Haplotype diversity in the gene DMPK in myotonic dystrophy sample of patients from the Republic of Sakha (Yakutia) and in populations of Northern Eurasia

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Summary

The analysis of six single-nucleotide polymorphisms substitutions (SNP) in the gene muscle protein kinase (DMPK) responsible for the development of myotonic dystrophy (MD). Six population-based of Northern Eurasia (N = 778) and samples of representatives of indigenous population of Yakutia, MD patients Given the population-genetic characteristics of the samples for the investigated locuses, the comparison of the samples from the frequencies of alleles, haplotypes, analyzed the structure of linkage imbalance in all the samples. Haplotypes associated with the disease were found, significant differences on the investigated between populations of the Ket, the Buryat, the Khanty and Russian were taped, coupling blocks in samples were shown.

Introduction

The aim of this work was the analysis of allelic and analysis haplotypic diversity in patients DM in the population of Yakutia and in 6 control samples on 6 locuses in the *DMPK* gene - *Dra III* (rs2070736), *Hha I* (rs572634), *Bpm I* (rs1799894), *Hph I* (rs527221), *Fnu4HI* (rs915915) and *Taq I* (rs10415988).

The myotonic dystrophy of Rossolimo – Steinert – Kirschmann - Batten (DM) is an autosomal-dominant, multisystem disease with a combination of clinical signs including myotonia, a muscular dystrophia, the defects of heart, subsequent iridescent of a cataract and endocrine disturbances [7]. It is known 3 types of the myotonic dystrophy. DM 1 type (about 98 % of all diseased) it is characterized by the beginning of muscular delicacy from distal muscles to proximal and has been described more 100 years ago in the European and North American populations. DM 2 type (exposed to approximately 2% of all cases) is characterized by the development of muscle weakness of proximal to distal, the mutation was found in 2001, was

described in 1998 in a big family in Minnesota [10]. The mutation leading to development MD of 3rd type has been found out in 2004 on 15 chromosome 15q21-q24 [1].

Range of the prevalence of myotonic dystrophy (DM 1) in the world ranges from 2.1 to average 14.3-4.0 -5.0 at 100 thousand [9]. In the Yakut population there is a high accumulation of MD -1: 4699, whereas the average prevalence of the disease in the world's population does not exceed 1: 10000. Range of the prevalence of DM in the regions of Yakutia is extremely wide and ranges from 1: 1 up to 818: 45455 [12]. One of the reasons for the accumulation of this pathology can be long geographic isolation of Yakut population. It is possible that the origin of the DM at the Yakuts is connected with the Caucasoid component of the gene pool, because the disease occurs mainly in Caucasoid populations [4].

It is supposed that all cases of myotonic dystrophy is based on one or a very small number of ancient mutation North-Eurasian origin with many media mutation or permutation. At DM I type the same mutation is found out in the myotonic dystrophy protein kinase (*DMPK*), located on a short shoulder 19 chromosomes in the field of 13.2.-13.3. The reason the disease is bound to expansion of number *CTG*- of repetitions in 3 ' – not broadcast of gene *DMPK* which is required for normal development and functioning muscle musculation [8]. There are a large number of publications for the study of *CTG*-repetition in the *DMPK* gene [3, 5, 11]. Studies of the same modular structure locus *DMPK* and its molecular genetic characteristics in population-based samples and in connection with myotonic dystrophy are not so much. In the literature and databases are fragmentary data on the frequencies of SNP and haplotypes in individual populations obtained on different sets of SNP [5], which exhibit significant interpopulation differences in the frequencies of haplotypes in the locus of *DMPK*.

This work presents the frequency characteristics of six polymorphic variants gene *DMPK* *Dra III* (rs2070736), *Hha I* (rs572634), *Bpm I* (rs1799894), *Hph I* (rs527221), *Fnu 4HI* (rs915915) and *Taq I* DNA polymerase (rs10415988), examined the genetic diversity of muscle protein gene in patients, healthy Yakuts and in five populations of Northern Eurasia, showing blocks of adhesion for each analyzed locus and analysis of associative observations haplotypic diversity in the above samples.

Materials and methods

In this work the DNA of 87 representatives of the indigenous population of Yakutia with confirmed diagnosis of "myotonic dystrophy", two control groups of Yakuts: 63 healthy relatives of patients and the population sample of 328 unrelated individuals living in central, vilusk, northern regions of RS (Y) similar to the patients and their relatives by age, sex and nationality were investigated. In addition to the Yakut population in the study included five

samples of Northern Eurasia (450 unrelated individuals, ethnicity which maternal line was posted to the 3-rd generation). It was the Russian population (100 people), buryats (100), kyrgyz (100), khants (100) and ket (50). The fence of a peripheral blood was made for DNA allocation only in the presence of the written informed consent. All the sick held clinical and instrumental researches, DNA-diagnostics on revealing of a heterozygotic carriage. DNA was excreted with a method phenol–chloroform extraction based on the standard methodology of peripheral blood lymphocytes. Study of polymorphic variants of specific stretches of gene investigated using the methods of polymerase chain reaction (PCR) and polymorphism analysis length polymorphism (RFLP), using the structure of the primers and the corresponding restriction enzymes that were described previously in the literature [6]. Products of restrictions were fractionated in 3% and 3.5% agarose gel. The DNA fragments were stained with ethidium bromide and visualized under UV light using a computer video on gel-documenting system «Bio-Rad». For polymorphic variant *Dra III* «T» an allele corresponded with amplificated fragment in length 183 p.o.,, «*G*» an allele was taped by two fragments in length 158 and 25 p.o. Fragment in length 25 p.o. is often not visible due to its relative ease and because of this, a quick exit from the gel. For a site of *Hha I "T*" allele corresponded with amplificated fragment in length 491 p.o., "C" allele was taped by two fragments in length 419 and 72 p.o. Fragment wasn't visible 72 p.o., as in the previous case, because its relative easiness. For a site *Bpm I "G*" allele had a length of 350 p.o., "C" allele was taped by two fragments in length 299 and 51 p.o. Fragment 51 p.o. was not also visible. *"T"* allele *Fnu 4HI* represented by two fragments – 127 and 155 p.n., «*G»* allele-Allele *Hph I* 282 p.n. These alleles presented by two fragments: *"T"*-124 +186 p.n., *"G"* -148 + 186 p.n. *Taq I* included alleles A and B, in length 676 and 574 p.n., respectively. Statistical analysis of results of the study was conducted using statistical software «Arlequin», «Haplowiew» and Fisher's exact test. Difference of the two compared values considered reliable the reliability of r > 0.95 if the probability of their identities have been less than 5%.

Results and discussions

Frequencies of genotypes and alleles, a heterozygosis and conformity to of Hardy-Weinberg balance on six studied SNP in gene *DMPK* are presented in table 1. Practically on all markers in all populations distribution of frequencies of genotypes corresponded to Hardy-Weinberg balance (an exception locuses *Dra III* and *Fnu 4HI* in population of kets and *HhaI*, *HphI* in population of Yakuts make). The least and greatest value of a heterozygosis was observed on different locuses in populations of kets, yakuts and the buryats.

Table №1

Frequencies of alleles studied locuses, the distribution of genotypes and heterozygosity in the studied samples

the researched group	of SNP in the DMPK gene	Frequency of minor allele	the observed heterozygosity (H_0)	expected heterozygosity (He)	Deviation of the X-B a $\chi 2$ value
Patient MD (N=87)	DraIII(rs2070736)	0.276	0..526	0.4	-
	HhaI (rs572634)	0.093	0.107	0.169	-
	BpmI(rs1799894)	0.42	0.733	0.487	-
	Hph I (rs527221)	0.086	0.118	0.156	-
	Fnu4HI(rs915915)	0.207	0.28	0.328	-
	TaqI(rs10415988)	0.441	0.776	0.493	-
Yakuts (N=328)	DraIII(rs2070736)	0.381	0.495	0.472	0.4474
	HhaI (rs572634)	0.107	0.117	0.192	**5.1128E-8(P<0.05)**
	BpmI (rs1799894)	0.127	0.197	0.222	0.0961
	Hph I (rs527221)	0.188	0.265	0.305	**0.0306(P<0.05)**
	Fnu4HI(rs915915)	0.235	0.331	0.36	0.2041
	TaqI(rs10415988)	0.136	0.227	0.234	0.7221
Buryats (N=100)	DraIII(rs2070736)	0.355	0.41	0.458	0.3772
	HhaI (rs572634)	0.153	0.204	0.259	0.0889
	BpmI (rs1799894)	0.278	0.414	0.401	0.9985
	Hph I (rs527221)	0.035	0.07	0.068	1.0
	Fnu I (rs915915)	0.283	0.343	0.406	0.1846
	TaqI(rs10415988)	0.245	0.41	0..37	0.4503
Kyrgyz (N=100)	DraIII(rs2070736)	0.337	0.388	0.447	0.2571
	HhaI (rs572634)	0.18	0.237	0.296	0.1056
	BpmI (rs1799894)	0.347	0.49	0.453	0.601
	Hph I (rs527221)	0.174	0.284	0.287	1.0
	Fnu4HI(rs915915)	0.335	0.402	0.446	0.429
	TaqI(rs10415988)	0.308	0.434	0.426	1.0
Russian (N=100)	DraIII(rs2070736)	0.24	0.42	0.365	0.2261
	HhaI (rs572634)	0.137	0.232	0.236	1.0
	BpmI (rs1799894)	0.449	0.535	0.495	0.5752
	Hph I (rs527221)	0.143	0.245	0.245	1.0
	Fnu4HI(rs915915)	0.336	0.402	0.464	0.2497
	TaqI(rs10415988)	0.435	0.49	0.492	1.0

Khanty (N=100)	DraIII(rs2070736)	0.311	0.337	0.429	0.054
	HhaI (rs572634)	0.107	0.153	0.191	0.1466
	BpmI (rs1799894)	0.459	0.469	0.497	0.6949
	Hph I (rs527221)	0.071	0.121	0.131	0.7792
	Fnu4HI(rs915915)	0.402	0.392	0.481	0.0954
	TaqI(rs10415988)	0.474	0.392	0.499	0.049
Kets (N=50)	DraIII(rs2070736)	0.15	0.14	0.255	**0.0131(P<0.05)**
	HhaI (rs572634)	0.36	0.48	0.461	1.0
	BpmI (rs1799894)	0.33	0.42	0.442	0.9045
	Hph I (rs527221)	0.38	0.52	0.471	0.7213
	Fnu4HI(rs915915)	0.46	0.16	0.497	**1.721E-6(P<0.05)**
	TaqI(rs10415988)	0.32	0.44	0.435	1.0

The observed values of genetic diversity (FST) on the populations studied are presented in table 2. The greatest variety of populations recorded in russian, buryat and khanty, while the smallest - in the population of the yakut.

Table 2

Genetic differentiation of populations

The Population	The value of the FST
Yakuts (Centre, North, vilyui.)	0,00956
Kets	0,010763
Russian	0,10841
Kyrgyz	0,010831
Buryats	0,10946
Khanty	0,10855

The analysis of structure of a disbalance on coupling shown on two small blocks of coupling in populations of russian (in the extent on 4 kb, includes on 2 SNP: rs2070736 - rs572634 and rs1799894 - rs527221), and kets (on 4 kb, are located in goniometrical and centromere areas of a gene and include on 2 SNP: rs2070736 - rs572634 and rs915915 – rs10415988). One is not extended (to 4 kb) to the coupling block it is taped in populations of northern, central yakuts and kirgiz. More extended block (5 kb), including 3 SNP (rs572634 - rs799894 - rs527221) was observed in population vilusk yakuts. In group of sick blocks of coupling was not taped.

At paired comparison population samples on frequencies of alleles the most frequent significant differences are taped between populations of kets and the buryats (on all to six investigated locuses). Pairs of kets and khants, the buryats and russian significantly differed in frequencies of alleles on five locuses. At comparison of sick yakuts with population sample of

yakuts observed significant differences in frequencies of alleles on three locuses: *Hha I, Hph I, Fnu 4HI.*

Also in work have been taped and analyzed possible haplotypes in all studied samples on six locuses. 15 general haplotypes registered in pair patients DM – population sample of yakuts (table 3), most frequent of which (TTCCGA) met in this pair frequency 0,242. The least frequency of occurrence of the general haplotype TGCGGA – 0,01. On five haplotypes (TTCCGA, GTCCGA, TTTCTC, GTCCTA, GTTCTC) significant differences on frequency of occurrence were observed. All five haplotypes are associated with disease, and protective are haplotypes TTCCGA, GTCCGA, and contributing to disease – haplotypes TTTCTC, GTCCTA GTTCTC.

Table 3

Haplotypes and their frequencies and results of paired comparison of patients DM with population sample of Yakuts

Haplotype	frequency of haplotype		Value P
	patients (N=87)	test (N=328)	
TTCCGA	0,068	0,289	**P<0.05**
GTCCGA	0,040	0,250	**P<0.05**
TTTCTC	0,384	0,065	**P<0.05**
GTCCTA	0,130	0,054	**P<0.05**
TTCCTA	-	-	P>0.05
TTCGGA	-	-	P>0.05
TGCGGA	-	-	P>0.05
TGCGTA	-	-	P>0.05
GTCGGA	-	-	P>0.05
GTTCTC	0,049	0,004	**P<0.05**
TTTCGA	-	-	P>0.05
TTTCTA	-	-	P>0.05
TTTCGC	-	-	P>0.05
TTCCTC	-	-	P>0.05
TGCCGA	-	-	P>0.05

It should be noted that in our previous study of two Yakutian samples (patients and the DM population sample [2]), was detected on the same haplotype TTTCTC, indicating an association with disease, suggesting a significant role of this haplotype in susceptibility to myotonic dystrophy.

Conclusions

Analysis of a sample of patients myotonic dystrophy, the Yakuts, a sample of their healthy relatives and six North Asian population samples (yakuts, khanty, ket, russian, buryat and kyrgyz) for the six SNP, located evenly throughout the muscle protein gene (*DraIII* (rs2070736), *HhaI* (rs 572 634), *BpmI* (rs 1799894), *HphI* (rs 527 221), *Fnu4HI* (rs 915 915) and *TaqI* (rs10415988), revealed the presence of specific haplotypes associated with disease, the most significant of which is haplotype. Further work on the study of muscle protein kinase gene requires linkage of polymorphic sites search with the number of *CTG*-repeats in the *DMPK* gene in populations of Yakuts living in the Republic of Sakha (Yakutia).

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Glutathione system state at valproate sodium toxic action

Effect of antiepileptic preparation depakin on the lipid peroxide oxidation and glutathione system of rats and possibility of combined use of sodium valproate and antioxidant – immunocal were studied. Depakin injection causes a decreased antioxidant defense and oxidative stress development. Immunocal contributing to the intracellular glutathione synthesis has a protective influence.

Key words: glutathione system, lipid peroxidation, sodium valproate, immunocal.

Introduction. Epilepsy is one of the most common diseases of the nervous system which requires the use of specific anticonvulsive in its therapy. They should be taken for a long time, often during the whole life in quite large doses. Up-to-date antiepileptic preparations have some side effects. The basic antiepileptic preparations with wide action spectrum that may be used in different forms of epilepsy are valproates.

A number of articles report about hepatotoxicity caused by taking sodium valproate, they providing some data on development of acute toxic liver necrosis with fatal outcome [6,8].