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Rapid Communication

Low variability at the *HLA-E* promoter region in the Brazilian population

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ABSTRACT

Little information has been reported regarding the regulatory region of the *HLA-E* gene. Only a proximal segment (300 bp immediately before exon 1) has been described at IMGT/HLA database. We aimed to evaluate the genetic diversity of the promoter region by PCR amplification and DNA sequencing. We observed a pattern of sequencing interruption in the same position in all samples, suggesting the presence of a secondary structure hampering the DNA polymerase sliding during the sequencing process, which was confirmed after bioinformatics analysis. Considering the promoter region evaluated (nucleotide −440 to −1), only three variation sites were found, including one new variation site at position −104, and the concomitant deletions already described at positions −187 and −186. We concluded that the promoter region of the *HLA-E* gene presents few and rare variable sites in this population sample; however, the double-stranded branch formation hampered the evaluation of the distant promoter by conventional sequencing.

The Brazilian population is one of the most heterogeneous worldwide, as a result of five centuries of integration among individuals from three continents: the European colonizers, mainly Portuguese, the African slaves and the Amerindians [1]. Hence, Brazil may be considered a rich repository of genetic variation and an excellent sampling for the characterization of the general variability of any potentially polymorphic genomic region. For example, most of the polymorphic sites described worldwide for the Human Leukocyte Antigen-G (*HLA-G*) and -E (*HLA-E*) genes considering 14 populations of the 1000Genomes project (phase 1) were also detected in Brazilians [2–6].

The non-classical class I human leukocyte antigen (HLA) genes, represented by the *HLA-G*, *HLA-E* and *HLA-F* loci, are located within the human Major Histocompatibility Complex and present a very low degree of variation in spite of being neighbor of the most variable genes at the human genome (the classical class I HLA genes) [7].

The non-classical HLA-E molecule plays a role both as a modulator of natural killer (NK) cell activity by interacting with the CD94-NKG2A receptor in the innate immunity pathway [8] and as an antigen-presenting molecule triggering a specific immune response [9]. The CD94 glycoprotein is expressed on the membrane of most NK cells and on a subset of T lymphocytes, forming a heterodimer with the NKG2A/B, NKG2C, NKG2E and NKG2H glycoproteins. HLA-E interactions with such CD94 heterodimers can activate, inhibit, or have no effect on NK cell-mediated cytotoxicity and cytokine production [10].

HLA-E is the least polymorphic of all class I HLA genes, presenting a much more limited polymorphism than the classical loci and even other non-classical genes. A number of worldwide populations from all continents, including two West-African population samples [11], the French Southeastern, Congolese Teke and Tswa African pygmies [12], Chinese [13], Brazilian Southeastern [4] and others [14–17] have already been evaluated for the *HLA-E* gene variability and usually only two distinct molecules were detected, encoded by a series of coding alleles presenting synonymous or intronic mutations and just one non-synonymous polymorphism, supporting the idea of restrict polymorphism at *HLA-E* [18]. So far, only seventeen *HLA-E* alleles coding for six different proteins and a truncated one were recognized worldwide (The International Genetics Database [IMGT/HLA], database version 3.21.1).

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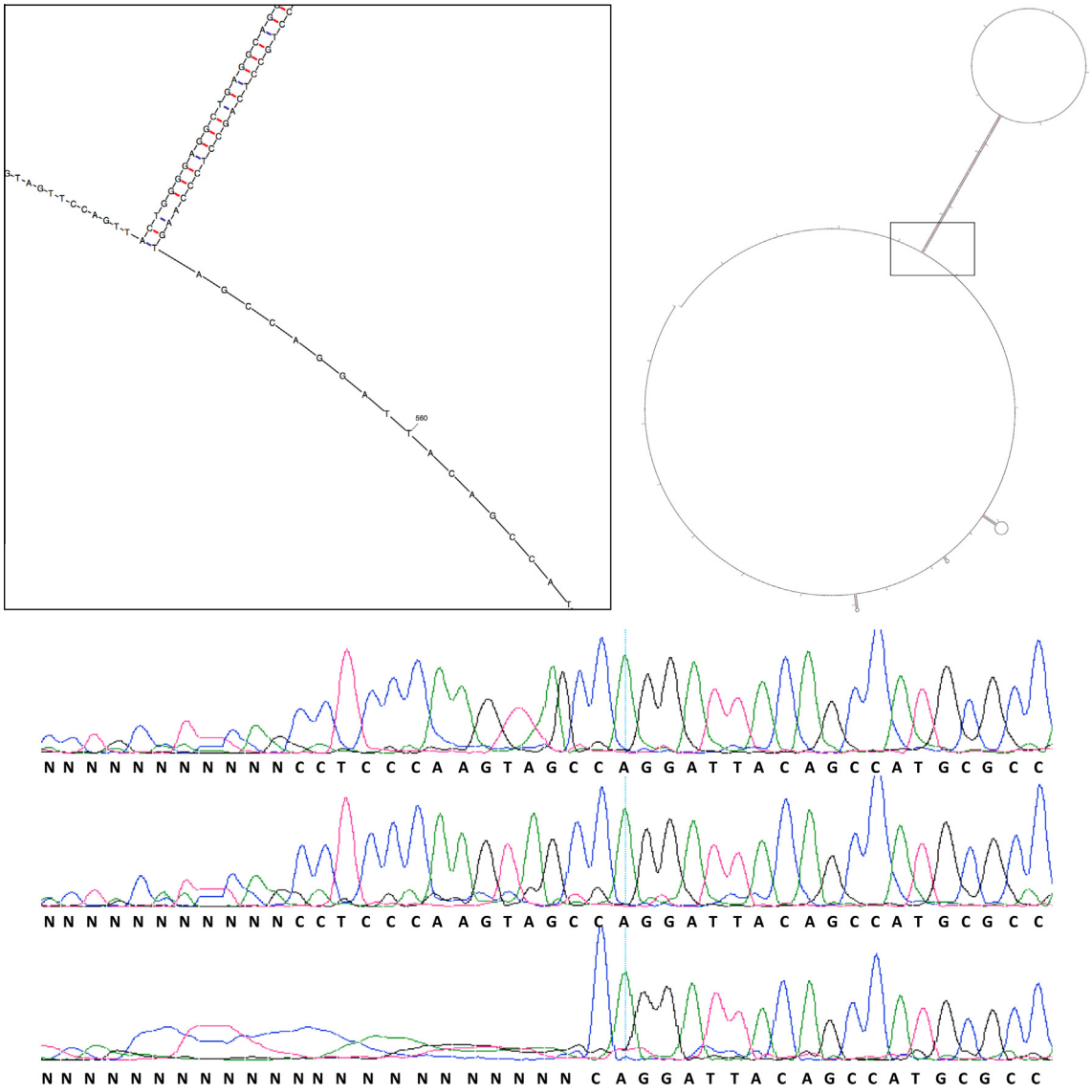

Fig. 1. Secondary structure of the *HLA-E* promoter region. The long annealed sequence shows that the molecule presents a repetitive and inverted sequence (Image generated by the mFold algorithm; Zuker, 2003 [25]). The large square is a zoom of the small square and do represent the exact point in which sequencing stops (bottom chromatograms).

Our group has evaluated the *HLA-E* coding region in the Brazilian population and found a low variability of this gene, corroborating what has been reported in other worldwide populations [4,5].

Little information has been reported in the literature regarding the *HLA-E* regulatory region. Only the proximal part (approximately 300 bp immediately before the beginning of exon 1) is considered by the IMGT/HLA database and is little polymorphic, with only two consecutive base deletions at positions −187 and −186 (considering the Adenine of the first translated ATG as +1), both detected in a single haplotype named as *E**01:01:01:02. In addition, this region has not been entirely evaluated in 10 of the *HLA-E* alleles (E*01:01:01:03, E*01:01:02, E*01:03:01:03, E*01:03:03, E*01:03:05, E*01:04, E*01:05, E*01:06, E*01:07 and E*01:08) (IMGT/HLA, database 3.21.1). Other HLA genes, such as the *HLA-G*, have already been evaluated and a high variability at the promoter region has been observed, with balancing selection increasing heterozigozity [19–21,2,6,22,23]. Few data has been reported regarding the promoter region of the HLA-E locus. In fact, only a recent study described the variability between nucleotides −118 and −1 in two African population samples [11]. Therefore, it is not clear if high heterozygosis at the regulatory regions is a characteristic of the non-classical genes or if it is restricted to *HLA-G*.

Considering that these molecules are potential natural immunosuppressors, the analysis of the variability and structure of the promoter region of *HLA-E* gene in an admixed population such as Brazilians can contribute for the understanding of the structure of its regulatory region and the mechanisms underlying its regulation. In this matter, we aim to evaluate the variability of the *HLA-E* promoter region in Brazilian samples.

A hundred and thirteen samples of healthy bone marrow donors from Ribeirão Preto, State of São Paulo, Brazil, with approximately 50% of men and women, and with no age restriction, were randomly selected and evaluated after approval of the local Ethics Committee (#9153/2008). These samples are expected to present ancestry contribution of 79% Europeans, 14% Africans and 7% Amerindians, according to previous studies conducted on similar population samples [24]. The *HLA-E* promoter region was amplified in a single PCR reaction, using primers EpromoF (5′-CCCGGCCTA TACGTTGTT-3′) (designed for this study) and HE01R (5′-CCTCTTA CCCAGGTGAAGCAGCG-3′) [16], producing an amplification