MOLECULAR AND EXPERIMENTAL BIOLOGY IN MEDICINE

THE STUDY OF HLA-B*44~C HAPLOTYPE POLYMORPHISM IN THE CROATIAN POPULATION

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Abstract: The aim of the study was to investigate the distribution of HLA-B*44 alleles and determine the common HLA-B*44~C haplotypes in the Croatian population.

The subjects included in this study (N=316) were randomly chosen B*44-positive healthy individuals previously typed for the HLA-A, -B, -C and -DRB1 loci. The high resolution level typing of HLA-B and -C loci was performed using the Polymerase Chain Reaction ï Sequence Specific Primers (PCR-SSP) method.

Among five detected HLA-B*44 alleles the most frequent one was HLA-B*44:02 (41.40%) followed by -B*44:03 (25.70%) and -B*44:27 (18.50%). The HLA-B*44:06 allele was detected only once (0.30%). The analysis of the HLA-C allelesô distribution showed a predominance of a single HLA-C allele in all four subgroups: HLA-C*05:01 for the HLA-B*44:02-positive individuals, HLA-C*04:01 among HLA-B*44:03-positive subjects, HLA-C*02:02 in the HLA-B*44:05-positive group and HLA-C*07:04 among individuals carrying HLA-B*44:27.

The potential application of these data can be found in pre-transplant management of HLA-B*44-positive patients in both hematopoietic stem cell and solid organ transplantation program.

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INTRODUCTION

The Human Leukocyte Antigen (HLA) complex, since its discovery in 1958, has for the past seven decades been the focus of research in countless studies and various fields, from population investigations, disease association studies, transplantation etc. The unique set of characteristics displayed by the HLA complex as well as the importance of its role in the immune processes is the reason why this system is among the most investigated genetic complexes in humans, with the abundance of available data and new information emerging daily.[1]

In addition to the great polymorphism of the HLA system, with more than 17,000 alleles described to date, one of the most interesting characteristics of this complex is the presence of linkage disequilibrium (LD) between the alleles of HLA loci, due to which two HLA alleles at different loci appear together at the same haplotype more frequently than it would be expected based on their individual frequencies.[2] Because of LD, the number of combinations of alleles at HLA loci (haplotypes) is in actuality much smaller than theoretically possible. This aspect of HLA has its advantages and disadvantages, especially in the field of transplantation. Namely, the matching of the HLA genes is of essential importance in both solid organ and hematopoietic stem cell transplantation (HSCT) outcome.[3, 4] The presence of LD reduces the number of possible HLA haplotypes and as a consequence raises the probability of finding a matched donor. Also, among partially typed available donors, it enables the assignment of priority status for those who, based on known LD, will more probably be matched for a

particular patient when all relevant HLA loci are typed. On the other hand, patients carrying unusual HLA haplotypes, or more precisely, HLA alleles between which LD is not commonly detected, will have a much smaller chance of finding a suitable donor.[5]

The LD is not equally strong throughout the entire HLA region which encompasses a 3Mbp stretch on chromosome 6p21. The strongest LD within the HLA region was detected between the following genes: HLA-B and -C, and HLA-DRB1 and -DQB1.[6] In addition, the strength of LD varies depending on the gene present at an HLA locus. In some cases, LD between certain HLA genes is so strong that they are found on a same haplotype almost exclusively. This, for example, is the situation with HLA-B*08:01 allele which will be found in association with HLA-C*07:01 allele in nearly all individuals of European descent. On the other hand, HLA-B*51:01 allele does not display such strong LD with a particular HLA-C alleles and can be commonly found on a same haplotype with HLA-C*01:02, -C*02:02, -C*14:02, -C*15:02 or -C*16:02 alleles. Finally, there are also differences in LD between HLA alleles of the same gene. For example, different alleles at HLA*B44 gene associate with different HLA-C alleles.[7]

The HLA-B*44 gene belongs in the group of more frequent HLA-B genes. The information about its frequency is available for numerous European populations and varies: from 3.5% (Italy-Sardinia) to 24.4% (Spain-Ibiza) [8]. Among Croatians, this gene is found with a frequency of 9.3%.[9] The IMGT/HLA database lists 388 alleles detected for the HLA-B*44 gene to date, however, despite such high number of alleles, in most European populations, including the Croatian population, only the following alleles are usually detected: HLA-B*44:02, -B*44:03, and -B*44:05.[8, 9] The variability of HLA-B*44 associations with HLA-C alleles as well as the fact that this gene can frequently be found among Croatians prompted the present study.

The aim was to investigate the distribution of HLA-B*44 alleles and determine the common HLA-B*44~C haplotypes in the Croatian population. This data would be of great benefit in planning unrelated donor search strategy for HLA-B*44-positive patients in HSCT program. Namely, identification of the common HLA-B*44~C associations would enable reducing the list of potential donors for whom HLA-C locus typing is available and HLA-B*44 high resolution typing needs to be performed.

MATERIAL AND METHODS

Subjects

The subjects included in this study (N=316) were chosen among healthy unrelated individuals. The criteria for inclusion was a positivity for the HLA-B*44 gene.

DNA Isolation

DNA was isolated from peripheral blood using a commercial isolation kit (MagNA Pure LC DNA, Roche Diagnostics GmbH, Mannheim, Germany).

HLA Typing

The subjects were previously typed for the HLA-A, -B, -C and -DRB1 loci using the Polymerase Chain Reaction ï Sequence Specific Oligonucleotide Probe Hybridization (PCR-SSO) method (Immucor Transplant Diagnostics Inc., Stamford, CT, USA) to achieve a low resolution typing at these loci.[10] This method uses kits for HLA-A ï B and -C, which cover exons 2 and 3, while kits for HLA-DRB1 cover exon 2. The 5ôends of upstream primers (included in kits) were labelled with biotin, and each PCR product was hybridized with probes complementary to the polymorphic sequences. After hybridization, amplicons were labelled with streptavidin-R-phycoerythrin, which is a specific fluorescent ligand of biotin and quantified on the Luminex LABScanTM 100 flow analyzer (Luminex Corporation, Austin, TX, USA). The high resolution level typing of the HLA-B and -C genes was performed using the Polymerase Chain Reaction ï Sequence Specific Primers (PCR-SSP) method (Olerup Inc, West Chester, PA, USA).[11]

Statistics

The allele and haplotype frequencies were determined by direct counting. Statistical significance of difference in haplotype frequencies was calculated using Fisherôs exact test with P value of 0.05 being considered significant.

RESULTS

The distribution of HLA-B*44 alleles among tested individuals is shown in Figure 1. Since three subjects were homozygous for the HLA-B*44 gene, the total number of analysed alleles was 319. Among five detected alleles, the most frequent one was HLA-B*44:02 (N=132, 41.38%) followed by -B*44:03 (N=82, 25.71%), -B*44:27 (N=59, 18.50%) and -B*44:05 (N=45, 14.11%). The HLA-B*44:06 allele was detected only once (N=1, 0.31%).

The analysis of the HLA-C allelesô distribution among subjects positive for one of the four HLA-B*44 alleles detected more than once in our study is shown in Figure 2. The predominance of a single HLA-C allele in all four subgroups is evident. Among HLA-B*44:02-positive individuals, the most frequent allele detected at the HLA-C locus was HLA-C*05:01 (107/131, 81.68%), while all other alleles failed to reach the frequency of 10%. Among individuals carrying HLA-B*44:03, HLA-C*04:01 was most frequently detected (59/81, 72.84%), however, in this case two other alleles were also present with a notable frequency; HLA-C*07:01 and -C*16:01 (28.40% and

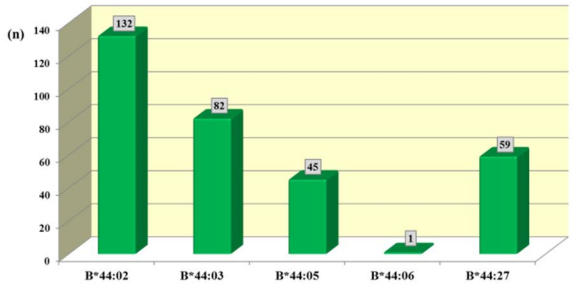

Figure 1. The distribution of HLA-B*44 alleles among tested individuals (N=316)

21.00%, respectively). In the group of HLA-B*44:05-positive subjects, the predominantly found HLA-C allele was HLA-C*02:02 with a frequency of 97.73% (43/44). Finally, among 59 individuals carrying HLA-B*44:27, HLA-C*07:04 allele was most repeatedly identified (59/59, 100%).

The comparison of haplotype frequencies of the above-mentioned combinations revealed that the predominance of a single HLA-C allele for four HLA-B*44 alleles (HLA-B*44:02~C*05:01, HLA-B*44:03~C*04:01, HLA-B*44:05~C*02:02, HLA-B*44:27~C*07:04) in comparison to remaining three HLA-B*44~C combinations is highly statistically significant (P<0.0001). Due to the fact that it was detected only once, HLA-B*44:06 allele was excluded from this comparison.

The distribution of HLA-C*16 alleles among HLA-B*44-positive individuals also displayed significant differences. The HLA-C*16:04 appeared only among HLA-B*44:02-positive subjects, while HLA-C*16:01 and -C*16:02 were detected almost exclusively for HLA-B*44:03-positive individuals. The P value of <0.0001 was calculated for the presence of HLA-C*16:01 among HLA-B*44:03-positive individuals, as well as for presence of HLA-C*16:04 among HLA-B*44:02-positive subjects, while the HLA-B*44:03~C*16:02 combination reached the P value of 0.0014.

DISCUSSION

The knowledge about the distribution of HLA alleles in general has numerous applications among which the planning of the typing and donor search strategies in the HSC transplantation program is one of the most important. The population studies focused on the investigation of HLA polymorphism result in data which can be used by registries in generation of donor search reports. Namely, the data about the distribution of HLA alleles and haplotypes is used in calculating the probability of a match when donorôs typing is incomplete or performed only at the HLA antigen level (low resolution typing). Examples of such search tools are HapLogic® used by the TraxisTM software in the National Marrow Donor Program (NMDP) and OptiMatch of Zentrales Knochenmarkspender-Register (ZKRD).[12] One of the main aims for all population studies performed on the HLA polymorphism among Croatians in our Centre is the application of obtained data in development of the Croatian Bone Marrow Donor Registry (CBMDR) search strategies and improvement of the reports issued by the CBMDR, all with a common goal to enhance the chance of finding a matched donor for patients in need of a HSC transplant.

The analysis of the HLA-B*44 allelesô distribution among Croatians in the present study revealed a pattern that has been observed in majority of reported European populations, with HLA-B*44:02 being the most frequently detected, followed by HLA-B*44:03 and -B*44:05 alleles. From the distribution of those HLA-B*44 alleles in worldwide populations, it can be deduced that their origin is indeed in Europe. More precisely, populations of Ireland and UK reported the highest observed frequency of HLA-B*44:02 (>10%), the highest reported frequency for HLA-B*44:03 (>10%) was found in Portugal, while the highest frequency for HLA-B*44:05 allele (3.60%) was reported for the Bulgarian population.[8] The HLA-B*44:06 allele which was detected only once in our sample, has not been so far reported in the population studies of Croatian population.[9, 13] The HLA-B*44:06 allele has thus far been reported only for a handful of European populations (Bulgaria, Czech Republic, Poland, Germany, Switzerland).[8] On a worldwide scale, this allele is found more frequently only in India.[14] For the HLA-B*44:27 allele, which was described in 2002 and differs for only one amino acid from HLA-B*44:02 to whom it is considered functionally identical, there is currently a lack of population data in the Allele Frequency Net database, with only a few reports from the Czech Republic, Germany, England, USA and South Africa.[8] However, a study focused on resolving several HLA ambiguities, among which was the B*44:02:01G ambiguity (HLA-B*44:02/44:02:01:02S/44:19N/44:27/44:55/44:118) gave valuable data about the distribution of HLA-B*44:02 and HLA-B*44:27 alleles in 9 European populations. Based on the results of this investigation, within the B*44:02:01G group, the HLA-B*44:27 allele is observed at a relative ratio frequency greater than 5% in Eastern European populations (except Greeks), Finnish, as well as in Portugal. In other populations, it is lower than 1%.[15]

There are reports of several HLA-B*44 alleles for Croatian individuals which were not detected in the present study. The only allele detected among CBMDR donors, but not in the present study was HLA-B*44:29, but this allele was seen only once in a sample of 744 HLA-B*44-positive individuals which would explain the fact that it was absent in the present sample.[9] Finally, a study of nonfrequent but well-documented, rare and very rare HLA alleles observed in the Croatian population has informed about a presence of HLA-B*44:21 and -B*44:16 among Croatians, with HLA-B*44:21 being classified as non-frequent allele and HLA-B*44:16 as a rare allele.[13]

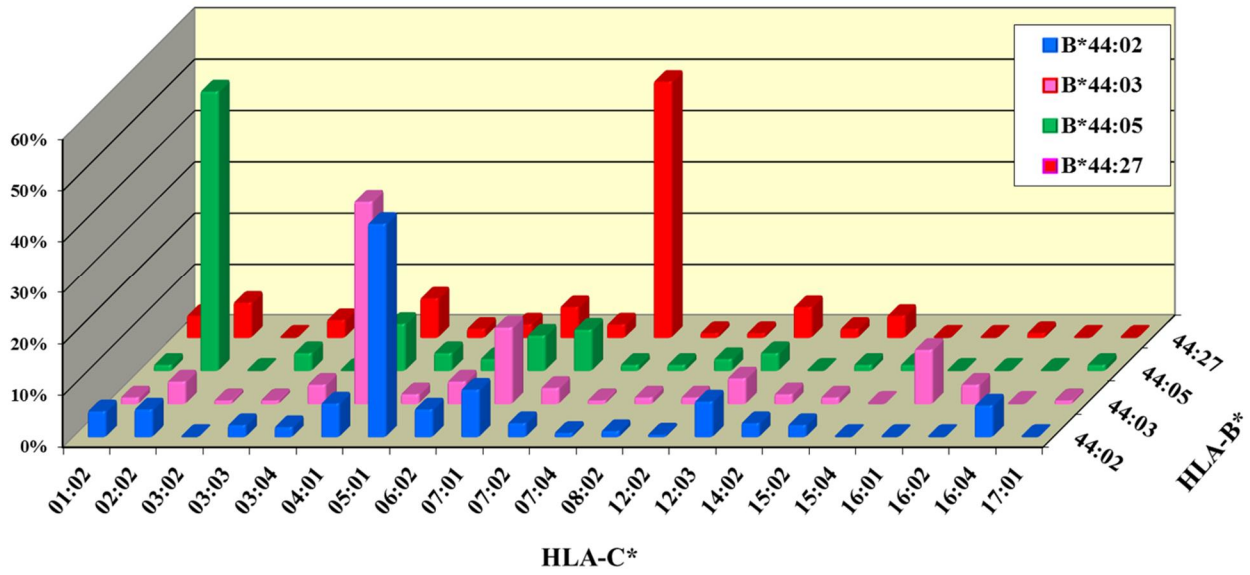

Figure 2. The distribution of HLA-C alleles among individuals positive for HLA-B*44:02 (n=131), -B*44:03 (n=81), -B*44:05 (n=44) or -B*44:27 (n=59) allele

The investigation of HLA-B*44~C haplotypes revealed several interesting and valuable results. The predominance of HLA-C*05:01 among B*44:02-positive individuals, HLA-C*02:02 for HLA-B*44:05-positive subjects and HLA-C*07:04 among individuals carrying HLA-B*44:27 should be taken into account when the typing strategy for patients carrying HLA-B*44 gene is being decided. In cases when multiple donors are typed for the HLA-C locus but lack the HLA-B high resolution typing, donors positive for HLA-C allele shown to be associated with HLA-B*44 allele carried by the patient should be given priority. Conversely, if donors are typed at high resolution level for HLA-B*44 but lack data for HLA-C, a mismatch for HLA-B*44 allele will not be equally predictive for a mismatch at HLA-C locus. More specifically, if the patient is mismatched with his donor for HLA-B*44:02 then it is more likely that the mismatch will also be present for the HLA-C than in cases of patients carrying HLA-B*44:03 for which association with several HLA-C alleles has been observed.

The information about associations of HLA-B*44:02 and -B*44:27 alleles with HLA-C are especially interesting. Namely, since the single difference in amino acid sequence of these alleles is located in ŭß domain, and the studies of their peptide binding features have shown that they share the same peptide motif, the general consensus today is that these two alleles have the same immune function and can be considered as a permissive mismatch in HSCT.[16] Despite that fact, since high resolution typing is mandatory in unrelated HSCT, a search specialist can, based on the donorŝ HLA-C typing results, narrow the list of HLA-B*44-positive potential donors who will have to be typed at high resolution level. Moreover, since the sequencing of HLA-B*44:27 in 2002, it has been shown that individuals previously typed as HLA-B*44:02 were in many instances incorrectly typed and were in fact carrying HLA-B*44:27 allele. As a consequence of this, the HLAB*44:02 result has been replaced by HLA-B*44:02:01G in numerous databases. The knowledge about different HLA-C associations of these two alleles could therefore help in choosing the individual with a preferred HLA-B*44 allele.

A possible application of the presented data could also be found in the solid organ transplantation program. Namely, the kidney allocation scheme of Eurotransplant currently allows matching of the patient and donor only at a low resolution level and only for the HLA-A, -B and -DRB1 loci. However, there are numerous reports about patients who have developed antibodies against HLA-C, -DQB1 and -DPB1 antigens.[17] In such cases, transplant centres require additional HLA-C, -DQB1 and -DPB1 typing of the donor in order to anticipate post-transplantation procedures if donor specific antibodies (DSA) are present. The presence of multiple associations of HLA-B*44 gene with HLA-C genes, which were demonstrated in the present study, suggest that, in cases of HLA-B*44-positive patients, HLA-C typing of patient/donor pairs should be considered in order to avoid a possible future emergence of antibodies against mismatched HLA-C specificities.

Finally, it should be noted that the HLA-C alleles that have been found to be predominant among individuals carrying specific HLA-B*44 allele are not exclusively associated with the HLA-B*44 alleles. On the contrary, these alleles can also be found in LD with other HLA-B alleles and are therefore not as reliable in predicting the HLA-B typing result at the gene level. More precisely, HLA-C*02:02 is frequently associated with HLA-B*27:05 and -B*40:02 alleles; HLA-B*35 and -B*53 genes are very frequently found in combination with HLA-C*04:01; HLA-C*05:01 is commonly detected in HLA-B*18-positive individuals. The only exception is HLA-C*07:04 allele which can indeed be found almost solely among individuals carrying HLA-B*44:27 allele.[18]

In conclusion, the presented data should be valuable in planning of HLA typing strategies in both HSCT and solid organ transplantation as well as in improvement of the HSC donor search protocols.

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