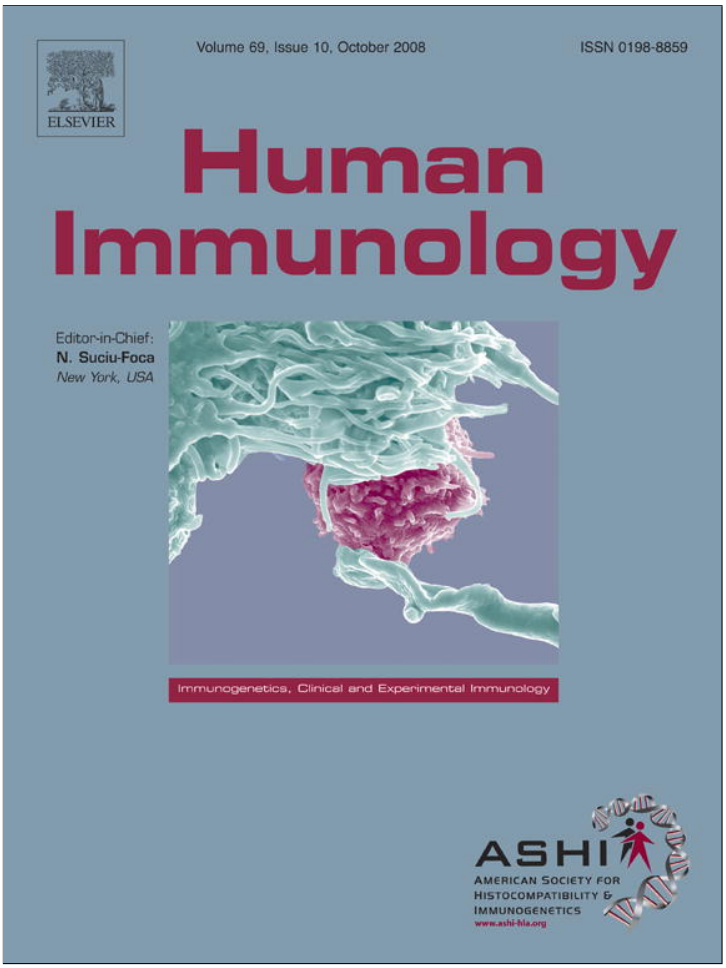

Human Immunology (2008) 69, 655-660

journal homepage: www.elsevier.com/locate/humimm

Allelic diversity of MICA gene and MICA/HLA-B haplotypic variation in a population of the Murcia region in southeastern Spain

Daniel Lucas, José Antonio Campillo, Ruth López-Hernández, Pedro Martínez-García, Manuela López-Sánchez, Carmen Botella, Gema Salgado, Alfredo Minguela, María Rocío Álvarez-López, Manuel Muro*

Immunology Service, University Hospital Virgen de la Arrixaca, Murcia, Spain

Received 23 May 2008; received in revised form 13 July 2008; accepted 18 July 2008

KEYWORDS
MICA;
HLA-B;
Polymorphism;
Linkage disequilibrium

Summary Major histocompatibility complex class I-related chain A (MICA) is located at 46 kb centromeric of HLA-B. It is highly polymorphic and interacts with NKG2D, its receptor on the surface of NK, T$\gamma\delta$ and T CD8 lymphocytes. Data on MICA polymorphism in different populations are still limited. Our aim was to establish allelic diversity of MICA gene and linkage disequilibrium with HLA-B in our population. DNA was obtained from 154 unrelated healthy individuals from the Murcia region in southeastern Spain. HLA-B genotyping was performed using polymerase chain reaction (PCR)-sequence-specific oligonucleotide probes and allele-specific PCR-sequence-specific primers, and MICA genotyping by using PCR-sequence-specific oligonucleotide probes. A total of 19 MICA alleles were detected on this study. MICA*008 was the most frequent allele (25.3%), followed by MICA*002 (16.1%), MICA*004 (14.9%), MICA*001 (7.8%), MICA*009 and MICA*016 (7.1%), and MICA*010 (4.6%). Eleven alleles had frequencies of $<$1%. In the haplotype analysis, MICA*008-B*0702 was found to be the most common, followed by MICA*004-B*4403 and MICA*001-B*1801, MICA*002-B*3501, MICA*008-B*4402, MICA*004-B*4901, MICA*008-B*0801, and MICA*002-B*3801. The frequency of MICA*010-B*1501, MICA*008-B*1302, MICA*015-B*4501, and MICA*008-B*4001 was remarkable inasmuch as these two last haplotypes have not been reported in Spanish population. Indeed, MICA*016 linked to B*1402 has also not been reported in the literature. In conclusion, the allelic diversity in our population is similar to other Caucasian populations; however we found a series of less frequent alleles, in addition to as-yet-undescribed haplotypic associations in other populations of Caucasian origin.

Introduction

On the 2-Mb class I segment, multiple major histocompatibility complex class I-related chain (MIC) loci have been identified [1]. The genes at these loci represent a second lineage of mammalian MHC class I genes. The MIC genes include seven members (MICA-MICG); five of these are pseudogenes and gene fragments, whereas MICA and MICB are functional genes closely related to each other. They code for stress-induced cell surface molecules, do not associate with β_2-microglobulin, and do not appear to present peptides [2].

MICA maps approximately 46 kb centromeric to the HLA-B locus and encodes a cell-surface glycoprotein of 383 amino acids. It is expressed in keratinocytes, fibroblasts, and gas-

The contribution by D. Lucas and J.A. Campillo is equal and the order of authourship is arbitrary.

* Corresponding author. Fax: (34) 968-349678.
E-mail address: manuel.muro@carm.es (M. Muro).

0198-8859/$ -see front matter
doi:10.1016/j.humimm.2008.07.011

ABBREVIATIONS

HLA	human leukocyte antigen
MHC	major histocompatibility complex
MIC	major histocompatibility complex class I-related chain
MICA	major histocompatibility complex class I-related chain A
PCR	polymerase chain reaction

trointestinal epithelium and in several other cell types [3], and its expression MICA is upregulated by TNF-α [4]. Its genome structure is similar to that of MHC class I genes. Exons 2, 3, and 4 of the gene encode the three extracellular domains (α_1, α_2, and α_3, respectively), and the exon 5 encodes the transmembrane domain.

In the same way as classical HLA, MICA display a high degree of allelic polymorphism within the nonclassical HLA gene loci, and the nomenclature describing the different alleles varies. At the moment, 63 MICA alleles are officially designated by the World Health Organization that result from different combinations of polymorphisms in the different exons. MICA polymorphic residues are positioned on the outer edge of an antigen-binding cleft, apparently bordering an invariant ligand-binding site, unlike in MHC class I molecules. The functional significance of these polymorphisms is unknown although certain changes in the amino acid sequence of the protein could influence the abnormal expression [5,6] or the affinity in the interaction with NKG2D/DAP10 (an activating immunoreceptor complex), its ligand on the surface of NK, T$\gamma\delta$, and T CD8 lymphocytes. MICA triggers the cytolysis mediated by these NKG2D-bearing cells. One report demonstrated that the presence of methionine or valine aminoacid at codon 129 of α_2 domain confers a strong or weak affinity, respectively [7].

Many tumors appear to express MICA on their surface [8,9]. Circulating soluble MICA also triggers the downregulation of NKG2D and impairs lymphocyte cytotoxicity in tumoral escape, as suggested [10–12], highlighting the therapeutic potential of anti-MICA antibodies to overcome immune suppression and to effect tumor destruction [13]. Indeed, MICA is implicated in outcomes of solid organ transplantations, as reported by the present and other authors [14,15], as MICA antigens elicit a powerful antibody response in organ allograft recipients. For this reason, some investigators have hypothesized that MICA could engage both adaptive and innate immunity [16]. In this sense, NKG2D ligand induction might participate in the amplification loop that leads to tissue damage during acute graft-vs-host disease [4]. MICA alleles may also be associated with susceptibility or resistance to autoimmunity development [4,17].

Information regarding MICA polymorphisms in different populations and ethnic groups is still limited, however. In this sense, our population shows particular characteristics reported in other marker genetic studies, due to its geographical place in southeastern Europe in close proximity to the African continent [18–20].

Our aim was therefore to establish the allelic diversity of the MICA gene and linkage disequilibrium with HLA-B (the closest HLA gene) in our population.

Subjects and methods

Subjects

Peripheral blood was obtained from 154 unrelated randomly selected healthy Murcian individuals, all of them Caucasian, recruited at the University Hospital "Virgen de la Arrixaca" from the Murcia region in southeastern Spain. Among these donors, 52.6% (81/154) were male and 47.4% (73/154) were female. Individuals are from different villages of the Murcia region and form a representative sample of the population of the Murcia region in southeastern Spain. The characteristics of the Murcia region, its location within the Iberian Peninsula, and its history have been previously published [18]. All subjects gave their informed consent before their inclusion in this study to the collection and storage of blood, isolation of DNA, and determination of the gene polymorphisms. The study was approved by the local medical committee.

MICA and HLA-B genotyping

Genomic DNA was extracted from peripheral blood lymphocytes with the Maxwell16 extractor (Promega, WI) according to the manufacturer's instructions, as previously described [21], and was used for the HLA-B and MICA genotyping.

For HLA-B typing, a two-step strategy, low- or intermediate resolution typing by polymerase chain reaction (PCR) with PCR-sequence-specific oligonucleotide probes, followed by allelic specific typing by PCR with sequence-specific primers (PCR-SSP), using commercial kits (OneLambda, CA). Amplification and detection were carried out according to the manufacturer's instructions.

MICA genotyping was performed using PCR-sequence-specific oligonucleotides for exons 2, 3 and 4 of the MICA gene by using luminex technology (OneLambda, CA). The specific alleles detected and their sequences are reported elsewhere (http://www.anthonynolan.org.uk/research/hlainformaticsgroup/nomenclature.htm). These alleles were assigned on the basis of sequence-specific oligonuclotide probe hybridization patterns predicted from known MICA alleles. Confirmatory typing was carried out for selected samples that deviated from expected HLA-B-MICA associations [22–25].

Statistical analysis

MICA and HLA-B gene frequencies were estimated by direct counting assuming that samples with a single allele were considered homozygous, and that allele was counted twice in the analysis. Allele and haplotype frequencies were calculated by using Arlequin population genetic software (Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland, http://cmpg.unibe.ch/software/arlequin3/). The maximum likelihood haplotype frequencies were imputed within population using an expectation-maximization algorithm approach for multilocus genotypic data when the gametic phase is not known, as implemented in the Arlequin program. The values of the linkage disequilibrium parameter D (measure of deviation from two-locus equilibrium) and the relative linkage disequilibrium parameter D' (D value standardized by the maximum value that it can take) were also estimated. We calculated the significance of the two-locus association (p) using for 2×2 contingency tables. Consistency with Hardy-Weinberg equilibrium of genotype frequencies at each locus was tested on a contingency table of observed versus predicted genotype frequencies using a modified Markov chain random-walk algorithm, as previously described [18,19].

Results and discussion

This study was designed to investigate genetic diversity in MICA and HLA-B genes in a population for which no previous MICA data were available. All 154 DNA samples from individuals in the Murcia region yielded unequivocal reaction patterns. HLA-B and MICA loci were observed to be in Hardy-Weinberg equilibrium in this population (MICA, $p = 0.092$) and, as expected from the short distance between these loci, strong linkage disequilibrium was also observed. The heterozygosity estimated by maximum likelihood for the MICA locus was 0.87.

A total of 19 MICA alleles were detected on the present study (Table 1), despite the high number of MICA alleles described. MICA*008 was the most frequent allele (25.3%), followed by MICA*002 (16.1%), MICA*004 (14.9%), MICA*001 (7.8%), MICA*009 and MICA*016 (7.1%), and MICA*010 (4.6%), similar to findings in other Caucasian populations [23-25,26-31]. However, in our study, MICA*008, known to be the most common MICA allele in individuals of Caucasian origin, was observed less frequently than in other European populations studied [30]. Eleven alleles showed frequencies of <1% (e.g., MICA*005, *007, *017, *023, *030, *033, *046, *050, and *052). Uncommon alleles, such as MICA*030, have also been observed in African Americans [28], and MICA*046 has been noted in Berbers [32]. Curiously, the MICA*052 allele was found to be frequent in a Thai population and to be associated with HLA-B*13 [33]. We also observed other particular characteristics in our population. For example, the MICA*007 allele is common in Caucasian populations [23,24] but was very infrequent in our population. Some alleles found in neighboring populations (*e.g.*, MICA*011, *012, *019, and *021) were also not detected. This may be the result of under-representation or of the simple absence of these alleles in this population.

Table 1 Distribution of allele frequencies of MICA alleles in 154 individuals from the Murcia region population in southeastern Spain.

Allele	*n*	Frequency
MICA*001	24	0.07856
MICA*002	50	0.16183
MICA*004	46	0.14998
MICA*005	1	0.00670
MICA*007	4	0.01241
MICA*008	78	0.25312
MICA*009	22	0.07126
MICA*010	14	0.04551
MICA*015	8	0.02513
MICA*016	22	0.07126
MICA*017	4	0.01241
MICA*018	8	0.02513
MICA*023	4	0.01241
MICA*027	8	0.02513
MICA*030	4	0.01241
MICA*033	4	0.01241
MICA*046	1	0.00670
MICA*050	1	0.00670
MICA*052	1	0.00670

MICA, major histocompatibility complex class I-related chain A.

Thus our population of Murcian individuals shows some characteristics in common with North African individuals (consisting mainly of a decreased frequency of MICA*010 and MICA*008 and an increased frequency of MICA*004), and these findings have been suggested as supporting the hypothesis of a common ancestral origin of Spanish and North African populations [34]. However our population also shows allele frequencies similar to those in populations of Portuguese and Spanish origin [23-25]. In this sense, comparison and analysis of MICA allele frequencies among populations of Murcian, non-Murcian Spanish [23,25], and Portuguese [24] origins show a closer relationship among all of these three populations in comparison with other populations from African origin [22,32,35] (data not shown).

On the other hand, Table 2 shows inferred haplotype frequencies between pairs of MICA and HLA-B alleles. Haplotype distribution showed a high degree of variation that was largely dependent on the distribution of HLA-B alleles. For example, MICA*008-B*0702 (9.4%) was the most common haplotype, followed by MICA*004-B*4403 and MICA*001-B*1801 (8.1%), MICA*002-B*3501 (6.7%), MICA*008-B*4402 (4.7%), and MICA*008-B*0801, MICA*004-B*4901, and MICA*002-B*3801 (4.5%). The frequency of MICA*010-B*1501, MICA*008-B*1302, MICA*015-B*4501, and MICA*008-B*4001 haplotypes is remarkable and these two last haplotypes have not been reported in Spanish population [23]. Indeed, MICA*015-B*4501 haplotype is found in our population, although B*4501 has been associated with MICA*009 in other sub-Saharan African ancestry populations [32,35]. MICA*010-B*1501, MICA*008-B*1302, and MICA*008-B*4001 haplotypes are also found in Americans of European origin, Brazilians, and Chinese [22,23,36]. MICA*002-B*5301 haplotype was also observed with low frequency, as B*5301 is found predominantly in populations of African origin [22,35]. Curiously, MICA*011 is detected in individuals from the Balearic Islands, in Berbers, and in Italians, and associated with B*1402 [23,32,37], but this allele was not found in Murcian individuals. However, in our population, B*1402 was associated with MICA*016 allele, which has also not previously been reported.

Overall, population-specific haplotypes are confined to those involving a population-specific HLA-B allele. For instances, the two dominant alleles MICA, alleles MICA*002 and *008, are both associated with several common HLA-B alleles. MICA*002 is associated with B*3501-, *3801-, *5301-, and *5801-related subtypes in a manner similar to that in African Americans [28], whereas MICA*008 is associated with B*0702, *0801, *1302, *4001, *4402, and *5001 alleles (Table 2). MICA*002-B*35 haplotype has also been reported in North American Caucasian, South American Indian, North African, and Japanese populations [28,32,38,39]. MICA*004 was also associated with *4101/02, *4403 and *4901 alleles.

However, very few HLA-B alleles showed multiple associations with MICA alleles, including some of the most common HLA-B alleles; for example, B*1801 was associated with MICA*001 and *018 (reported in Berbers), and B*1501 was associated with MICA*010 and *016. In our population, we did not find the B*4801 allele, which is known to be associated with a MICA null allele caused by a deletion involving the entire MICA gene [39].

Table 2 Distribution of most common MICA–HLA-B haplotypes in the Murcia region population in southeastern Spain, estimated by maximum likelihood.

Haplotype		Haplotype frequency	*D*	*D′*	*p* value
MICA*001	B*1801	0.08121	0.0617	0.8764	<0.001
MICA*002	B*3501	0.06701	0.0430	0.8516	<0.001
MICA*002	B*3801	0.04522	0.0382	0.8599	<0.001
MICA*002	B*5301	0.01451	0.0192	0.6671	<0.001
MICA*002	B*5801	0.01451	0.0183	0.5728	<0.01
MICA*004	B*4101/02	0.01451	0.0189	0.5213	<0.001
MICA*004	B*4403	0.08123	0.0621	0.9204	<0.001
MICA*004	B*4901	0.04522	0.0373	0.8134	<0.001
MICA*008	B*0702	0.09451	0.0696	0.9981	<0.001
MICA*008	B*0801	0.04522	0.0387	1.0000	<0.001
MICA*008	B*1302	0.02521	0.0222	0.9217	<0.001
MICA*008	B*4402	0.04747	0.0373	0.4519	<0.001
MICA*008	B*4001	0.02011	0.0218	0.9123	<0.001
MICA*008	B*5001	0.01451	0.0181	0.4524	<0.001
MICA*009	B*3503	0.01451	0.0172	0.4762	<0.001
MICA*009	B*5101	0.02521	0.0213	0.5767	<0.001
MICA*009	B*5201	0.02011	0.0207	0.6132	<0.001
MICA*010	B*1501	0.03350	0.0394	0.2816	<0.001
MICA*015	B*4501	0.02521	0.0261	0.4084	<0.001
MICA*016	B*3502	0.01451	0.0177	0.7240	<0.001
MICA*016	B*1402	0.01451	0.0183	0.5628	<0.001
MICA*016	B*1501	0.01451	0.0174	0.4454	<0.001
MICA*016	B*1503/10/16	0.01451	0.0186	0.6145	<0.01
MICA*017	B*5701	0.01451	0.0192	0.3271	<0.001
MICA*018	B*1801	0.01451	0.0181	0.6712	<0.001

HLA, human leukocyte antigen; MICA, major histocompatibility complex class I-related chain A.

Our data show moderately high MICA diversity in our population. The observed MICA polymorphism, however, has limited variation across racial/ethnic groups, with little evidence of active microevolution occurring after the separation of racial groups, which has been clearly observed at the HLA loci. Although 19 MICA alleles were detected in our population, a large number of these alleles were found only sporadically (11 alleles had a frequency of <1%). The same group of three alleles (MICA*008, *002, and *004) accounted for more that 56% of the allele frequencies in our population, and these alleles have also been commonly observed in other populations [22,23,25,32]. Individual MICA alleles that have high frequencies showed multiple relationships with HLA-B alleles, whereas individual HLA-B alleles, including the common B*0702 and *0801 alleles, were generally observed with only a single MICA association. This "one-way" relationship suggests that the common alleles MICA are very old, predating major branches of HLA-B alleles, as suggested by Gao *et al*. [22]. For example, MICA*001 and *018 are identical except for a nonsynonymous mutation in exon 3, codon 125, with MICA*018 retaining the MICA consensus codon 125. The two alleles are both linked to B*1801, indicating that MICA*001 might have been generated from MICA*018 through a point of mutation, as previously suggested [22].

On the other hand, HLA-B*15 (*1501, *1503, *1510, and *1516) encoding alleles displayed stable MICA association patterns with MICA*016 in our population. However, B*15 alleles have been associated with MICA*010 in other populations [25]. In our population, the MICA*010-B*1501 haplotype only was observed.

Conversely, linkage relationship between MICA and HLA-B genes also reflect the evolutionary history of HLA-B alleles. For example, HLA-B*35, *53, and *58 alleles share a high degree of sequence homology and are considered to have been generated from the same progenitor allele [22]. The fact that they are all linked with the same MICA*002 allele, consistently detected both in the present work and in previously reported populations around the world [22,28,35,40], further supports the notion of a common ancestry.

In addition, individual MICA alleles also tend to associate with serologic HLA-B groups. One of the few exceptions is B*44, where the two more common B*44 subtypes, B*4402 and B*4403, are found to be exclusively associated with different MICA alleles (MICA*008 and *004, respectively). These observations may reflect the long and distinctive evolutionary histories of the two B*44 alleles and their extended MHC haplotypes, even though they still share the same serologic determinants, as previously suggested [22]. These data confirm the observation that a common evolutionary origin could be more related to the primary structure of alleles than to serologic similarities. Indeed the B*4404 subtype was also observed with MICA*004 allele in our population (data not shown).

These data are of interest because it has been reported that the allelic diversity at the MICA locus affects binding

between the MICA and NKG2D pairs, potentially affecting natural killer cell activation and the modulation of T-cell responses [7,41]. Alleles at the MICA locus can be defined as strong or weak binders on the basis of their capacity to bind NKG2D, which has been suggested to be attributed to the methionine versus valine aminoacid substitution in position 129 of the MICA protein. The strong NKG2D binding alleles share methionine in position 129, whereas weak binding alleles have valine in this position. In this regard, high-affinity alleles include MICA*001, *002, *007, and *017, whereas low-affinity alleles include MICA*004, *006, *008, *009, and *010. Although the significance of high versus low affinity for NKG2D in terms of immune activation is unclear, knowledge of specific haplotypes may provide valuable information with regard to modern population ancestries, admixtures, and the selective pressures maintaining such linkages. These data may be important in future studies of the potential role of MICA in allogenic stem cell and solid organ transplantation, in addition to tumoral, infectious and autoimmune disease susceptibility in Caucasian populations [13,15,31,42]. In addition data may be useful for future comparisons with other populations in our geographic area. MICA is also an important candidate gene for a number of clinically significant disease conditions including diabetes as well as rheumatoid and other autoimmune diseases [16]. It is increasingly clear that knowledge of population-specific allelic and haplotypic structure is critical for genotype–phenotype association studies [35,43].

In conclusion, our data indicate that the allelic diversity in our Murcian population is similar to that in other Caucasian populations, although we found a series of alleles that are less frequent, in addition to other, as-yet-undescribed haplotypic associations in other populations from Caucasian origin. Thus the data will inform future studies not only in anthropology but in donor–recipient matching in clinical transplantation and linked disease associations.

Acknowledgments

This work was supported by the 05748/PI/07 Project from Foundation Seneca of the Murcia region and from CIBER*ehp* (FIS), Ministerio de Sanidad y Consumo, Spain. The authors also thank Maria J. Sanchis and Jose M. Alemany for help in processing of the samples in this study.

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