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Impact of population admixture on the distribution of immune response co-stimulatory genes polymorphisms in a Brazilian population

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ABSTRACT

Co-stimulatory molecules are essential in the orchestration of immune response and polymorphisms in their genes are associated with various diseases. However, in the case of variable allele frequencies among continental populations, this variation can lead to biases in genetic studies conducted in admixed populations such as those from Brazil. The aim of this study was to evaluate the influence of genomic ancestry on distributions of co-stimulatory genes polymorphisms in an admixed Brazilian population. A total of 273 individuals from the north of Brazil participated in this study. Nine single nucleotide polymorphisms in 7 genes (*CD28*, *CTLA4*, *ICOS*, *CD86*, *CD40*, *CD40L* and *BLYS*) were determined by polymerase chain reaction-restriction fragment length polymorphism. We also investigated 48 insertion/deletion ancestry markers to characterize individual African, European and Amerindian ancestry proportions in the samples. The analysis showed that the main contribution was European (43.9%) but also a significant contribution of African (31.6%) and Amerindian (24.5%) ancestry. *ICOS*, *CD40L* and *CD86* polymorphisms were associated with genomic ancestry. However there were no significant differences in the proportions of ancestry for the other SNPs and haplotypes studied. Our findings reinforce the need to apply AIMs in genetic association studies involving these polymorphisms in the Brazilian population.

1. Introduction

The development of an immune response depends on a complex network of cells and is essential to protect humans against infectious agents and the appearance of autoimmune diseases and tumors. T cells have a critical role in the development of the immune response however these cells require two independent signals for them to become completely activated. The first signal is triggered by the binding of the T cell receptor (TCR) to an antigenic peptide presented by a major histocompatibility complex molecule (MHC). The second signal is provided by co-stimulatory molecules; the binding of the CD28 receptor to CD80 and CD86 molecules is essential for the activation of T cells. However, another molecule called CTLA-4 can also bind to CD80 and CD86 molecules which, instead to providing a positive stimulation, exert a regulatory role by reducing the generated response. This process is crucial for homeostasis and immune tolerance [1].

Another stimulatory receptor expressed on the surface of T cells is called *ICOS*; the gene of this molecule is located close to the *CD28* and *CTLA4* genes in the 2q33 chromosomal region. The interactions between B cells and activated T cells, mediated by CD40/CD40L signaling, also indirectly acts on T cell activation, but this signaling

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pathway is critical to B lymphocyte activation and so, in the development of the humoral immune response. Another molecule, *BLyS*, expressed on the surface of T cells and also in soluble form, modulates the survival and proliferation of B cells through three different receptors: BR3, TACI and BCMA [2].

An adequate immune response must maintain a balance between the ability to respond to infectious agents and to suppress autoimmunity. Thus, polymorphisms associated with the modulation of gene expression of co-stimulatory molecules can influence the development of several diseases. In recent years, several studies have shown associations between polymorphisms in co-stimulatory genes and diseases [3–14]. However, studies in different populations have failed to reproduce the results [15–19]. One of the reasons may be due to variable allele frequencies in different populations, which result in a lack of statistical power. For example, geographical gradients in the distribution of *CTLA4* alleles have been well documented [20]. Population structure also has been presumed to cause many of the unreplicated disease-marker associations reported in the literature, particularly in admixed populations.

Brazil has one of the most diverse populations in the world resulting from five centuries of interethnic breeding between Europeans, Africans and Amerindians. It has been shown that due to the intense miscegenation of the Brazilian population, indicators of physical appearance, such as skin color, are poor indicators of genomic ancestry [21,22]. Some studies have shown that the distribution of pharmacogenetic polymorphisms in the Brazilian population is best characterized using ancestry informative markers (AIMs) instead of self-declaration of ethnicity [23,24]. In fact, nowadays it is recognized that ethnicity can be better studied with AIMs, which enable a better understanding of the relationship between the various ethnic components and the variability of these co-stimulatory genes. Thus, the objective of the present study was to describe the allele frequencies of nine SNPs distributed across seven co-stimulatory genes (*CD28*, *CTLA4*, *ICOS*, *CD86*, *CD40*, *CD40L* and *BLYS*) and assess the impact of Brazilian population admixture on the distribution of these polymorphisms using AIMs.

2. Materials and methods

2.1. Sample

The sample of this study was composed of 273 (175 men and 91 women) unrelated subjects from the town of Goianésia do Pará (03°50′33″ S; 49°05′49″W), located in the southeastern region of the State of Pará in the north of Brazil. All the participants signed informed consent forms. The project was approved by the Research Ethics Committee of the Medicine School in São José do Rio Preto (FAMERP 45992011). The DNA was extracted from peripheral blood samples using the Easy-DNA™ extraction kit (Invitrogen, California, USA).

2.2. Genotyping

The following SNPs were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP): rs35593994 and rs3116496 in the *CD28* gene; rs5742909 and rs231775 in the *CTLA4* gene; rs4404254 in the *ICOS* gene; rs1129055 in the *CD86* gene; rs3092945 in the *CD40L* gene; rs1883832 in the *CD40* gene and; rs9514828 in the *BLYS* gene. All PCR reactions were performed in a final volume of 25 μL containing 1× Buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 pmol of each primer and 0.5 U of Taq DNA Polymerase Platinum (Invitrogen, São Paulo, Brazil). Amplifications were made in a MasterCycler DNA thermal cycler (Eppendorf, Hamburg, Germany) under the following conditions: an initial step of 5 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at an annealing temperature depending on the primer and 1 min at 72 °C, and a final step of 10 min at 72 °C. The sequences of the primers as well as the annealing temperatures used in each reaction are shown in Table 1. The amplification products were viewed in agarose gel

Table 1
Location of SNPs, primers, annealing temperatures, restriction enzymes used for genotyping and length of fragments resulting from PCR-RFLP.

SNP	Gene (Chromosome region)	Location	Primer 5′–3′ (forward)	Primer 5′–3′ (reverse)	Annealing temperature (°C)	Restriction enxyme	Fragments length (pb)
rs35593994	*CD28* (2q33)	204570826	TTCTCATTCTGTTGCCCTGGC	CACCATCCCCTTAGGGCACAT	62	HinfI	G: 468 + 78 A: 546
rs3116496	*CD28* (2q33)	204594512	GAAACACCTTTGTCCAAGTC	CTCAATGCCTTCTGGGAAATC	52	AciI	T: 333 C: 193 + 140
rs5742909	*CTLA4* (2q33)	204732347	GGGATTTAGGAGGACCCTTG	GTGCACACACAGAAGGCACT	48	MseI	C: 244 T: 179 + 65
rs231775	*CTLA4* (2q33)	204732714	CTGAACACCGCTCCCATAAA	CACTGCCTTTGACTGCTGAA	50	BbvI	A: 215 G: 159 + 56
rs4404254	*ICOS* (2q33)	204819570	TTACCAAGACTTTAGATGCTTTCTT	GAATCTTTCTAGCCAAATCATATTC	55	AluI	T: 385 + 339 + 99 C: 339 + 289 + 99 + 96
rs1129055	*CD86* (3q21)	121838319	CTGTTCCAATGGCAACCTCT	GGTTGCCCAGGAACTTACAA	56	CviKI-1	G: 79 + 75 + 58 + 54 A: 154 + 58 + 54
rs3092945	*CD40L* (Xq26)	135729609	ATCTTCACAGCAACCTAC	CACTAAACTCAATGAAAGCC	56	LweI	T: 251 + 195 C: 446
rs1883832	*CD40* (20q12-q13.2)	44746982	GAAACTCCTGCGCGGTGAAT	GAAACTCCTGCGCGGTGAAT	56	StyI	C: 133 + 96 + 74 T: 207 + 96
rs9514828	*BLYS* (13q32-q34)	108921373	TGGCTCTTGTGTGATCAAGG	GCCTGGTCTCAGCTTTTCTG	50	MbiI	C: 162 + 48 T: 210

Chromosome positions were referred to the sequence of NCBI database (GRCh37).