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The Molecular Genetic Features of Patients with Juvenile Arthritis in Yakutia

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Abstract

The research objective was to conduct a retrospective study of patients with Juvenile arthritis (JA) in association with the carriage of the *HLA-B27* allele.

Materials and Methods: A total of 73 patients (39 boys and 34 girls aged from 1 to 16 years, mean age of 10.28±4.24 years) living in Yakutia with Juvenile Chronic Arthritis (JCA), Juvenile Ankylosing Spondylitis (JAS), Juvenile Psoriatic Arthritis (JPsA), and reactive arthritis (RA) were examined. Among them, 62(84.9%) children were of Yakut nationality, and 11(15.1%) – the Russian nationality. The control group included 85 Yakuts without clinical diagnosis of arthritis. Testing for the *HLA-B27* allele was performed according to Dominquez et al. (1992) as modified by Steffens-Nakken et al. (1995).

Results: According to the genotyping results, in 30 of 73 examined samples an association was found between the *HLA-B27* allele carriage and JA. The *HLA-B27* allele was diagnosed in 24 (38.7%) Yakuts and 6 (54.5%) ethnic Russians. For further analysis, all patients (Yakuts, Russians) were divided into diagnosis-related groups. Diagnoses of JAS (n=10) and JCA (n=9) prevailed in Yakuts. In the Russian children, RA was more common (n=4). In population sampling of Yakuts, the frequency of the *HLA-B27* allele was 32.9%. A comparison of the frequencies of the *HLA-B27* allele among the Yakut patient groups and the control group found a statistically significant association with JAS. The carriage of the *HLA-B27* allele in Yakut females did not increase the risk of JAS development, whereas in male Yakuts this risk increased by 5.6 times.(**International Journal of Biomedicine. 2019;9(2):121-124.**)

Key Words: Juvenile ankylosing spondylitis • HLA-B27 • Yakuts

Introduction

Juvenile arthritis (JA) is a group designation of the numbers of rheumatic children's diseases presented in ICD-10, heading M.08 and M.09, including Juvenile Rheumatoid Arthritis (JRA), Juvenile Ankylosing Spondylitis (JAS), Juvenile Chronic Arthritis (JCA) not otherwise specified, Juvenile Psoriatic Arthritis (JPsA), and arthritis with inflammatory bowel diseases (Crohn's disease, Whipple's disease, non-specific enterocolitis).

JA is an umbrella term used to describe the many autoimmune and inflammatory conditions that can develop in children under the age of 16.[1-3] Characteristics associated with these conditions are familial susceptibility, existence of the pathogenetic or associated markers of the disease predisposition, the variability of clinical implications depending on gender and age, lower level of coincidence on a disease at monochorial twins, and others.[4]

The human leukocyte antigen (HLA) class I molecule *HLAB27* was the first genetic risk factor identified as associating with JAS and remains the most important risk locus for this archetypal spondyloarthropathy.[5] The important role of *HLA-B27* in JA pathogenesis has been known for a long time. The number of the first immunogenic works revealed the

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upregulation of *HLA-B27* among children with JA.[6.7]

According to contemporary views, the basis of pathogenesis of immune inflammatory rheumatic diseases is the complex combination of genetically determined and acquired defects ("imbalance") of immune regulatory mechanisms, limiting pathological activation of the immune system in response to potentially pathogenic factors of the external environment.[8-11] With rheumatic diseases, under the influence of the IL-23 excess production, the *folding* of a *HLA-B27* heavy chain appears to be *slower* than other HLA-alleles, leading to misfolding.[12] In the presence of endoplasmic reticulum stress there is an abnormal accumulation of misfolded heavy chains leading to activation not only of the unfolded protein response, but also of a nuclear factor of NF-kB—a key transcriptional regulator of synthesis of pro-inflammatory cytokines, including IL-17 and TNFα, which also play an important role in the development of inflammation. There are data showing that an adjournment of β2m caused by a high rate of dissociation between a *HLA-B27* heavy chain and β2m occurs also in a synovial tissue and can lead to chronic inflammation.[13-15]

Numerous studies have found a close connection between *HLA-B27* and diseases of this group. Detection of the carrier state of *HLA-B27* is one of modern approaches in preliminary diagnosis and the choice of treatment. Well-timed definition of *HLA* antigens before the emergence of symptoms allows identifying a risk group for the development of a particular disease. Thus, studying the prevalence of the *HLA-B27* gene and features of a clinical aspect of inflammatory joint diseases among the children's population of Yakutia will help to develop effective preventive programs, which, carried out, will reduce the disease burden among children and teenagers. The research objective was to conduct a retrospective study of patients with JA in association with the carriage of the *HLA-B27* allele.

Materials and Methods

Genotyping of HLA-B27 was performed in the laboratory of molecular genetics at Yakut Science Center of Complex Medical Problems. A total of 73 patients (39 boys and 34 girls aged from 1 to 16 years, mean age of 10.28±4.24 years) living in Yakutia with JCA (30 Yakuts and 3 Russians), JAS (15 Yakuts and 1 Russians), JPsA (2 Yakuts), and reactive arthritis (RA) (15 Yakuts and 7 Russians) were examined. Among them, 62(84.9%) children were of Yakut *nationality,* and 11(15.1%) – the Russian nationality. The control group included 85 Yakuts without clinical diagnosis of arthritis. The ethnic origin was considered to the third generation.

For specification of the clinical diagnosis and for the purpose of *HLA-B27* identification, we conducted a molecular and genetic analysis of 73 children from 73 families. From each patient, 2 mL of peripheral blood were drawn into an EDTA tube. Genomic DNA was isolated from the peripheral blood leukocytes using standard phenol–chloroform extraction technique (Maniatis et al., 1982)

Testing for the *HLA-B27* allele was performed according to Dominquez et al. (1992) as modified by Steffens-Nakken et al. (1995). Primers are amplifying codons 91-136, E91S (5'-GGG TCT CAC ACC CTC CAG AAT-3') and 136AS (5'-CGG CGG TCC AGG AGC T-3') (amplificate length 135 bp). For internal control, the β-globin gene was genotyped by primers PCO4 (5' - CAA CTT CAT CCA CGT TCA CC-3') and GH20 (5' - GAA GAG CCA AGG ACA GGT AC-3') (amplificate length 268 bp). All primers described above were synthesized in MNPK Biotekhindustriya. (Moscow, Russia).[16-18]

The reaction mixture (20 μL) contained : 50 ng of DNA, 0.15-0.9 mmol/l. of primers (MNPK Biotekhindustriya), 200 mmol/L of each nucleotide triphosphate (NTP) (Sileks, Russia), 2 units of DNA polymerase (Sileks, Russia), 10×PCR buffer (500 mmol/l KCl, 100 mmol/l. Tris-HCl (pH=8.3), gelatin 1 g/l, 11 mmol/l $MgCl_2$) (Sileks, Russia).

PCR was conducted in the MJ Mini Gradient Thermal Cycler (BioRad). The DNA was amplified using the following thermocycling steps: 94°C for 100 sec, 94°C for 1 min, 57 °C for 1 min, 30 cycles of 72 °C for 2 min, 72 °C for 10 min.

PCR products were analyzed on 2% agarose gels after staining with ethidium bromide and were visualized using a UV transilluminator (Vilber Lourmat, France) (Fig.1).

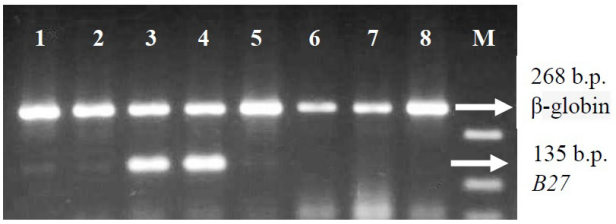

Fig.1. *Electrophoretogram of the PCR products on a 2% agarose gel.*

Statistical analysis was performed using the Statistica 8.0 software package (StatSoft Inc, USA). Differences in the HLA-B27 allele distribution between the two groups were assessed by χ^2- test with 1 degree of freedom (df) or Fisher's exact test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

According to the genotyping results, in 30 of 73 examined samples an association was found between the *HLA-B27* allele carriage and JA. We did not find the HLA-B27 allele in 43 patients with JA. The *HLA-B27* allele was diagnosed in 24 (38.7%) Yakuts and 6(54.5%) ethnic Russians. For further analysis, all patients (Yakuts, Russians) were divided into diagnosis-related groups. Diagnoses of JAS (n=10) and JCA (n=9) prevailed in Yakuts. In the Russian children, RA was more common (n=4).

For comparison, population sample of healthy Yakuts (without JA and family burden for JA) (n=85) was tested. All DNA samples of population sampling were genotyped for the carriage of the *HLA-B*27 allele. The frequency of the HLA-B27 allele varies widely across populations, from 0.4% to 39.6%—the lowest frequency in Japanese from the southern regions of

Japan, the highest frequency in Koryaks from settlements of the Koryak Autonomous Area (Tymlat and Voyampolka).[19] In populations of Europe, the frequency of the *HLA-B27* allele is from 4% to 8%, in ethnic Russians - 10.4%.[20,21] Studies conducted on different populations of the world have shown that the highest and lowest frequency of *HLA-B27* is detected in populations belonging to the Mongoloid race (Fig.2). In our research, in population sampling of Yakuts, the frequency of the *HLA-B27* allele was 33%. It can possibly be defined by similarity of profiles of the HLA system and ethnogenetic bases of populations of the Arctic mongoloids and Yakuts. The distribution of Yakut patients by gender showed an insignificant prevalence of male patients (54.8%). In sampling of Russians, this distribution appeared approximately identical, probably because of a small number of patients (n=11).

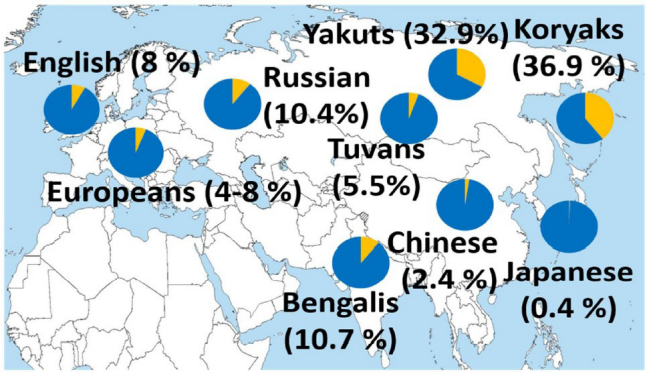

Fig. 2. *The frequency of the HLA-B27 allele in the different populations.*

While comparing the frequency of the *HLA-B27* allele in the group of patients (JA and RA) of the Yakut nationality (38.7%) with population sampling of Yakuts (32.9%), an association with the *HLA-B27* allele carriage was not found (Table 1). A similar analysis for the Russian patients was impossible due to the lack of comparative control. The Yakut patients were divided into diagnosis-related groups: JAS (n=15), JCA (n=30), JPsA (n=2) and RA (n=15). A comparison of the frequencies of the HLA-B27 allele among the Yakut patient groups and the control group found a statistically significant association with JAS (Table 1).

Table 1.

The frequency of the HLA-B27 allele in the studied groups of Yakut patients

№	Group	*HLA-B27* n (%)	OR (95% CI)	*P*-value
1	JAS (n=15)	10 (66.7)	4.071 (1.270-13.052)	0.0182
2	JCA (n=30)	9 (30)	0.872 (0.354-2.151)	0.7670
3	JPsA (n=2)	0 (0)	0.000	1.0000
4	RA (n=15)	5 (33.3)	1.018 (3.217-3.263)	0.9762
Total (n=62)		24 (38.7)	1.286 (0.650-2.544)	0.4704
Control group (Yakuts) (n=85)		28 (32.9)	-	-

We found also a statistically significant association with the carriage of the *HLA-B27* allele in the male subgroup (OR=5.6471, 95% CI: 1.3474-23.6676; *P*=0.018) (Table 2). Thus, the carriage of the *HLA-B27* allele in Yakut males increases the risk of JAS by 5.6 times.

Table 2.

The frequency of the HLA-B27 allele in the studied groups of Yakut patients depending on gender

№	Group	Male				Female			
		n	HLA-B27 n (%)	OR (95% CI)	*P*	n	HLA-B27 n (%)	OR (95% CI)	*P*
1	JAS	12	9 (75)	5.647 (1.347- 23.668)	0.018	3	1 (33.3)	1.136 (0.093-13.886)	0.920
2	JCA	12	4 (33.3)	0.941 (0.247-3.582)	0.930	18	5 (27.8)	0.874 (0.250-3.056)	0.833
3	RA	9	3 (33.3)	0.941 (0.209-4.242)	0.938	6	2 (33.3)	1.1364 (0.180-7.152)	0.892
4	JPsA	1	0	0	1	1	0	0	1
Total		34	16 (47.1)	1.6732 (0.684-4.092)	0.260	28	8 (28.6)	0.909 (0.308-2.688)	0.863
Control group (n=85)		49	17 (34.7)	-	-	36	11 (30.6)	-	-

In conclusion, the population frequency of the *HLA-B27* allele in Yakuts was 32.9%. In the Yakut population, the association between the carriage of the *HLA-B27* allele and JAS was not found, which might be due to a highly heterogeneous sample of patients. The carriage of the *HLA-B27* allele in Yakut females did not increase the risk of JAS development, whereas in male Yakuts this risk increased by 5.6 times.

Competing Interests

The authors declare that they have no competing interests.

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