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HLA-G Genetic Polymorphism in 57 Portuguese White Families Studied by PCR-RFLP and PCR-SSOP

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FROM STUDIES on tissue-specific expression and interaction with T and NK cells, it was suggested that HLA-G may have an important role in immunologic tolerance.

The recognition of HLA-G polymorphism, its association with other classical HLA class I loci, and eventual variation among different populations may prove to be useful in understanding its immunologic functions and evolution of human populations.

Although the level of polymorphism in HLA-G has been considered low, it was hypothesised that large differences concerning the nature, site, and frequency of HLA-G polymorphisms do exist in different ethnic groups. In whites, the limited and conservative nature of nucleotide and amino acid substitutions, seems to diverge significantly from the changes found at the antigen-binding domain of black samples.[1,2]

The HLA-G locus also shows a strong and selective linkage *disequilibria* with most (but not all) HLA-A alleles.[3] This nonrandom association is due to the presence of a highly polymorphic region between the two genes, producing a true variation in physical distance of the A–G segment carried by different haplotypes.[4,5] The most common association found in all studied populations, A*2-G*01011, is probably the oldest A/G linkage disequilibrium present in human genome that is shared with other nonhuman primates.[3]

On the basis of this theoretical background, we studied

Table 2. HLA-G Allelic Frequencies

Alleles	Portuguese Whites (n = 117)	Whites[3] (n = 105)	Orientals[3] (n = 103)
G*01011	0.744	0.742	0.534
G*01012	0.627	0.590	0.291
G*01013	0.348	0.114	0.291
G*0103	0.023	0.038	0.000
G*0104	0.255	0.238	0.514

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Table 1. Primer and Probe Sequences Used for HLA-G Typing (PCR-RFLP and PCR-SSOP)

Oligonucleotides		Sequences (5′-3′)	Specificities
Primers	G23	CTG GGC CGG AGT TAC TCA CT	HLA-G exon 2 forward primer
	G25	TCC ATG AGG TAT TTC AGC GC	HLA-G exon 2 reverse primer
	G33	GGT ACC CGC GCG GCG CTG CAG CA	HLA-G exon 3 forward primer
	G35	CAC ACC CTC CAG TGG ATG AT	HLA-G exon 3 reverse primer
Exon 2 probes	G-2.1	GTG GAC TCG CAG TTC	HLA-G*0103
	G-2.2	GTG GAC GAC ACG CAG TTC	HLA-G*01011, 01012, 01013, 0102, 0104, 0105N
	G-2.3	GAG GGG CCA GAG TAT TGG	HLA-G*01012, 01013, 0104, 0105N
	G-2.4	GAG GGG CCG GAG TAT TGG	HLA-G*01011, 0102, 0103
Exon 3 Probes	G-3.1	TCC GAC GGT CGC CCC CT	HLA-G*01013
	G-3.2	TCC GAC GGA CGC CTC CT	HLA-G*01011, 01012, 0102, 0103, 0104, 0105N
	G-3.3	GAA CGA CTG CGC TCC	HLA-G*0105N
	G-3.4	AAC GAG GAC CTG CGC TCC	HLA-G*01011, 01012, 01013, 0102, 0103, 0104
	G-3.5	CGC CTC ATC CGC GGG TAT	HLA-G*0104
	G-3.6	CGC CTC CTC CGC GGG TA	HLA-G*01011, 01012, 01013, 0102, 0103, 0105N

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Table 3. HLA-G*0105N Allelic Frequencies in Different Populations

Population	Frequency	Reference
Portuguese whites	0.000	This study
Spanish	0.061	9
American blacks	0.074	2
American Hispanics	0.029	2
American whites	0.000	2
Germans and Croatians	0.023	1
Danish	0.006	1

the HLA-G polymorphism in Portuguese white families, evaluated the level of association between HLA-G and HLA-A alleles, assigned extended MHC haplotypes, and compared results with studies from other populations.

MATERIALS AND METHODS

Samples

The present study was performed with a sample of 135 individuals, belonging to 57 families, previously typed for HLA-A, -B, -Cw, and -DRB1 loci by PCR-SSOP and/or ARMS-PCR SSP. These families attended the Centre of Histocompatibility to evaluate identical siblings for bone marrow transplantation.

Genomic DNA was isolated from EDTA anticoagulated blood by a salting-out procedure.[6]

PCR Amplification

The analysis of polymorphic differences in the HLA-G locus was performed by RFLP and SSOP typing methods, upon PCR amplification of exons 2 and 3, using locus-specific primers (Table 1).

Fifty microlitres PCR reaction mixtures consisted of: 67 mmol/L Tris-HCl (pH 8.8 at 25°C) (Sigma, St Louis, Mo); 16 mmol/L $(NH_4)_2SO_4$ (Sigma); 7.5% glycerol (BDH Chemicals, Poole, UK); 2 mmol/L $MgCl_2$ (BDH); 250 μmol/L each of dATP, dCTP, dGTP, dTTP (Pharmacia Biotech); 133 μg/mL cresol red (Sigma); 200 ng DNA; and 0.5 U Biopro DNA polymerase (Bioline, London, UK). PCR amplifications were carried out in a GeneAmp PCR 9600 system (Perkin-Elmer Cetus, Norwalk, Conn). A touchdown amplification method was used with the following conditions: a first denaturation step at 96°C for 1 minute; and five cycles of a three-step cycling program (25 seconds at 96°C, 45 seconds at 70°C and 30 seconds at 72°C), followed by 21 cycles (25 seconds at 96°C, 45 seconds at 65°C and 30 seconds at 72°C), and four times (25 seconds at 96°C, 1 minute at 55°C and 2 minutes at 72°C), with a final extension of 10 minutes at 72°C.

Table 5. HLA-G, -A, -Cw Haplotype Frequencies

Haplotypes	Portuguese
G*01011-A*02-Cw*0501	0.063
G*01011-A*03-Cw*15	0.042
G*01012-A*01-Cw*0602	0.042
G*01012-A*68-Cw*07	0.042

Restriction Fragment Length Polymorphism (RFLP) Typing

Amplified DNA was digested with *Msp*I, *Hinf*I, and *Acy*I endonucleases,[5] according to the manufacturer's recommendations (Boehringer Mannheim, Germany). Electrophoretic separation was done in a 2% NuSieve agarose gel (FMC, Rockland, Me) and visualised under UV light after ethidium-bromide staining. The resulting banding pattern allowed the detection of four HLA-G alleles, G*01011, G*01012, G*01013, and G*0103.

Sequence-Specific Oligonucleotide Probe (SSOP) Typing

Five-microlitre aliquots of PCR products were immobolised onto replicate nylon membranes. After a prehybridisation step of 30 minutes at 42°C, hybridisation with digoxigenin-11-ddUTP-labelled probes (Table 1) was performed at 42°C for 40 minutes, followed by stringency washes at empirically determined optimal temperatures. Hybrids were detected by alkaline-phosphatase (AP) reaction after conjugation with AP-antidigoxigenin (Boehringer Mannheim), using a chemiluminescent substract (CDP Star, Boehringer Mannheim) followed by X-ray film exposure.[7]

Controls

Reference DNA-typed samples, kindly provided by A. Arnaiz-Villena (Madrid), were included for the correct interpretation of SSOP patterns.

Nomenclature

HLA-G alleles were designated according to the WHO Nomenclature Committee for factors of the HLA system.[8]

RESULTS AND DISCUSSION

When compared with the values reported for white populations,[3] the HLA-G allele frequencies registered in our

Table 4. HLA-A/G Haplotype Frequencies in Different Populations

Haplotypes	Portuguese	Spaniards[3]	Chinese[3]	Japanese[3]	Asian Indians[3]
A2-G*01011	0.196	0.178	0.214	0.171	0.183
A1-G*01012	0.109	0.093	—	—	—
A24-G*01012	0.087	—	—	—	—
A24-G*0104	0.000	0.076	—	—	—
A11-G*01013	0.065	0.034	—	0.300	—
A33-G*0103	0.000	0.017	0.142	0.042	0.200

Table 6. HLA-G and Ancestral Haplotypes (AH)

AH	HLA-G	HLA-A	HLA-Cw	HLA-B	HLA-DRB1
18.1	01012	25	1203	18	15
18.2	01011	02	0501	18	03
51.1	01012	01	14	51	04
51.2	01013	11	0702	51	04

Alleles found in this study associated with commonly described ancestral haplotypes (AH) are underlined.

sample were not significantly different (Table 2) HLA-G*0101 is the most frequent allele, followed by G*0104, and, with smaller values, by G*0103. HLA-G*0105N was not found in our sample. This null allele, recently reported in both American black[2] and Spanish[9] populations, has been found in several populations with different ethnic origins. In Caucasoid populations, it has been found in strong linkage disequilibrium with HLA-A*30. On the basis of this information, a common genetic background for the G*0105N allele in Spanish and central European populations was hypothesised, which probably introduced into the Spanish HLA pool by Arab invaders in the eighth century.[10] The decreasing allelic prevalence with increasing geographic distance from Spain is consistent with a northwestward dissemination of the mutation through migration and admixture of populations (Table 3).[1]

The absence of the G*0105N allele in this study, contrasting with the frequency described in Spanish population, corroborates previous studies that suggested a certain degree of genetic isolation of Portuguese.[11] In addition, HLA-A*30, found in 2% of Portuguese whites,[12] was not present in these samples.

As it has been already reported for all studied populations, we found strong linkage *desequilibria* between HLA-G and HLA-A alleles. The strongest A/G associations observed were: G*1011-A02; G*01012-A03; G*01012-A01; G*01012-A24, and G*01013-A11 (Table 4).

The most common HLA-G, -A, -Cw haplotypes were HLA-G*01011-A*02-Cw*0501, HLA-G*01011-A*03-Cw*15, HLA-G*01012-A*01-Cw0602, and HLA-G*01012-A*68-Cw*07 (Table 5).

Nonclassical HLA genes, namely HLA-G, could allow a better definition of some MHC ancestral haplotypes (AH), providing, eventually, haplospecific markers. In this study, individuals carrying AHs were analysed in order to establish specific alleles that could contribute to understand the mechanisms of MHC recombination and evolution (Table 6).

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