Human Immunology 77 (2016) 1187–1193

Contents lists available at ScienceDirect

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

MICB gene diversity and balancing selection on its promoter region in Yao population in southern China

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ARTICLE INFO

Article history:
Received 17 March 2016
Revised 9 August 2016
Accepted 30 August 2016
Available online 31 August 2016

Keywords:
MICB
Gene diversity
Haplotype
Balancing selection

ABSTRACT

To comprehensively examine the MICB gene polymorphism and identify its differences in Chinese Yao population from other ethnic groups, we investigated the polymorphism in the 5′-upstream regulation region (5′-URR), coding region (exons 2–4), and the 3′-untranslated region (3′-UTR) of MICB gene by using PCR-SBT method in 125 healthy unrelated Yao individuals in Guangxi Zhuang Autonomous Region. Higher polymorphism was observed in the 5′-URR, nine single nucleotide polymorphisms (SNPs) and a two base pairs deletion at position −139/−138 were found in our study. Only five different variation sites, however, were detected in exons 2–4 and three were observed in the 3′-UTR. The minor allele frequencies of all variants were greater than 5%, except for rs3828916, rs3131639, rs45627734, rs113620316, rs779737471, and the variation at position +11803 in the 3′-UTR. The first nine SNPs of 5′-URR and rs1065075, rs1051788 of the coding region showed significant linkage disequilibrium with each other. Ten different MICB extended haplotypes (EH) encompassing the 5′-URR, exons 2–4, and 3′-UTR were found in this population, and the most frequent was EH1 (23.2%). We provided several evidences for balancing selection effect on the 5′-URR of MICB gene in Yao population.

1. Introduction

Human major histocompatibility complex class I chain-related B (MICB) molecule is a stress-inducible ligand for NKG2D, the activating receptor, expressed on the NK cells, CD8αβ T cells, and γδT cells, which plays an important role in innate immunity [1,2]. At the transcriptional level, MICB seems to be widely expressed, but the expression of MICB gene is highly restricted in normal tissues in order to prohibit the activation of immune cells expressing NKG2D receptors [3,4]. Up-regulation of MICB gene expression can be induced by certain stimuli such as viral infection [5], cancer [6], and heat shock [7]. Up-regulation of gene expression and high soluble serum level for MICB may contribute to disease progression and present an attractive target for the treatment of cancer or infection [8–12].

MICB gene is located 141.2 kb centromeric to HLA-B gene, with a full length of 12,930 bp [13,14]. It is composed of six exons that encode the leader peptide (exon 1), three extracellular α1, α2, and α3 domains (exons 2–4), transmembrane region (exon 5), and the cytoplasmic tail (exon 6) [14]. A limited MICB gene polymorphism has been observed, with only 42 different alleles officially named so far (http://hla.alleles.org/data/micb.html, Release 3.24.0). Most of MICB gene SNPs are either exonic or synonymous, yielding 28 unique protein sequences (two null alleles: MICB*009N and MICB*021N). However, higher degree of polymorphism is observed in the 5′-upstream regulation region (5′-URR) and 3′-untranslated region (3′-UTR). The 5′-URR contains various regulatory elements such as heat shock elements (HSE), a CCAAT box, and a GC box [15–17]. The polymorphism of 5′-URR and 3′-UTR of MICB gene may be involved in the regulation of MICB gene expression. Rodriguez-Rodero et al. demonstrated that rs3842620 (a deletion of 2 base pairs at position −139/−138: CT/−) results in a remarkable down-regulation of MICB gene expression as this deletion decreased the transcriptional activity [15]. The 3′-UTR of MICB is usually a selective target for miRNAs, such as HCMV-miR-UL112, miR-10b, miR-20a, and miR-93 [18–20]. These miRNAs specifically reduce MICB expression by binding to 3′-UTR of MICB mRNA.

The Yao ethnic group, with a population of about 3 million, is one of the 55 ethnic minorities in China. They mainly live in Guangxi Zhuang Autonomous Region. Data on MICB polymorphism

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http://dx.doi.org/10.1016/j.humimm.2016.08.015
0198-8859/

in different populations is accumulating [21–30], however, there is a lack of information regarding MICB genetic variation in Yao ethnic population. In this study, we investigated the polymorphism of the 5′-URR, exons 2–4, and 3′-UTR of the MICB gene and its haplotype structure in Yao population.

2. Materials and methods

2.1. Subjects

A total of 125 healthy unrelated individuals of Yao ethnic minority were collected from Guangxi Zhuang Autonomous Region, southern China. Each subject received an interview on their overall health conditions and body functional abilities. Subjects without significant illness, such as cancer, chronic diseases, and genetic association diseases, were considered as healthy. At least three generations of each participant were Yao ethnic minority. Review Committee for Medical Institution of local authorities approved the protocol of our study, and all blood samples were taken with each individual informed consent.

2.2. MICB 5′-URR, exons 2–4, and 3′-UTR sequence based typing

An 886 bp MICB 5′-URR and exon 1 fragment was amplified using the primers as previously published [31] and sequenced by using the reverse primer for analysis of positions −588 to −1 (positions in accordance with what was described in IMGT/HLA database). For samples with deletion variation, sequencing was performed by using both direction primers. Exons 2–4 of MICB coding region were amplified and sequenced using the methods as described in our previous studies [24,25]. The 3′-UTR fragment of MICB gene was amplified and sequenced based on the methods by Pan [32].

2.3. Statistical analysis

Arlequin 3.5 (http://cmpg.unibe.ch/software/arlequin35/) [33] was used to compute allele frequencies, Hardy-Weinberg tests for each variation site and the Ewens-Watterson homozygosity test. Pairwise global linkage disequilibrium (LD) analysis was evaluated by a likelihood ratio test using Arlequin 3.5 as well. Given the position association but unknown gametic phase of the genotype data, the probable haplotype constitution of each sample was estimated by a bayesian statistical method using PHASE 2.1 [34]. The parameters used in PHASE 2.1 were as follows: (1) number of interactions: 1000, (2) thinning interval: 1, and (3) burn-in value: 100. The reliable performance for haplotype estimation was evaluated by the mean probability of the inference, which was defined as average of the probabilities of the haplotype pair with the highest probability value for each sample [35]. Tests on nucleotide diversity, haplotype diversity as well as Tajima's D, Fu, and Li's neutrality were performed by using DNAsp 5.0 (http://www.ub.edu/dnasp) [36]. Nei's standard genetic distances

Table 1
Frequencies and *p* values of Hardy-Weinberg test of 18 variation sites in MICB gene.

SNP				Frequency		
Gene region	Position	rs number	Allele	(2n = 250)	MAF[a]	HWE
5′-Upstream regulation	−481	rs2534673	C	0.196	0.267	0.566
			G	0.804		
	−413	rs2534672	C	0.196	0.267	0.567
			G	0.804		
	−334	rs6915833	T	0.220	0.152	0.606
			C	0.780		
	−310	rs2534671	T	0.244	0.338	1.000
			G	0.756		
	−245	rs3828912	C	0.476	0.495	0.591
			A	0.524		
	−176	rs3828913	A	0.224	0.219	1.000
			C	0.776		
	−152	rs3828914	C	0.476	0.495	0.593
			T	0.524		
	−139	rs3842620	Deletion	0.196	0.267	0.567
			CT	0.804		
	−72	rs2516498	C	0.232	0.157	0.128
			G	0.768		
	−56	rs3828916	G	0.048	0.071	1.000
			C	0.952		
Coding region (exons 2–4)	+7576	rs3131639	A	0.044	0.071	1.000
			G	0.956		
	+7591	rs1065075	G	0.196	0.267	0.567
			A	0.804		
	+8029	rs1051788	A	0.196	0.267	0.567
			G	0.804		
	+8914	rs45627734	A	0.004	0.024	1.000
			G	0.996		
	+9086	rs41273040	A	0.180	0.081	0.231
			G	0.820		
3′-Untranslated region	+11800	rs113620316	G	0.044	–	1.000
			A	0.956		
	+11803	rs-	AA	0.044	–	1.000
			Deletion	0.956		
	+12677	rs779737471	Deletion	0.004	–	1.000
			TATTA	0.996		

rs- representing the 2-bp deletion at position +11803, which has not been listed in the SNP database.
[a] The MAF (minor allele frequencies) for MICB SNPs in Han population in southern China from the 1000 genome project.