Kh.A. Kurtanov, A.L. Danilova, A.E. Yakovleva, A.D. Savvina, N.R. Maksimova

MOLECULAR AND GENETIC TESTING OF HLA II CLASS GENES IN CELIAC DISEASE PATIENTS IN YAKUTIA

ABSTRACT

The article presents the results of molecular genetic study based on typing of class II HLA genes (*DRB1, DQA1, DQB1*) in patients in age from 8 months to 18 years with a referral diagnosis of celiac disease or with suspected celiac disease. The study of the gene HLA class II with three-locus haplotypes *DRB1-DQA1-DQB1* revealed carriers of the haplotypes associated with celiac disease. The researchers found a high frequency of carriers of the haplotypes *DRB1*04 - DQA1*03:01 - DQB1**03:02 (*DQ8* type) in the Yakuts compared to the Russians.

Keywords: celiac disease, *DRB1, DQA1, DQB1*genes, haplotype.

INTRODUCTION

Celiac disease is one of the common medical-social problems of modern gastroenterology. High frequency of complications and development of associated diseases causing the disability testify to necessity of studying this pathology and search of the latest technologies for detecting groups of risk and rehabilitation of patients [1].

Until recently the disease has been observed rarely (1:5000 – 10000). However, nowadays the disease is known to be identified in the majority of countries (from 1:184 to 1:500). According to the researches conducted in Europe and the USA the prevalence of celiac disease among children was 1:80 – 1:300. In Russia no clinical-epidemiological researches on this pathology have been conducted, its prevalence approximately estimated 1:1000 [1]. Due to a significant role of celiac disease in the etiology of many systematic diseases there is uncertainty of its diagnostics, treatment and rehabilitation that would promote treatment from celiac disease and diseases connected with it, prevention of oncological intestinal diseases [2].

Celiac disease and its complications have multifactorial pathogenesis detected by genetically associated features of metabolism, immune system and gluten hypersensitivity. Of all the theories of celiac disease pathogenesis the genetic theory is considered the most significant

one according to the World Society of Gastroenterologists. The disease progression is connected with genes *HLA-DQ2 (A1*0501 and B1*0201)* diagnosed at 90-95% of patients and *HLA DQ-8 (A1*03 and B1*0302)* at 5-10% of patients [3, 4]. But existence of these genes not always leads to the progression of celiac disease, as non-HLA genes can be evidence of predictive celiac disease as well [3].

The concept of molecular genetics concerning association of polymorphic genetic markers with predisposition or resistance of the pathology is noted to be one of the approaches of studying genetic risk factors at multifactorial diseases including celiac disease. These markers typical to concrete pathology can be revealed long before its clinical manifestation that will allow to detect risk groups, to organize their monitoring, and if necessary to appoint preventive therapy. Studying of candidate genes with a product of their expression (enzyme, hormone, receptor) involved directly or indirectly in the development of pathological process is of special interest [2].

Due to contemporary achievements in the field of molecular genetics, the study of impact of candidate genes on the disease progression, its clinical manifestations, variability of quantitative signs of immunity and metabolism in the formation of complications appears to be perspective and conductive to the search of risk criterion of celiac disease [1].

In the Republic of Sakha (Yakutia) the prevalence of celiac disease among children in 2008 was 1:1660, and 1:884 among the children's population in Yakutsk city [2]. The absence of unified diagnostic criteria in Yakutia, especially concerning the subclinical forms causing the higher rate of complications and shortcoming of molecular genetics foundations of celiac disease predetermine the expediency of carrying out this research. The topicality of studying of the haplotyping variety of genes HLA II *(DRB1,DQA1,DQB1)* among patients of various ethnic groups living in the territory of RS(Ya) is connected first of all with the necessity of accumulation of database on specific genotypes of patients with celiac disease in this region. The numerous researches conducted in the field of 'HLA and diseases' testify to some distinctions in the clinical course of the disease and development of the immune response depending on the patient's genotype. Detection of such correlations will allow to find out certain treatment of celiac disease by an individual pathogenetic approach in the nearer future and significantly reduce a number of oncological and other complications as well as improve quality of life of patients.

Thus, the aim of this work was the molecular and genetic research and haplotypic variety of celiac disease on the basis of typing genes *HLA II (DRB1, DQA1, DQB1*) in Yakutia.

MATERIALS AND METHODS

This research included DNA samples of the sick children taken by a gastroenterologist of the municipal establishment "Children's City Hospital" of Yakutsk. An informed consent for the genetic research was received from each participant of the research. In total 37 patients with initial diagnosis of celiac disease or with suspicion on celiac disease aged from 8 months till 18 years living in RS (Ya) have been investigated. Of them 24 (64.9%) were the Yakuts, 11 (29.7%) Russians, 1 (2.7%) Evenk and 1 (2.7%) Kyrgyz. On a sexual sign patients were divided on 18 (48.6%) female and 19 (51.4%) male patients (Tab. 1).

DNA purification was conducted from 0.5–0.6 ml of venous blood by means of commercial sets for DNA purification Extra – Gene I (BAG Health Care GmbH, Germany). All examined patients were genotyped on genes of *DRB1, DQA1 and DQB1* by means of commercial sets for HLA tissue typing of HISTO TYPE SSP alleles (BAG Health Care GmbH, Germany).

RESULTS AND DISCUSSION

Totally 37 people were genotyped with the initial diagnosis of celiac disease or with suspicion on celiac disease. As a result of genotyping the haplotypes associated with celiac disease (tab. 2) were found in 17 of 37 investigated. At the rest 20 people no haplotypes associated with celiac disease were revealed, 4 patients of them having a clinic of celiac disease. At Yakuts haplotypes associated with celiac disease were revealed at 11 people, the Russians had 6 people, at the Evenk and the Kyrgyz no haplotype was revealed (Tab. 2). The first haplotype *(DRB1*04 – DQA1*03:01 – DQB1*03:02)* was diagnosed at 6 patients (30%), of them 5 Yakuts (38.4%) and 1 Russian (14.3%). The second haplotype in the amount of 3 *(DRB1*03 – DQA1*05:01 – DQB1*02:01)* was found in equal quantities at both Yakuts and Russians, 6 patients as the whole (30%). The amount of the third haplotype *(DRB1*07 – DQA1*02:01 – DQB1*02:02)* made 5 (25%), of them 3 (15%) were found at Yakuts and 2 (10%) at the Russians, and the fourth haplotype *(DRB1*11–DQA1*05:05 – DQB1*03:01)* rated at 3 patients (15%), of them 2 were the Yakuts (10%) and 1 Russian (5%). As we see in the table 2, the *DQ2* type is presented with 3 haplotypes, and DQ8 type with 1 haplotype. The DQ8 type is found in 5 cases (31.1%) at the Yakuts and in 1 case at the Russians (11.6%). The DQ2 type rated 8 at Yakuts (49.8%) and 6 at Russians (69.3%).

The DQ8 type is noted at Yakuts 3 times (31.1%) more frequently than at Russians (11.6%). Three patients (the Yakuts – 2 people, the Russians – 1 people) are carriers of two celiac disease associated haplotypes (tab. 2). The first patient (the Yakut) has *DRB1*04 – DQA1*03:01 – DQB1*03:02 and DRB1*07 – DQA1*02:01 – DQB1*02:02* relating to DQ8 and DQ2 types, respectively. The second patient (the Yakut) has *DRB1*03 – DQA1*05:01 – DQB1*02:01 and DRB1*07 – DQA1*02:01 – DQB1*02:02* haplotypes (both DQ2 types), and the third person (the Russian) has *DRB1*04 – DQA1*03:01 – DQB1*03:02 and DRB1*03 – DQA1*05:01 – DQB1*02:01* (DQ8 and DQ2 types, respectively). The last patient (*) having DQ8 and DQ2 types at the same time has very high risk of celiac disease.

In various regions of the world the incidence rate of these alleles at celiac disease has specific features and changes from 50 to 97%. On the basis of E.N. Kasatkina's research [6] carried out among a group of children in Moscow 97.2% of patients with celiac disease had gluten enteropathy associated alleles. Thus the main share (88.6%) is presented by the molecule of DQ2 and 8.6% by DQ8 haplotype. In our work specific alleles are noted in 80.9% of cases low the data given above. At the Russians the DQ2 type meets in 69.3% of cases, and DQ8 type in 11.6%. At the Yakuts the DQ2 type is found in 49.8%, and DQ8 type in 31.1% that is 3 times higher, than at Russians. Comparing to the results received at the research of various population groups it is to be noted that there are no similar frequency rates of these alleles at Caucasian and Mongoloid races. So if in Europe the frequency rate of DQ2 amounts for 90-95%, at people of Mongoloid race it is much lower. For example, in A.T. Kamilova's research carried out in Uzbekistan DQ2 type was identified at 69,2% (according to the established allelic loci associated with DQA1*0501 \DQB1*0201 celiac disease – DQ2 type), and at 62% in T.K. Isabekova's works (Tab. 3) [6].

Despite the significant association of celiac disease with *DQ2 (DQA1*05-DQB1*02)* and *DQ8 (DQA1*03-DQB1*0302*) genes, there are data testifying to existence of other genes of HLA system causing the progression of celiac disease. According to the European researchers, 61 patients with celiac disease of 1008 (6. 05%) carried neither DQ2 nor DQ8 heterodimer. In our research, 4 patients of 21 are both DQ2 and DQ8 negative amounting for 19.1%.

At the same time certain Russian researchers specify that the genotype of patients in various regions can have the features and lack of alleles, characteristic for the European population, doesn't exclude the development of the disease. [2]

In this regard, the results obtained require carrying out further researches for establishing genotype features of patients with celiac disease in our region.

CONCLUSION

Thus, according to the contemporary researchers' points of view the existence of *HLA-DQ2 and HLA DQ-8* genes is the basic, but insufficient factor of the development of celiac disease. Lack of the genes almost excludes the diagnosis of celiac disease. If pathological alleles are identified in combination with serologic markers it is likely to predict celiac disease.

When typing the patients with celiac disease and suspicion of celiac disease on HLA II genes by *DRB1-DQA1-DQB1* three loci haplotypes 17 carriers of haplotype associated celiac disease are found out. 4 persons have no these haplotypes, but have the clinic of celiac disease. Three patients (Yakuts – 2 people, Russians – 1 person) are 2 haplotype carriers at once. The high frequency of the haplotype *DRB1*04 – DQA1*03:01 – DQB1*03:02 (DQ8* type) is found out at the Yakuts (31.1%) in comparison with the Russians (11.6%) and with earlier conducted researches (5–10%) that requires more thorough population and genetic research of the Yakut population on *DRB1 – DQA1 – DQB1* HLA II genes.

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Table 1.

Patients: ethnicity and sex, (%)

#	Ethnicity	Patients (%)	Sex	
			Male	Female
1	Yakuts	24 (64.9)	13 (54.2)	11 (45.8)
2	Russians	11 (29.7)	5 (45.5)	6 (55.5)
3	Evenks	1 (2.7)	1 (100.0)	-
4	Kyrgyz	1 (2.7)	-	1 (100.0)
5	Total	37 (100.0)	19 (51.4)	18 (48.6)

Table 2

The distribution of haplotypes associated with celiac disease in patients in Yakutia (Sakha Republic)

	Celiac disease: haplotypes (HLA genes) *DRB1 – DQA1 – DQB1*	Type	Yakuts (%)		Russians (%)		All haplotypes found (%)	Risk	
1.	*DRB1**04 – *DQA1**03:01 – *DQB1**03:02	DQ8	5***	31.1	1*	11.6	6 (30.0)	high	very high
2.	*DRB1**03 – *DQA1**05:01 – *DQB1**02:01	DQ2	3**		3*		6 (30.0)	high	
3.	*DRB1**07 – *DQA1**02:01 – *DQB1**02:02	DQ2	3**, ***		2		5 (25.0)	low	
4.	*DRB1**07 – *DQA1**02:01 – *DQB1**02:02 / *DRB1**11–*DQA1**05:05 – *DQB1**03:01	} DQ2	2	49.8	1	69.3	3 (15.0)	high	

- DRB1*03-DQA1*05:01-DQB1*02:01 and DRB1*04-DQA1*03:01-DQB1*03:02; **- DRB1*03-DQA1*05:01-DQB1*02:01 and DRB1*07-DQA1*02:01-DQB1*02:02; *- DRB1*04-DQA1*03:01-DQB1*03:02 and DRB1*07-DQA1*02:01-DQB1*02:02.*

Table 3

The frequency of HLA haplotypes in different populations

Populations	Haplotypes	frequency of occurrence, %
Finland	DQ2 and/or DQ8	97.0
Northern Europe	DQA1*0501 DQB1*0201	98.9
Israel	DQA1*0501 DQB1*0201	80.0
Kazakhstan	DQA1*0501 DQB1*0201	62.0
Uzbekistan	DQA1*0501 DQB1*0201	69.2
Russia, Tomsk	DQ2 and/or DQ8	70.0
The results of this study	DQ2 and/or DQ8	80.9

Data is taken from the article [Zakkarova, Borovik, Roslavceva et al., 2011].

Information about authors:

FGBNU YSC CMP:

Hariton A. Kurtanov - junior researcher Division of Molecular Genetics, Yakutsk, postal address: 977019, Sergelyakh Highway 4, Yakutsk, Russia, e-mail: hariton_kurtanov@mail.ru;

Anastasia L. Danilova - PhD, researcher Division of Molecular Genetics FGBU YSC CMP, Yakutsk, postal address: 977019, Sergelyakh Highway 4, Yakutsk, Russia, e-mail: ana-danilova@yandex.ru;

EDUCATIONAL AND RESEARCH LABORATORY "GENOMIC MEDICINE" MI AMMOSOV NEFU:

Alexandra E. Yakovleva - junior researcher;

Vilena V. Gerasimova – researcher;

Nadezhda R. Maximova – MD,Head of the Lab;

Anastasia D. Savvina - PhD, Head of Dep. GBU Sakha (Yakutia), "Children's City Hospital".