmental factors by 71-78%. Environmental factors make a significant contribution to the development of encephalitis in patients.

2. Investigation of immunity genes

Genetic studies of VE have been initiated with immunity genes. In the work of V. V. Fefelova (1996) analysis of HLA class 1 markers using serological reactions showed a slight statistically significant increase of the HLA-b15 variant in patients with VE [7].

Genotyping of alleles of HLA 2 class (-dp, -dq, -dr) on variants of DQA1 gene was carried out in patients with VE, which revealed an ethnic difference in frequency of allele 0301 ($p < 0,05$) between patients of Yakut and European origin. At the same time, the frequency of DQA1'0301 in patients with VE was higher than in healthy Yakut patients [9]. This gives grounds for talking about the participation of immunity genes in the development of the pathogenesis of VE disease.

At a next stage of research to search for new immunity candidate genes predisposing to the VE, 7 inflammatory genes were analyzed. These are the genes of the chemokine receptors *CCR2 / CCR5,* interferon gamma, interleukins 4, 6, 10 and the stromal factor (CDF) and cytokine Rantes [8]. 17 single nucleotide substitutions (SNPs) were analyzed. As

the results of the typing of SNPs showed, none of the 17 SNPs of the 7 inflammatory genes was associated with the required disease with the required reliability [8]. Differences were revealed in the analysis of additional SNPs of the interferon gamma gene. It was shown that, the four of the eight SNPs of the interferon gamma gene were polymorphic for representatives of the Yakut population, i.e. they are specific for the Yakut ethnos. In the case-control study, a significant association of two of these alleles was shown (SNPs: rs2069718, p = 0.003, relative risk is 5.44 (OR> 1) rs2069727, p = 0.003, relative risk 7.78 (OR> 1). The strong positive association found in the group of patients aged 60-69 years [1]. IFNg mutations - (SNP; rs2069718, rs2069727, rs2430561) are located in non-coding region of a gene intron, basically 5'UTR region of the gene, which performs an important function of gene expression control. These results also note the peculiarity of the genetics of the ethnos immunity.

Clinical polymorphism of VE disease appears to be associated with a difference in the level of IFNg production. As shown in the IFNg SNP (+874) T / A gene variants (rs2430561), the low production of the cytokine IFNg binds to the variant of the gene A allele. The genotypes of TA and AA genes can be considered as factors that reduce the severity of the disease course, promoting the development of the chronic form and increase of life expectancy of the patients. The TT genotype of the patients with encephalopathy can be considered as a predisposition factor to inflammatory processes and therefore the patients carriers of this genotype are more prone to acute disease [2].

These data indicate that the pathology of VE is associated with a special genotype of the immunity of the Yakut genome and gives grounds to talk about the immunogenetic nature of the disease of the VE.

Exome sequencing of VE patients' DNA

For detail investigation of patients' genome whole exome sequencing was performed by two groups of researchers [3, 5].

In first study exome sequencing data of 12 representatives of the Yakut ethnic group was analysed. Analyzed persons lived in rural areas of Vilyui district of Yakutia, were not close relatives. Seven persons (3 men and 4 women) have VE diagnosis and five (1 woman and 4 men) were healthy. The average age of individuals included in the analysis, was 47 years old (ranged from 27 to 66 years). Exome sequencing was held in Beijing Institute of Genomics, (BGI, Beijing Genomics Institute) using the Illumina HiSeq2000 system [5].

Resulting analyses of genetic material of VE patients exome 2507 allele variants were identified, which led to non-synonymous amino acid substitutions or a shift of the reading frame or the appearance of stop codons were identified. 437 from these variants were new and may be specific for Yakut genome. For dominant model 11 variants were selected. In test of pathogenic foretelling using several computer programs it was reveal that 4 variants may be pathogenic for VE by more than two algorithms: *TMPRSS11E, SCUBE2, PPP1R36* and *PSMB6.*

SCUBE is the gene of signal peptide *CUB.*

TMPR SS11E is the gene of transmembrane serin protease.

PPP1R36 is the gene of regulator protein subunit (36) serin\treonin phosphoprotein phosphotase PP1. Protein subunit interacts with phosphotase PP1. Biological function is negative regulation of phosphotase activity.

PSMB6 is representative of B type proteasome family, subunit B6. It has endopeptidase activity of treonin type. Multi-subunit proteolitic complex is an enzyme of non lysosome degradation of proteins in the cytoplasm and nucleus of cells. Product of this gene take part in many cellular processes (metabolism, apoptosis, immunity, disease).

PPP1R36 and *PSMB6* variants are new and not present in dbSNP (the SNP NCBI database), it is typical for representatives of the Yakut population. Therefore *PPP1R36* (NM_172365:c.G1096A:p.G366R) and *PSMB6* (NM_002798:c.A541C:p.M181L) genes were selected for analyses of possible associations with VE. But significant association with VE was not revealed in group of VE patients comparing with healthy control group.

By second group of researchers sequencing was carried out at the National Institutes of Health Intramural Sequencing Center, Bethesda, Maryland, USA (4 patients) and Cornell University Sequencing Center, New York, USA (5 patients) using Illumina platform [3]. Within the framework of this project, DNA exome sequencing was performed on two patients with subacute VE and 7 patients with a documented diagnosis of chronic VE.

Analysis of the data using first a recessive model revealed no homozygous variants thus excluding the recessive inheritance of VE in studied patients. Analysis of 9 patients based on the dominant model also failed to identify rare heterozygous variants. Using largely softened parameters, two promising variants were identified in *AURKC* and *HLA-DRB1* genes, although they were also present in two patients with Spastic paraplegia living in Yakutia, and the population frequency of these variants was about 25 % (ncbi.nlm.nih.gov). This characterizes the variants as common polymorphisms, probably not related to VE. Another genetic variant in the regulatory region of *NMI* was found in 8 of 9 VEM patients studied, but again the population frequency is 5 to 24 % in different populations. Seven of 9 VE patients show variants in the coding regions of two other interesting genes-*ADAMTS14* having population frequency of zero to 0.5 %, and *SOX30* with a frequency of 2.1 % that is even higher in Asian populations, 9-12 %. The heterozygous variant of the *ADAMTS14* gene is most promising in the pathogenesis of the VE, particularly the participation of its product (enzyme metalloloproteinase) in microvascular pathology.

Analysis of the data obtained on the basis of the recessive model did not reveal rare homozygous variants, which excludes the possibility of recessive inheritance of RE in the patients studied. The next attempt to analyze the results of the exomial sequencing was done using the dominant model. But this time it was not possible to identify rare (less than 5% in the population) heterozygous variants, which would be present in each of the nine patients studied. Using the softened parameters, two promising variants in the *AURKC* and *HLA-DRB1* genes, which were present in all nine patients with RE, were identified. However, the population frequency of variants in the *AURKC* and *HLA-DRB1* genes was extremely high - about 25% (ncbi.nlm.nih.gov), which characterizes them as often occurring polymorphisms, probably not related to RE. Another variant in the regulatory part of the *NMI* gene was found in eight patients with VE from the nine investigated, but the population frequency in this case is from 5 to 24% in different populations. In seven of the nine patients studied, variants were found in the coding parts of two other interesting genes *ADAMTs14* with a population frequency from zero to 0.5% and *SOX3O* with a population fre-

quency of 2.1%, which in Asian populations is up to 9-12%. The heterozygous variant of the *ADAMTS14* gene is most promising in the pathogenesis of the VE, particularly the participation of its product (enzyme metalloloproteinase) in microvascular pathology.

CONCLUSION

Thus, the results of genetic studies of genes of immunity and analysis of exom sequencing in VE patients reveal specific features of Yakut genome, predisposing to immunity dysfunction and functional insufficiency of proteolitic and phosphatase activity. Revealed variants have not statistically significant association with VE possibly due insufficient amount of studied patients and clinical heterogeneity of disease. Nevertheless these factors may take part in induction of intracellular autophagy of neurons and atrophy of brain tissues. In condition of extreme environmental factors these peculiarities may led to the development of dystrophic processes in brain with the formation of a special type of encephalopathy as basis of VE pathogenesis.

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