MOLECULAR ANALYSIS OF A NOVEL HLA-DQB1 ALLELE AND ITS GENE FREQUENCY AMONG SARDINIANS

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The genetic mechanism of MHC polymorphism generation is a controversial issue and it has been supposed to vary from locus to locus [1,2]. Recombination mechanisms (gene duplication, gene conversion, inter-allelic/inter-locus sequence exchange) and point mutations are thought to have played different roles during the evolution of the various HLA class II loci [3-5]. In this respect, recombination events promoted by conserved χ-like putative recombination signals, found in HLA-DRB1 and -DPB1 genes between the β-sheet and α-helix domains, are believed to have significantly contributed to polymorphism generation at these loci [5,6]. By contrast, the fact that χ-like sequences have not been observed at the DQB1 locus has been put forward to explain why recombination-like events are less frequently seen at this locus where point mutations are considered to have played the major role in polymorphism generation [1]. We indicate that a χ-like sequence is also present within the DQB1 locus (5'-GCTGGGG-3', nucleotide position 156-162) and that this motif may have contributed, through intralocus gene conversion, to the generation of the novel DQB1*0305 allele.

DNA of 1000 randomly selected newborns of Sardinian descent, referred to our centre for Guthrie screening programs, was extracted by the chelex method starting from dried blood spots [7]. Amplification of the polymorphic second exon of DQB1 gene and dot blot analysis of amplified DNA with sequence specific oligonucleotide probes (SSO) was carried out using the procedures and oligonucleotide probes already described by others [8-10].The samples having a novel hybridization pattern were sequenced using the asymmetric PCR method [11] First, DNA was amplified with DQB130 (5'-AGG-GAT-CCC-CGC-AGA-GGA-TTT-CGT-GTA-CC-3') and DQB131 (5'-TCC-TGC-AGG-GCG-ACG-ACG-CTC-ACC-TCC-CC-3') ampli-primers that specifically amplify DQB1 second exon avoiding the

co-amplification of the DQB2 locus. Next, an aliquot of amplified material was subjected to another amplification using 50 pmol of DQB130 and 0.5 pmol of DB86 (5'-CTG-CAG-GGT-CGT-GCG-GAG-CTC-CAA-CTG-3') ampli-primers and vice versa. The single strand DNA was purified in microconcentrator Centricon 100 (Amicon) and directly sequenced with the Sanger method on both strands. The sequence pattern of the novel allele, formally indistinguishable from a double crossing over, can most likely be explained by an unidirectional gene conversion event between the DQB1*0302 (recipient) and DQB1*0402 (donor) alleles. While both the 5' and 3' sequences of the DQB1*0305 allele belong to the DQB1*0302, those comprised between these two stretches are derived from the DQB1*0402 allele. In particular all nucleotides 5' to position 26 derive from the DQB1*0302 allele, those comprised between position 63 and 114 unequivocally belong to the DQB1*0402 allele, while the sequence 3' to position 164 is again that of the DQB1*0302 allele. Due to the sharing of the sequences between the donor and the recipient alleles from position 27 to 62 and from 115 to 163 the cross-over points cannot be unequivocally identified. Considering the sequence pattern of DQB1*0305, to achive a better discrimination of this allele, we suggest the use of the following additional SSO probe to the traditional panel used for the typing of DQB1 locus : DQ-3.5 (5'-GG-ACC-GAG-CGC-GTG-CGG-GGT-3', codons 20-26; washing temperature 72 °C).

Sardinians are an isolated ethnic group believed to be one of the oldest and genetically most deviant population in Europe [12]. The DQB1*0305 allele was found in 10 out of 1000 samples (gene frequency of 0.005) suggesting that it is not an uncommon variant in this population. It is interesting to note that the gene frequency of DQB1*0305 was obtained starting from dried blood spots commonly used for neonatal screening programs and stored for 1-2 years. This approach may offer a simple method to establish the frequency and the intra-regional distribution, from a large number of samples, not only of HLA genes but also of other genetic markers in epidemiological studies

The recognition of the DQB1*0305 alleles has not only implications in evolutionary studies but also for forensic studies, as well as for bone marrow transplantation (BMT) considering also that BMT from unrelated donors is becoming a possible therapeutic option

for different haematological disorders [13]. Since single aminoacid substitutions may have a profound effect on peptide binding and T-cell recognition [14] accurate molecular typing, including the use of the SSO DQB-3.5 probe we suggested in this study, should be mandatory, before BMT from unrelated donors is performed.

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