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A NOVEL DRB1 ALLELE WITH A HYBRID SEQUENCE OF DRB1*13 AND *16

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HLA DRB1-typing of the family of a Dutch renal transplant patient resulted in the identification of a possible variant DRB1*13 by PCR-SSP. The typing pattern seemed most closely related to the DRB1*1305 pattern. SSOP typing by 12th workshop oligonucleotides revealed an unusual typing pattern, but clearly different from DRB1*1305.

Subsequent sequence analysis was conducted by solid phase direct DNA sequencing on a Pharmacia Alfexpress and typing was performed with the SBTyper software. Typing resulted in a sequence which did not match the database and therefore a new allele was suspected. From codon 19 to 37 the sequence was concordant with different DRB1*03, *13 and *14 alleles. From codon 57 to 89 the nucleotide sequence changed to the exact sequence shared by DRB1*1601 or DRB5*01. Segregation analysis could be demonstrated for the new allele in this 10 member family.

Although variant patterns were recognized by SSP and SSOP typing, the direct determination of the nucleotide sequence by SBT identified this new allele.

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A NEW HLA DRB1 ALLELE (DRB1*1322) DESCRIBED IN TWO UNRELATED CAUCASIAN INDIVIDUALS.

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Using several molecular biology techniques, HLA DRB1 DNA typing of two unrelated individuals presented the same discordant results. Using PCR-SSP assay, both DNAs appeared to be DRB1*01 homozygous. PCR-RFLP assay showed an uncharacterized pattern. Meanwhile reverse dot blot gave the following result : DRB1*0101, DRB1*1308. Although, the DRB1*13 specific primers used for PCR-SSP amplify the DRB1*1308 allele. Furthermore, BstNI profile digestion gave a 123 bp fragment never associated with a DRB1*1308 allele. The new allele sequence (DRB1*1322) is identical to DRB1*1317 with the exception of codon 13 where TCT has been substituted for GGT and codon 16 where CAT has been substituted for TAT. This allele seems to have a higher frequency than expected.

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A NEW DRB1*14 ALLELE (DRB1*1423)

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A german bone marrow donor was typed DRB1*04,*13, *14 by DRB generic oligotyping. After DR3,5,6,8 group specific amplification and oligohybridization DRB1*14 could be assigned, but the reaction pattern was not compatible with the DRB1*14 subtypes so far known (DRB1*1401-*1421). By DR4 specific amplification and oligotyping DRB1*0401 was found, which was confirmed by sequencing. By direct sequencing after separation of the tw DRB1* haplotypes a new DRB1*14 allele could be identified. DRB1*1423 is identical with DRB1*1414 except codon 86 (GGT → GTG). This nucleotide exchange leads to an aminoacid exchange: GGT = Glycin → GTG = Valin. As it is known that the aminoacid exchange in position 86 is of biological significance because of different peptide binding specificity we think it is necessary to include those variants in our testing systems, even if they are possibly very rare.
The nucleotide sequence data have been submitted to the EMBL Data Library and have been assigned the accession number X91640. The name DRB1*1423 has been officially assigned by the WHO Nomenclature Committee in November 1995.
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DRB4 ALLELES IN A NORTHERN ITALIAN POPULATION
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DRB4 gene encodes the DRw53 specificity in DR4, 7 and 9 alleleic variants. We analized distribution of DRB4 alleles in 35 subjects from a panel of 101 samples typed for HLA class II loci. We made DRB4 typing by PCR amplification (Dynal set).
The 35 DRw53 typed for DRB1 locus resulted: 23 DRB1*0701, 10 DRB1*04, 1 DRB1*0404/0701 and 1 DRB1*0404/0407. No DR9 was present. Nine DR4 samples are DRB4*0103 and one DRB4*0102. Within DR7 group, DRB4*01011 was found in eleven out of 19 DRB1*0701/DQB1*0202 and in one sample DRB1*0701-DQB1*0303. The remainders DRB1*0701-DQB1*0202 were DRB4*0103. DRB4*01012N was always associated with DRB1*0701-DQB1*0303 with one exception. In DRB4*01012N individuals a single point mutation at the splice site results in lacking of protein expression. The unusual haplotype DRB1*0701-DRB4*01011-DQB1*0303 could represent an evolutionary step among the three many DR7 haplotype observed. In two samples (DRB1*0404/0701 and DRB1*0404/0407) it was not possible to assign a definitive DRB4 typing since the two heterozigous combinations DRB4*0103/0103 and DRB4*01012N/0103 yield unique amplification pattern.

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HLA-DQB1*0202 IS ASSOCIATED WITH DRB1*0701
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The DQB1*0202 allele is known to differ from DQB1*0201 by a single nucleotide in the sequenced part of the third exon. DQB1*0202 has a guanine in second position of the 135th espressed codon, whereas the DQB1*0201 has an adenine, like all the other alleles at this position. We choose ten homozigous cell lines, from XI Workshop, and fortytwo samples, from an Italian panel of 101 individuals DNA typed, all DQB1*0201. To selectively amplify the DQB1*0202 allele we designed two sequence-specific primers.
Twenty subjects were DR3+ and 25 were DR7+ (3 of them were heterozygous DRB1*0301/DRB1*0701); our results show that all the DQw2+ samples carrying the DRB1*0301 allele, were found to have the DQB1*0201 specificity, while DQw2/DR7+ were found to have the DQB1*0202 allele. The three heterozygous individuals were typed as DQB1*0201/DQB1*0202. These results were completely confirmed in homozygous cell lines group. We can conclude that the DQB1*0202 allele is associated with DRB1*0701, and the DQB1*0201 allele is associated with DRB1*0301.

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NEW VARIANT OF SSP-DRB1 AND DQA1 GENOTYPING.

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We developed a fast, reliable but less labour-intensive variant of SSP technique as compared to the classic one. We called it mSSP (mixed SSP). Its characteristic feature is using of primer mixture in specific amplification. Here a product of certain length corresponds to each specifcity. It allows to significantly reduce both the number of tubes and lanes on gel as the possible products can be easily identified on the same lane due to their length difference.

The developed set of primers amplifies all the presently reported DRB1 alleles so that it is possible to identify to which of the following DRB1 groups they belong: DR1, DR2, DR3, DR4, DR5(11), DR5(12), DR6(13), DR6(14), DR7, DR8, DR9, DR10; DQA1*101, -102, -103, -201, -301, -401, -501, -601.The typing procedure consists of two phases. The first one - standard generic amplification of the second exon of corresponding gene. The second - groups specific amplification with two different primer mixes. The groups are identified by the fragmental length in two corresponding lanes of polyacrilomide gel. The whole procedure (after DNA extraction) using our technique can be performed within three hours.

mSSP has undergone quality control in frame of XII IHWC (Dr.S.Caillat-Zucman). MSSP has been effectively implied in clinical transplantology (over 200 donor-recipient pairs of kidney allografts were typed using it) as well as in HLA and diseases problem (IDDM in particular)