

A PCR-SSP BASED MICA AND MICB TYPING SYSTEM

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Introduction

MICA and MICB are emerging as important additional loci within the MHC. As their extensive polymorphism and immune function is unfolding the relevance of these loci in transplantation, disease and population analyses is increasingly being evaluated.

We have developed a typing system, compatible with our routine HLA-A,B,C PCR-SSP technique, which will allow us to identify and differentiate the majority of the MIC alleles.

Method

IMGT/HLA Database sequence alignments were used for MICA. MICB sequences were aligned from GenBank and MICB nomenclature was based on Ahmad et al. (Tissue Antigens 2002, 60, 164).

We designed 69 primers in 67 mixtures for MICA and 36 primers in 29 mixtures for MICB. This allowed the detection and differentiation of the 54 MICA and 17 MICB alleles except 5 MICA alleles, 3 within allele families, e.g. 00901/00902 and 3 MICB alleles, 2 of which are intronic variations.

Helmberg's SCORE software, using version 1.14 of the IMGT/HLA Database, confirmed the MICA amplification reactivity of each SSP mixture and evaluated the typing set for MICA allele combination ambiguities. Due to sequence similarity between MICA and MICB SSP mixtures were carefully checked manually to ensure absolute specificity for alleles at one locus.

MICA and MICB types were allocated manually by two independent operators. SCORE was also used as an aid to MICA assignment.

As part of the validation procedure a total of 83 reference DNA samples were used: 51 from IHW B-cell lines (38 typed for MICA and MICB, 12 for MICA only and 1 for MICB only) and 32 MICA typed reference samples from two laboratories. This included a sample which has a 100-kb deletion resulting in the entire loss of the MICA gene

We also typed 166 contiguous blood donors from the Welsh Bone Marrow Donor Registry and determined carriage, gene and haplotype frequencies for HLA-B/MICA/MICB.

Results

The reference material identified 27/54 MICA alleles and 14/17 MICB alleles validated 48/67 MICA and 29/36 MICB primer mixtures. These included all alleles with a Caucasoid gene frequency of >1% and also included some rarer alleles such as MICA*027, MICA*046 and MICB-0112 and -0113.

Eight reference sample types did not concur. Of these 5 were resolved in favour of our typing, 3 are currently unresolved.

The MICA and MICB allele frequencies (Tables 1 and 2) and HLA-B/MICA/MICB linkage disequilibrium parameters and haplotype frequencies of the 166 blood donors concurred with other published data on UK subjects.

Table 1. MICA carriage and gene frequencies in 166 blood donors

MICA allele	Carriage frequency (%)	Gene frequency
001	1.81	0.00904
00201/20	18.67	0.09639
00202	0.60	0.00301
004	9.04	0.04518
005	0.00	0.00000
006	0.60	0.00301
00701/26	7.23	0.04217
00801/03	78.31	0.53012
009	15.06	0.07831
010	7.83	0.04217
011	3.01	0.01506
012	3.61	0.01807
016	1.20	0.00602
017	9.04	0.04819
018	6.63	0.03313
019	3.01	0.02108
027	1.20	0.00602
029	0.60	0.00301

Table 2. MICB carriage and gene frequencies in 166 blood donors

MICB allele	Carriage frequency (%)	Gene frequency
0104	45.18	0.256
0105	9.04	0.048
0106	27.11	0.154
01021	58.43	0.355
01022	1.81	0.009
01022v	0.60	0.003
0103101/02	28.31	0.157
0103101v	3.01	0.018

Comments

This system is a simple, reliable and rapid technique for typing MICA and MICB alleles. However, although it is easily updated as new alleles are identified it requires a continuing validation review until all possible MICA and MICB alleles have been identified.