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CD80 and *CD86* polymorphisms in populations of various ancestries: 5 new *CD80* promoter alleles

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ABSTRACT

CD80 and *CD86* are closely linked genes on chromosome 3 that code for glycoproteins of the immunoglobulin superfamily, expressed on the surface of antigen-presenting cells. These costimulatory molecules play essential roles for stimulation and inhibition of T cells through binding to CD28 and CTLA-4 receptors. In this study, *CD80* promoter and *CD86* exon 8 polymorphisms were analyzed to investigate the genetic diversity and microevolution of the 2 genes. We genotyped 1,124 individuals, including Brazilians of predominantly European, mixed African and European, and Japanese ancestry, 5 Amerindian populations, and an African sample. All variants were observed in Africans, which suggests their origin in Africa before the human migrations out of that continent. Five new *CD80* promoter alleles were identified and confirmed by cloning and sequencing, and *promoter 2* is most likely the ancestral allele. Nucleotide -79 is monomorphic in 4 Amerindian populations, where the presence of the -79 G allele is probably the result of gene flow from non-Amerindians.

1. Introduction

CD80 and *CD86*, also called *B7.1* and *B7.2*, are closely linked genes on chromosome 3 (3q21) that code for structurally related type 1 glycoproteins, members of the immunoglobulin superfamily. CD80 and CD86 are transmembrane molecules expressed on the surface of antigen-presenting cells (APC), and play essential roles in T-cell modulation. The first signal required for the activation of T cells is antigen specific, based on recognition of the human leukocyte antigen (HLA)/peptide complex on APC via the T-cell receptor. The best characterized costimulatory signal derives from the interaction between B7 molecules CD80 and CD86, on APC, and the CD28 receptor on the T cell. The interaction of B7 with the CTLA-4 receptor gives the negative regulatory signal, which promotes peripheral T-cell tolerance [1,2].

Although both CD80 and CD86 are able to costimulate T-cell proliferation, they exhibit different patterns of expression, biochemical features, and oligomeric states. CD86 is thought to be the major CD28 ligand, whereas CTLA-4 has a higher affinity for CD80 than for CD86 [3]. In addition, whereas CD80 favors a T helper 1 response, CD86 favors T helper 2 type cytokine production. They also have distinct intracellular domains and may mediate differential signal transduction in APC [4–6].

Allelic frequencies of genetic variants may differ within human populations. Patterns of linkage disequilibrium (LD) may also vary among populations with different evolutionary histories, especially between modern Africans and populations distantly related to them [7]. Comparing the diversity observed within continents, Africa usually has the highest levels of polymorphism and the lowest values of LD. In addition, populations that went through bottleneck events, such as Amerindians [8], usually exhibit the highest values of LD and the lowest genetic diversity [9].

Although *CD80* and *CD86* play a crucial role in the immune system, the polymorphisms analyzed in this study have been poorly investigated. In this work Africans, Guarani and Kaingang Amerindians, and Brazilians of Japanese, European, and mixed African and European ancestry were analyzed. The frequencies of *CD80* promoter and *CD86* exon 8 variants and the LD between them were described to access information about the diversity and evolution of these polymorphisms.

2. Subjects and methods

2.1. Population samples

A total of 1,124 individuals from 4 urban and 5 Native American populations were genotyped for *CD80* and *CD86* polymorphisms.

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The urban Brazilian population sample, composed of genetically unrelated healthy individuals from the general population, was collected in Curitiba, in the state of Paraná (25°25′S, 49°16′W). The population is admixed but still retains some structure regarding the ancestry of individuals. Therefore, the sample was subdivided according to predominant ancestry into European (EU-B, $n = 158$), mixed African and European (AF-B, $n = 274$), and Japanese (JA-B, $n = 101$). In 2 previous papers as well as unpublished data from our group, the admixture of our population was estimated using HLA class I and II as ancestry markers. EU-B and AF-B had 87 and 50% of European, 8 and 44% of sub-Saharan African, and 5 and 6% of Amerindian ancestry, respectively [10,11]. JA-B reported exclusively Japanese ancestry. Also, a sample of 16 genetically unrelated African individuals was collected in Curitiba: 7 from Guinea-Bissau, 7 from Angola, 1 from Ghana, and 1 from Cameroon.

The Amerindian populations were Guarani Kaiowá (GKW, $n = 161$) from Limão Verde (23°12′S, 55°06′W) and Amambai (23°06′S, 55°12′W), Guarani Ñandeva (GND, $n = 84$) from Amambai and Porto Lindo (23°48′S, 54°30′W), all from the state of Mato Grosso do Sul, and Guarani M'byá (GRC, $n = 89$) from Rio das Cobras (25°18′S, 52°32′W) in Nova Laranjeiras, in the state of Paraná. These 3 Guarani subgroups differ in various aspects of their culture. Kaingang were from Rio das Cobras (KRC, $n = 110$) and from Ivaí (24°30′S, 51°40′W) in Manoel Ribas, also in the state of Paraná (KIV, $n = 131$). Guarani populations are from the linguistic family Tupi-Guarani, which belongs to the Tupi branch, whereas Kaingang are from the linguistic family Macro-Jê, Jê branch. Although nowadays they live close to each other in many indigenous areas, they remain remarkably distinct in culture and genetic polymorphism. A common feature of the Kaingang and Guarani groups is the isolation from non-Amerindian populations. More detailed description of these populations can be found in previous publications [12,13].

The individuals gave their informed consent and the study was approved by the Committee for Ethics in Human Research from the Federal University of Paraná.

2.2. Genotyping

Genomic DNA was obtained from peripheral blood by salting-out [14] and phenol–chloroform–isoamyl alcohol [15] methods. Six single-nucleotide polymorphisms (SNPs), *g.-454C>A* (rs68180496), *g.-387T>C* (rs1880661), *g.-232G>A* (rs41271393), *g.-79G>C* (rs16829984), *g.-7T>C* (rs16829980), and *g.5C>A* (rs41271391); 1 indel (insertion/deletion polymorphism), *g.*-557_*-561insCATGA* (*-558ins*), in the promoter region of the *CD80* gene; and 1 SNP in exon 8 of the *CD86* gene, *g.*1057*G>A* (rs1129055), were analyzed by polymerase chain reaction (PCR) followed by hybridization with sequence-specific oligonucleotide probes.

A fragment encompassing 690 bp of the *CD80* promoter region was amplified with the following PCR conditions: 80 ng of genomic DNA, 1× PCR buffer, 0.2 mM dNTP, 0.4 μM of each primer, and 0.75 U of Tth DNA polymerase (Biotools, Madrid, Spain) in 40 μL of reaction mixture. PCR started with 95°C for 5 minutes; followed by 9 cycles of 95°C for 30 seconds, 60°C for 45 seconds, 72°C for 45 seconds, and 26 cycles of 95°C for 30 seconds, 65°C for 45 seconds and 72°C for 45 seconds; with a final step at 72°C for 10 minutes. A segment of 166 bp of exon 8 of the *CD86* gene was amplified in a 10-μL reaction mixture set up to contain 20 ng of genomic DNA, 1× PCR buffer, 0.2 mM dNTP, 0.4 μM of each primer, 3 mM $MgCl_2$, and 0.2 U of Platinum Taq DNA polymerase (Invitrogen Corporation, Carlsbad, CA). PCR conditions were 95°C for 3 minutes; followed by 34 cycles of 95°C for 15 seconds, 56.7°C for 30 seconds, and 72°C for 40 seconds; with a final step at 72°C for 10 minutes.

PCR products were denatured and cross-linked on a nylon membrane. Dot blot hybridization was performed with biotin-labeled probes as previously described [16]. Probes used in genotyping (Table 1) were designed based on the sequence of the human *CD80* and *CD86* genes (GenBank). Primers were described previously [17].

Table 1
Oligonucleotide probes and high-stringency washing temperatures optimized for detection of the *CD80* promoter and *CD86* exon 8 polymorphisms by PCR with sequence-specific oligonucleotide probes

	Sequence (5′–3′)	T (°C)
CD80		
c.-557_*-561insCATGA*	*del*: GGTGGCAACCTAGTT	46
	ins: TGG**CATGA**CAACTTA	42
c.*-454C>A*	C: TGTCCAG**C**GTGGCTG	48
	A: TGTCCAG**A**GTGGCTG	52
c.*-387T>C*	T: GGTGGTC**T**TGTGAGC	48
	C: GGTGGTC**C**TGTGAGC	48
c.*-232G>A*	G: ACACAGCAA**G**GCTAG	40
	A: ACACAGCAA**A**GCTAG	38
c.*-79C>G*	G: AAAAGCAA**G**TAGAAGAA	42
	C: AAAAGCAA**C**TAGAAGAA	42
c.*-7T>C*	T: TACTGAG**T**GAACTCA	36
	C: TACTGAG**C**GAACTCA	42
c.5C>A	C: CTCAAAC**C**CTCTGTA	38
	A: CTCAAAC**A**CTCTGTA	36
Control	TCACTCACAGGGGCT	42
CD86		
c.*1057G>A*	A: TGATGAA**A**CCCAGCG	48
	G: TGATGAA**G**CCCAGCG	50
Control	GACATCTTCATGCGA	48

2.3. Cloning and sequencing

The new *CD80* promoter alleles were confirmed by cloning and sequencing the segment encompassing the promoter polymorphisms, from nucleotide -637 to 24 (chr3: 119278409-119279098). PCR products were cloned in *Escherichia coli* DH5-α competent cells using the PTZ57R/T vector (InsTAclone, Fermentas, Ontario, Canada) or TOPO (Invitrogen), and *E. coli* cells were grown in Luria broth–agar medium (Invitrogen Corporation, Carlsbad, CA) containing ampicillin (200 μg/mL). Plasmid vectors were extracted using the QIAprep Spin Miniprep kit (Qiagen Inc., Duesseldorf, Germany). The sequencing reaction was performed using M13 or Universal primers and chromatograms were visually analyzed in Bioedit [18] to confirm the DNA sequences.

2.4. Statistical analysis

Allelic and genotypic frequencies were estimated by direct counting. Hardy–Weinberg equilibrium (HW) was accessed using a modified version of the Markov-chain random walk algorithm [19]. Haplotypic frequencies were obtained using the ELB algorithm. Comparisons of the allele frequencies between the population samples were performed using contingency tables in RXC [20]. The p value of 0.05 was adopted as the significance limit for all statistical tests. These analyses, the estimates of the LD parameters D', r^2, and p, and the analysis of molecular variance were performed using the Arlequin 3.1 software package [21]. The relationship between haplotypes was assessed with the median-joining algorithm in Network 4.516 software. The dendrogram was constructed with the neighbor-joining method [22] using the modified Cavalli–Sforza chord measure for genetic distances [23] in the DISPAN software [24].

3. Results and discussion

3.1. Polymorphisms

Allelic and genotypic frequencies are presented in Table 2. *CD80* alleles -557_*-561*del, -454*C, -232*G, -79*C, -7*T,* and *5*C* were the most common alleles in all populations. The *CD80 -387*C* allele was the most frequent in EU-B and in Amerindians, whereas in AF-B and JA-B the *-387*T* allele was the most common.

Nucleotide -79 in the *CD80* promoter is polymorphic all over the "Old World," as indicated by the presence of this SNP in Africans,