Ambiguities Resolution of HLA Genotypes in Macedonian Population

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Abstract

A total of 850 persons from the Republic of Macedonia, divided in the four groups, were DNA-HLA genotyped: 1. Donors of the Macedonian Donor Registry (220); 2. Patients for bone marrow transplantation and related potential donors (350); 3. Patients with rheumatoid arthritis, tuberculosis, cardiomyopathies and other diseases (150); and 4. Ambiguity resolution of the genotypes (130). Class I HLA genes (A, C, B, and DQβ1) were typed by Reverse Line Strip Typing (RLS); Class II HLA gene (DRB1) were typed by Sequence Based Typing (SBT). The main results of the project are: a) Creation and legislation of Macedonian Donor Registry, and Macedonian Human DNA Bank; b) HLA typing of unrelated healthy persons for class I HLA-A, -C, and –B, and class II HLA-DRB1; c) HLA genotyping of blood related samples for Bone Marrow Transplantation; d) Association analysis of HLA alleles with Rheumatoid Arthritis (HLA-DRB1, and HLA-B27), and with Pulmonary Tuberculosis (HLA-DQB1); e) Evaluation of allele and genotype ambiguities for HLA class I, and HLA class II, as well as resolution of HLA class I and class II ambiguities; f) Introduction and establishment of sequence specific primers (SSP) method for resolving HLA class II ambiguities in patients with Rheumatoid Arthritis.
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Project Objectives: The original aims of the project were: 1) Creation of Macedonian Donor Registry (MDR) and connection with the European and World Marrow Donor Registries; 2) HLA typing of unrelated healthy persons using methods already established in our laboratory: RLS for Class I HLA-A, -B, and -C typing, and SBT for Class II HLA-DRB1 typing; 3) HLA typing of patients for bone marrow transplantation and related potential donors (brothers, sisters, and parents); 4) HLA typing of samples from the patients with rheumatoid arthritis, tuberculosis, cardiomyopathies, and other diseases associated with HLA alleles; 5) Evaluation of allele and genotype ambiguities for Class I and Class II typings and definition of strategy for their resolution; 6) Introduction and establishment of sequencing based methods for typing of Class I HLA -A, -B, and -C loci; and 7) Introduction and establishment of method that uses sequence specific primers (SSP) for resolving the ambiguous results for Class II typing.

Project Results: Macedonian Donor Registry (MKDR) was legislated in August, 2004 as a foundation according to the Macedonian Low for Non Governmental Organizations and Foundations, 1998. The samples collected from Macedonian population inside and outside of Macedonia are parts of the Macedonian Human DNA Bank (hDNAMKD, http://www.hdnamkd.org.mk). As of December 31, 2006, a total number of 3439 DNA samples were isolated and stored in hDNAMKD. In the anthropology and blood related patents projects were classified 1579 and 707 samples, respectively. In the unrelated patient’s project were classified 1153 samples. During the 2004-2006 a total number of 1117 DNA samples were isolated.

The data for HLA-A, -B, -C and -DRB1 allele frequencies in Macedonian population are deposited in the Allele frequency database (http://www.allelefrequencies.net). The HLA-DRB1 alleles are submitted as high resolution (4-decimal) data (Macedonian population 1, n=158), while for HLA class I alleles (e.g. -A, -B and -C) part of the results are shown as 4-decimal, and part of them are shown as group-specific (Macedonian population 4, n=216). The differences arise from the fact that the ambiguities (undistinguishable results) have been clarified for HLA-DRB1 locus and not for the class I loci, what remains to be done in the future.

We genotyped 356 DNA samples for Bone Marrow Transplantation (BMT), most of them for the Clinic of Hematology, Clinical Center, Faculty of Medicine, Skopje (296), smaller number for the Clinic of Pediatrics, Clinical Center, Faculty of Medicine, Skopje (43), the rest of the samples for Hematologie, Innere Medicin, Basel, SCH (13), and Regional Paediatric Nephrology Unit, Leeds, UK, as well as Pediatric Hemato-Oncology, Silvestrini Hospital, Perugia, IT (4).

From 316 genes from healthy individuals and 124 genes from patients with Rheumatoid Arthritis (RA), a total 40 HLA-DRB1 alleles were identified. In Macedonian RA patients, compared with healthy controls, significantly higher incidences were found of HLA-DRB1*0404 allele (p=0,029), HLA-DRB1*0405 allele (p=0,025), HLA-DRB1*0101 allele (p=0,037) and HLA-DRB1*1407 allele (p=0,024). All B*27xx alleles were found significantly associated to RA patients (12% in RA vs. 3.7% in C.; p=0,003). HLA-B*2702 and B*2704 were significantly more frequent in RA patients considering them to be the most common alleles responsible for RA in Macedonian population. HLA DNA typing for HLA-DQB1 gene was performed on 42 patients (84 alleles) with PTB and compared with control group of 208 (416 alleles) healthy unrelated Macedonian volunteers. Of the obtained 18 DQB1 alleles at patients with PTB, statistical analyses revealed HLA-DQB1*0502 (10.71% in PTB patients vs. 17.94% in controls; p<0.05) significantly more frequent in a control group. It is possible that it appears as protective allele against PTB at Macedonian population. The occurrence of specific DQB1 alleles may be linked to susceptibility/resistance to PTB in Macedonian patients.

Since in a total of 214 samples examined, ambiguous allele combinations were found in only one sample for HLA-A locus, the observed resolution of RLS for HLA –A locus at the allele group level was 99.5%. The observed resolution for HLA-C locus, with ambiguous combinations found in 12 samples of the 214 examined, was 94.4%, and was 91.1% for HLA-B locus, with ambiguous combinations in 19 of 214 examined samples. After one cycle of PCR amplification and DNA sequencing of the second exon of the DRB1 gene, unique (unambiguous) result for the DRB1 genotype was achieved for 38 samples (24.1%), considering as ambiguous the differences at the fifth decimal position which account for a silent mutation and do not alter the amino acid sequence. By eliminating this type of ambiguities by setting the typing result to four decimal positions (while remaining the high
resolution of the analysis) the percentage of unambiguously typed samples rose to 73 (46.2%). This leaves 85 (53.8%) samples for which an additional activity was needed, and this proportion is comparable to other similar studies. We solved most of the ambiguities by high resolution sequence specific primer (SSP) method.

The results clearly indicate that in Macedonian population there are ambiguous HLA class I and HLA class II genotypes and alleles for the most frequent genes, and we need strategy for simple, efficient and fast methods for their resolution.

**Project Publications (full papers):**

**Project URL (WEBSITE):**
[www.ibhg.ukim.edu.mk](http://www.ibhg.ukim.edu.mk)