Immunology Letters 176 (2016) 65–71

Contents lists available at ScienceDirect

Immunology Letters

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

HLA-G coding region and 3′untranslated region (3′UTR) in two Chinese Han populations

Wen Yi Wang[a], Wei Tian[a,b,*], Xue Xiang Liu[a], Li Xin Li[b]

[a] *Immunogenetics Research Group, Department of Immunology, College of Basic Medical Sciences, Central South University, Changsha, Hunan, China*
[b] *Laboratory of Cellular and Molecular Biology, College of Basic Medical Sciences, Central South University, Changsha, Hunan, China*

ARTICLE INFO

Article history:
Received 10 March 2016
Received in revised form 2 May 2016
Accepted 30 May 2016
Available online 2 June 2016

Keywords:
Sequence-based typing
Linkage disequilibrium
3'Untranslated region
Chinese Han population
HLA-G

ABSTRACT

In this study, exons 2–4 and 3′untranslated region (3′UTR) of human leukocyte antigen (HLA)-G gene were investigated for 201 and 104 healthy unrelated Han samples recruited from Hunan Province, southern China and central Inner Mongolia Autonomous Region, northern China, respectively, using sequence-based typing and cloning methods. Totally 12 *HLA-G* alleles in the coding region, 9 variable sites in 3′UTR, 8 3′UTR haplotypes and 15 *HLA-G* extended haplotypes (EHs) incorporating the coding region and 3′UTR were observed. Very strong linkage disequilibrium (LD) was observed between *HLA-A* and *HLA-G*, and between *HLA-G* coding region and 3′UTR in each population (all global $P = 0.0000$). Seven *HLA-A-G* haplotypes showed significant LD in both populations. Three *HLA-G* alleles in the coding region, 4 polymorphic sites in the 3′UTR, 3 3′UTR haplotypes and 4 *HLA-G* EHs differed significantly in their distributions between the 2 Chinese Han populations (all $P \leq 0.0001$). There was evidence for balancing selection acting on *HLA-G* 3′UTR positions +3010, +3142 and +3187 in the two populations. The NJ dendrograms demonstrated the existence of two basic *HLA-G* lineages and indicated that, *HLA-G*01:01:01*, the most common *HLA-G* allele, formed a separate lineage from other alleles. Our results shed new lights into *HLA-G* genetics among Chinese Han populations. The findings reported here are of importance for future studies related to post-transcriptional regulation of *HLA-G* allelic expression and the potential role of *HLA-G* in disease association in populations of Chinese ancestry.

© 2016 European Federation of Immunological Societies. Published by Elsevier B.V. All rights reserved.

1. Introduction

The gene encoding human leukocyte antigen (HLA)-G maps about 110 kb telomeric to the HLA-A locus on chromosome 6p21.3 and belongs to the family of non-classical HLA class I genes [1]. It is preferentially expressed at the feto-maternal interface during pregnancy, attenuating the maternal immune response against the fetus [2]. Under physiological conditions HLA-G gene is expressed in adult immune privileged organs such as cornea and in cells of the hematopoietic lineage such as erythroblasts [3,4]. HLA-G molecule suppresses a wide range of immune responses by binding to inhibitory receptors [5,6]. HLA-G ectopic expression is observed in various types of malignancies, facilitating the escape of tumor cells from immune surveillance [7].

Currently, 51 HLA-G alleles are officially named (http://hla.alleles.org/data/txt/g_nuc.txt) [8]. Despite the relatively limited variability in the *HLA-G* coding region, abundant polymorphisms exist in the 5′ upstream regulatory region (URR) and 3′ untranslated region (UTR) of the *HLA-G* gene [9,10]. Variable sites in the *HLA-G* 3′UTR, particularly the 14-bp insertion/deletion dimorphism (Indel) and +3142C/G single nucleotide polymorphisms (SNPs), are believed to affect *HLA-G* expression through modifying mRNA stability or disrupting microRNA binding sites [11,12]. Sequence variations located at *HLA-G* coding region or *HLA-G* 3′UTR are associated with a variety of diseases such as cervical squamous cell carcinoma, preeclampsia and transplantation outcome [13–15].

Previous studies on *HLA-G* genetics in Chinese populations have focused on the 14-bp insertion/deletion dimorphism [16,17; among others]. In this study, we performed sequence-based typing combined with cloning to determine the allelic distributions of the *HLA-G* coding region (exons 2–4) and 3'untranslated region (3′UTR) in two Chinese Han populations recruited from southern and northern mainland China, respectively.

* Corresponding author at: Department of Immunology & Laboratory of Cellular and Molecular Biology, College of Basic Medical Sciences, Central South University, Changsha, Hunan, China.
E-mail addresses: tianwei6@csu.edu.cn, tianwei3@yahoo.com (W. Tian).

http://dx.doi.org/10.1016/j.imlet.2016.05.017
0165-2478/© 2016 European Federation of Immunological Societies. Published by Elsevier B.V. All rights reserved.

2. Materials and methods

2.1. Subjects

The first group comprised 201 local residents of Han ethnicity in the Hunan Province, southern China [16,18] (abbreviated as HNH). This sample panel comprised 59.2% men (119/201) and 40.8% women (82/201). The age of HNH samples ranged from 30 to 75 (45.4 ± 6.5 years). The second group consisted of 104 Han individuals recruited from Baotou Prefecture, central Inner Mongolia Autonomous Region, northern China [16] (abbreviated as IMH). The IMH group comprised 40.38% men (42/104) and 59.62% women (62/104). The age of IMH samples ranged from 18 to 82 (45.0 ± 10.5 years). Ethnicity and ancestry was reported by each participant. A blood sample was taken with each person's informed consent. All protocols were approved by the Institutional Review Committee of local authorities.

2.2. Typing of HLA-G coding region

Polymerase chain reaction-sequence-based typing (PCR-SBT) and cloning was used to examine exons 2, 3 and 4 of *HLA-G* gene. Because polymorphisms have been documented in the area of *HLA-G* sequence (http://www.ncbi.nlm.nih.gov/gene/3135), where most of the previously reported PCR or sequencing primers were located [19,20], degenerate base was introduced into our primers to avoid any possible allelic drop-out. The sequence and location of each primer, as well as the nucleotide position of each exon are given in Table 1. PCR was carried out in a 40 μl volume, containing 1X PCR buffer, 200 μM dNTP, 10 pmol of each primer, 1 unit of Taq DNA polymerase (Promega, Shanghai, China) and 150 ng of genomic DNA. Amplification was performed using a MyCycler (Bio-Rad).The reaction mixture was subjected to 1 cycle of denaturation at 95 °C for 2 min, followed immediately by 30 cycles of 95 °C for 1 min, 63 °C for 1 min (exon 2), or 66 °C for 1 min (exon 3), or 56 °C for 30 s (exon 4) and 72 °C for 1 min, and a final extension at 72 °C for 5 min before cooling to 15 °C. The PCR product was purified using the AxyPrep™ DNA Gel Extraction kit. Sequencing was performed using the sequencing primers listed in Table 1 and the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) on an ABI 3730XL DNA analyzer. The sequence chromatograms were analyzed using Chromas Lite 2.01 with raw sequencing data manually reviewed and heterozygous positions annotated using IUB (Degenerate Bases) code as previously described [21]. To assign each *HLA-G* genotype, the following strategy was used (i) the haplotypic phase of variable sites in each exonic segment was determined on the basis of *HLA-G* gene sequence database (http://hla.alleles.org/data/txt/g_nuc.txt); (ii) any potential sequence variation in homozygous state was searched for by blastn analysis of both DNA stretches against an EMBL-ENA Release database; (iii) for sequence chromatogram with ambiguous *HLA-G* allele combinations, the maximum likelihood genotype was determined using both the expectation-maximization (EM) algorithm as implemented in the program ARLEQUIN3.5 [22] and the Bayesian statistical method as implemented in program PHASE 2.1 [23], taking all of the variable sites and all of the samples in the study population into consideration. Any samples with discordant genotype probability yielded by the two programs were further PCR-cloned using a PUCM-T cloning kit (Sangon Biotech, Shanghai, China) and sequenced to determine the haplotypic phase of SNPs in the *HLA-G* genomic segment of interest; and (iv) for samples showing an unexpected association with *HLA-A*, the *HLA-G* typing was repeated in at least one additional PCR-SBT.

2.3. Typing of HLA-G 3′UTR

We chose the genomic interval from nucleotide position +3703 to +4057 (aligned with *HLA-G* consensus sequence under accession number J03027) as the *HLA-G* 3′UTR for genetic analysis, reasoning that transcription of the *HLA-G* sequence into messenger RNA (mRNA) terminates at +4057 (http://www.ncbi.nlm.nih.gov/nuccore/NC_000006.12?report=genbank&from=29826979&to=29831122).This segment was amplified by PCR using the sense primer (5′-3′:ACATAgCTgTgCTATgAggT) and antisense primer (5′-3′:CCCCATCTACTCTCCTCTCC). The sense primer corresponds to the genomic segment from nucleotide position +3586 to +3605 and the antisense primer is complementary to genomic segment from nucleotide position +4094 to +4113, both aligned with J03027. The PCR amplicon is 528-bp in length when there is a 14-bp deletion in the 3′UTR. PCR was carried out in a 40 μl volume, containing 1X PCR buffer, 200 μM dNTP, 16 pmol of each primer, 1 unit of Taq DNA polymerase (Promega, Shanghai, China) and 150 ng of genomic DNA. Amplification was performed using a MyCycler (Bio-Rad). The reaction mixture was subjected to 1 cycle of denaturation at 95 °C for 2 min, followed immediately by 30 cycles of 95 °C for 1 min, 62 °C for 45 s, and 72 °C for 45 s, and a final extension at 72 °C for 5 min before cooling to 15 °C.The PCR product was purified using the AxyPrep™ DNA Gel Extraction kit. Sequencing was performed using the PCR primers and the BigDye Terminator v3.1 cycle sequencing kit on an ABI 3730XL DNA analyzer. The sequence chromatograms were analyzed using Chromas Lite 2.01 with raw sequencing data manually reviewed and heterozygous positions annotated using IUB (Degenerate Bases) code as previously described [21]. Blastn analysis was then performed against the *HLA-G* consensus sequence (under accession number J03027) to search for potential sequence variation in homozygous state. The maximum likelihood haplotype of *HLA-G* 3′UTR was reconstructed using both the programs ARLEQUIN3.5 and PHASE 2.1. As for the *HLA-G* coding region, any samples showing discordant genotype probability between the two programs were further PCR-cloned and sequenced to determine the haplotypic phase of *HLA-G* 3′UTR.

2.4. Data of HLA-A locus

HLA-A data have been previously reported for the 2 populations [16,18]. Generic typing of *HLA-A* locus for the majority of the samples was performed by PCR-sequence-specific priming method (PCR-SSP), using a commercial typing kit (Genovision, Westchester, PA). A small portion of the samples were typed for *HLA-A* locus using the 12th International Histocompatibility Workshop ARMS-PCR protocol [24]; any apparent homozygote observed in this process was further confirmed using the Genovision typing reagents. The *HLA-A* frequencies were used here (with permissions from Elsevier Inc and John Wiley and Sons) to compute linkage disequilibrium (LD) with *HLA-G* alleles in our current work.

2.5. Statistical analysis

Comparisons of allele as well as haplotype frequencies between populations were carried out using program CLUMP (http://www.smd.qmul.ac.uk/statgen/dcurtis/software.html) [25]. Tests of Hardy-Weinberg equilibrium (HWE) and Ewens-Watterson homozygosity on *HLA-G* locus were performed using the program ARLEQUIN3.5. Before the inference of haplotype, a pairwise global linkage disequilibrium (LD) analysis that aims to characterize the total association between all of the haplotypes at two loci rather than a specific LD between two particular alleles at two loci, was performed using the program ARLEQUIN3.5. Due

Download English Version:

https://daneshyari.com/en/article/6116971

Download Persian Version:

https://daneshyari.com/article/6116971

Daneshyari.com