

Genetic Variability of Inflammatory Genes in the Brazilian Population

Marcelo dos Santos,¹ Elaine Stur,¹ Lucas Lima Maia,¹ Lidiane Pignaton Agostini,¹
Gabriela Tonini Peterle,¹ Suzanny Oliveira Mendes,¹ Eloiza Helena Tajara,²
Marcos Brasilino de Carvalho,³ Íuri Drumond Louro,⁴ and Adriana Madeira Álvares Silva-Conforti⁵

Inflammatory gene variants have been associated with several diseases, including cancer, diabetes, vascular diseases, neurodegenerative diseases, arthritis, and others. Therefore, determining the population genetic composition of inflammation-related genes can be useful for the determination of general risk, prognostic and therapeutic strategies to prevent or cure specific diseases. We have aimed to identify polymorphism genotype frequencies in genes related to the inflammatory response in the Brazilian population, namely, *IκBL* –62AT, *IκBL* –262CT, tumor necrosis factors alpha (*TNFα*) –238GA, *TNFα* –308GA, lymphotoxin-alpha (*LTα*) +80AC, *LTα* +252AG, *FAS* –670AG, and *FASL* –844TC, considering the white, black, and Pardo ethnicities of the São Paulo State. Our results suggest that the Brazilian population is under a miscegenation process at the current time, since some genotypes are not in the Hardy–Weinberg equilibrium. In addition, we conclude that the Pardo ethnicity is derived from a complex mixture of ethnicities, including the native Indian population.

Introduction

THE BRAZILIAN POPULATION is a remarkably heterogeneous population. When Brazil was discovered by the Portuguese in 1500, there were ~2 million native Indians living in the Brazilian territory. Since then, continuous migratory waves from different countries worldwide have brought together a wide variety of ethnicities that have ultimately composed the contemporary Brazilian population. Migratory waves included a constant influx of Portuguese, Africans, and Europeans who were brought to Brazil during the 19th and 20th centuries (Callegari-Jacques *et al.*, 2003).

The new genetic pool greatly contributed to a high degree of variability, directly affecting most genetic polymorphic traits, such as genes related to the inflammatory response. Among these, we can attribute special importance to tumor necrosis factors alpha (*TNFα*) (Warren, 1990), lymphotoxin-alpha (*LTα*) (Ruddle and Homer, 1988), nuclear factor kappa-B inhibitor-like (*NFκBIL1*, also known as *IκBL*) (Castiblanco and Anaya, 2008), and *FAS/FASL*, also known as *CD95/CD95L* (Müllauer *et al.*, 2001).

Polymorphic variants of inflammatory genes have been associated with several diseases, including cancer (Schwartz-

burd, 2003; Philip *et al.*, 2004), diabetes (Willerson and Ridker, 2004), vascular diseases (Gan *et al.*, 2004; Hansson, 2005), neurodegenerative diseases (Perry, 2004; Nagatsu and Sawada, 2005), arthritis (Arend and Gabay, 2004), and others. An accurate description of the population background of genes that are important to the inflammatory response can be useful for the determination of general risk, prognostic and therapeutic strategies to prevent or cure specific diseases.

The present study aims to identify single-nucleotide polymorphism (SNP) genotype frequencies in genes related to the inflammatory response in the Brazilian population (*IκBL* –62AT [rs2071592], *IκBL* –262CT [rs2071592], *TNFα* –238GA [rs361525], *TNFα* –308GA [rs1000629], *LTα* +80AC [rs2239704], *LTα* +252AG [rs909253], *FAS* –670AG [rs1800682], and *FASL* –844TC [rs763110]), considering the white, black, and Pardo (brown) ethnicities of the São Paulo State.

Materials and Methods

Ethics

This study was approved by the Committee of Ethics in Research of the Heliópolis Hospital on 10/06/2008 (CEP no. 622; 637) and an informed consent was obtained from all patients enrolled.

¹Programa de Pós Graduação em Biotecnologia, Universidade Federal do Espírito Santo, Vitória, Espírito Santo, Brazil.

²Departamento de Biologia Molecular, Faculdade de Medicina, São José do Rio Preto, São Paulo, Brazil.

³Laboratório de Biologia Molecular, Hospital Heliópolis, São Paulo, Brazil.

⁴Núcleo de Genética Humana e Molecular, Departamento de Ciências Biológicas, Universidade Federal do Espírito Santo, Vitória, Espírito Santo, Brazil.

⁵Departamento de Biologia, Universidade Federal do Espírito Santo, Alegre, Espírito Santo, Brazil.

TABLE 1. SINGLE-NUCLEOTIDE POLYMORPHISM PRIMER SEQUENCES

SNP	S	Primers	Annealing temperature (°C)	Restriction enzyme
<i>IκBL</i> -62 rs2071592	F	F 5' CACAGTTCCTCCGTCCTCCAGC 3' ^a R 5' CCTGTGTTAAGAAGCTCGG 3' ^a	58	<i>PvuII</i>
<i>IκBL</i> -262 rs3219184	F	F 5' CCTCTCTCTGCCAAGTTAGAGGAGGCGCG 3' ^a R 5' GGGCCGTCTGAAACCAGAAGACTGG 3' ^a	58	<i>AciI</i>
<i>TNFα</i> -238 rs361525	F	F 5' CACTCCCCATCTCCCTGGTC 3' R 5' GGTCTACACACAAATCAGT 3'	61	<i>AvaII</i>
<i>TNFα</i> -308 rs1000629	F	F 5' AGGCAATAGGTTTTGAGGGCCAT 3' R 5' TCCTCCCTGCTCCGATCCCG 3'	58	<i>NcoI</i>
<i>LTα</i> +80 rs2239704	R	F 5' GAGAGACAGGAAGGGAACAGAG 3' R 5' GTGCTTCGTGCTTTGGACTACCGCTC 3' ^a	60	<i>BseYI</i>
<i>LTα</i> +252 rs909253	R	F 5' GAGAGACAGGAAGGGAACAGAG 3' R 5' GTGCTTCGTGCTTTGGACTACCGCTC 3' ^a	60	<i>NcoI</i>
<i>FAS</i> -670 rs1800682	R	F 5' CTACCTAAGAGCTATCTACCGTTC 3' R 5' GGCTGTCCATGTTGTGGCTGC 3'	54	<i>MvaI</i>
<i>FASL</i> -844 rs763110	F	F 5' CAGCTACTCAGGAGGCCAAG 3' R 5' GCTCTGAGGGGAGAGACCAT 3'	62	<i>BsrDI</i>

^aModified primer sequence to create restriction site.

S, sense; F, forward; R, reverse; *TNFα*, tumor necrosis factors alpha; *LTα*, lymphotoxin-alpha; SNP, single-nucleotide polymorphism.

Samples

Samples were collected by the Head and Neck Genome Project (GENCAPO), a collaborative consortium created in 2002 with more than 50 researchers from nine institutions in São Paulo State, Brazil, whose aim is to develop clinical, genetic, and epidemiological analysis of head and neck squamous cell carcinomas. In this study, 252 DNA samples were obtained and used for polymorphism genotyping, from patients treated at the Heliópolis Hospital, São Paulo, Brazil, during the period of January 2001 to December 2009. Selected patients had no history of cancer, alcohol or tobacco addiction, occupational diseases, immunodeficiencies, mental disorders, or neurological diseases. Inability to answer the questionnaire was an exclusion criterion.

Among the 252 analyzed individuals, age varied from 29 to 91 years, with a mean of 54 years (SD ± 11 years), 220 (87.3%) were men and 32 (12.7%) women. Ethnicity was determined

by a patient auto-description, which was 146 (57.9%) white, 77 (30.6%) Pardos, and 29 (11.5%) blacks.

Genotyping

Genomic DNA was extracted from peripheral blood samples of 252 individuals as previously described (Miller *et al.*, 1988). Genotypes were determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). PCR conditions were 25 µL reaction mixture containing 200 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200 µM of each deoxyribonucleoside 5' triphosphates, 1.5 mM MgCl₂, 1 U Taq DNA polymerase (Life Technologies, Inc.[®], Rockville, MD), and 25 pmol of each primer. Cycling conditions were 5' at 94°C, 35 cycles of 1' at 94°C, 1' at annealing temperature (Table 1), and 1' at 72°C. PCR products were digested overnight with restriction endonucleases described in Table 1, following the manufacturer's recommendations (New England Biolabs[®],

TABLE 2. HARDY-WEINBERG EQUILIBRIUM ANALYSIS

SNP	HWE ^a								F _{st}
	Whole population		White		Pardo		Black		
	χ ²	p	χ ²	p	χ ²	p	χ ²	p	
<i>IκBL</i> -62AT	0.298	0.584	2.345	0.125	0.913	0.339	0.247	0.619	0.038
<i>IκBL</i> -262TC	24.652	≤0.05	16.871	≤0.05	1.546	0.213	20.000	≤0.05	0.034
<i>TNFα</i> -238GA	0.001	0.973	0.677	0.410	0.417	0.233	0.106	0.743	0.009
<i>TNFα</i> -308GA	2.407	0.120	1.381	0.239	0.973	0.323	0.106	0.743	0.065
<i>LTα</i> +80AC	15.296	≤0.05	9.024	≤0.05	6.428	≤0.05	0.960	0.327	0.008
<i>LTα</i> +252AG	2.063	0.150	2.544	0.110	0.372	0.541	0.464	0.495	<0.001
<i>FAS</i> -670AG	0.554	0.456	0.659	0.416	2.164	0.141	2.666	0.102	0.031
<i>FASL</i> -844TC	0.268	0.604	0.164	0.685	0.048	0.825	0.158	0.690	0.030

^aEquilibrium was assumed when *p* > 0.05.

χ², chi-square; *p*, significance value; F_{st}, genetic distance coefficient; HWE, Hardy-Weinberg equilibrium.

Beverly, MA). Restriction fragments were resolved on a 1%–3% agarose gel.

Statistical analysis

Genotypic frequencies were tested for Hardy–Weinberg equilibrium (HWE). The χ^2 and Fisher exact tests were used for population difference analysis and confirmation was obtained by the Lilliefors test (significance considered when $p < 0.05$). The F-statistic model was used to evaluate genetic differentiation among subpopulations. Statistical calculations were performed using Epi Info® v3.4.3, 2007 and Statsoft Statistica® v7.0.61.0 software.

Results

Except for *IκBL* –262TC and *LTa* +80AC, all polymorphisms analyzed were at HWE in the Brazilian population. HWE calculations were also applied to each ethnic group separately, showing a result similar to the population as a whole. However, the Pardo group showed disequilibrium at the *LTa* +80AC polymorphism and *IκBL* –262TC was at disequilibrium in the black group (Table 2). The genetic distance coefficient (F_{st}), a measure of population differentiation based on genetic polymorphisms, is described in Table 2.

Homozygous variants –238AA and –308AA of the *TNFA* gene were rarely found in the general population, the –238AA

TABLE 3. GENOTYPIC DISTRIBUTION

SNP genotype	Population								White vs. Pardo p	Pardo vs. Black p	Black vs. White p
	General		White		Pardo		Black				
	No.	f	No.	f	No.	f	No.	f			
<i>IκBL</i> –62AT											
AA	80	0.428	49	0.454	20	0.345	11	0.524	0.197	0.299	0.411
AT	82	0.439	42	0.389	31	0.534	9	0.429			
TT	25	0.134	17	0.157	7	0.121	1	0.048			
N/a	65										
<i>IκBL</i> –262TC											
TT	126	0.783	75	0.806	33	0.688	18	0.900	0.191	0.046 ^a	0.217
TC	24	0.149	12	0.129	12	0.250	0	—			
CC	11	0.068	6	0.065	3	0.063	2	0.100			
N/a	91										
<i>TNFA</i> –238GA											
GG	203	0.871	119	0.869	63	0.875	21	0.875	0.356	0.833	0.616
GA	29	0.124	18	0.131	8	0.111	3	0.125			
AA	1	0.004	0	—	1	0.014	0	—			
N/a	19										
<i>TNFA</i> –308GA											
GG	190	0.815	112	0.818	57	0.792	21	0.875	0.651	0.280	0.362
GA	43	0.185	25	0.182	15	0.208	3	0.125			
AA	0	0.000	0	—	0	—	0	—			
N/a	19										
<i>LTa</i> +80AC											
AA	14	0.106	9	0.123	2	0.057	3	0.125	0.520	0.586	0.668
AC	87	0.659	49	0.671	24	0.686	14	0.583			
CC	31	0.235	15	0.205	9	0.257	7	0.292			
N/a	120										
<i>LTa</i> +252AG											
AA	104	0.491	68	0.548	29	0.460	7	0.280	0.257	0.231	0.006 ^a
AG	95	0.448	52	0.419	29	0.460	14	0.560			
GG	13	0.061	4	0.032	5	0.079	4	0.160			
N/a	40										
<i>FAS</i> –670AG											
AA	44	0.208	29	0.236	10	0.159	5	0.192	0.473	0.041 ^a	0.016 ^a
AG	111	0.524	66	0.537	37	0.587	8	0.308			
GG	57	0.269	28	0.228	16	0.254	13	0.500			
N/a	40										
<i>FASL</i> –844TC											
TT	50	0.236	28	0.228	12	0.190	10	0.385	0.781	0.048 ^a	0.096
TC	102	0.481	59	0.480	30	0.476	13	0.500			
CC	60	0.283	36	0.293	21	0.333	3	0.115			
N/a	40										

^aSignificant genotypic differences.

N/a, not available, not included in statistical calculations; *f*, frequency; *p*, significance value.

genotype was found only once (0.4%) and the -308GA genotype was not observed at all. Frequencies of 1%–10% were observed for *IκBL* -262CC (6.8%) and *LTa* +252GG (6.1%) homozygous variants. Frequencies higher than 10% were found for *IκBL* -62TT (13.4%), *LTa* +80CC (23.5%), *FAS* -670GG (26.9%), and *FASL* -844CC (28.3%, Table 3) homozygous variants.

Genotypic frequencies were also calculated for each ethnical group separately. Nonsignificant differences were observed for *IκBL* -62AT; white and black ethnicities showed a higher frequency of the -62AA genotype, whereas the Pardo group showed a higher frequency of the -62AT heterozygote variant. Additionally, the black group presented the lowest frequency of the -62TT variant. Polymorphism *IκBL* -262TC showed similar frequencies among all groups. Nonetheless, a statistically significant difference was observed between the Pardo and black groups ($p=0.046$), 90% of the blacks were of the -262TT genotype. This difference was not observed between the black and white groups (Table 3).

Polymorphisms *TNFa* -238GA, *TNFa* -308GA, and *LTa* +80AC showed no differences among the three groups (Table 3). In contrast *LTa* +252AG presented a significant difference between the black and white groups. White individuals showed 55% frequency of the +252AA genotype, as compared to 28% of blacks. In comparison, 16% of blacks presented the +252GG variant, which was detected in only 3% of whites (Table 3).

FAS -670AG genotypic frequencies were similar between individual groups and the general population. However, 50% of blacks presented the -670GG genotype, whereas 30% showed the -670AG genotype. The same genotypes were observed at 25% and 55% frequencies in white and Pardo populations. The different frequencies observed in blacks

were statistically significant when compared to whites ($p=0.016$) and Pardos ($p=0.041$, Table 3).

FASL -844TC polymorphism showed significant differences between Pardos and blacks ($p=0.048$). This difference was not observed between the whites and blacks or between the whites and Pardos (Table 3).

Variants of *IκBL* -62AT, *IκBL* -262TC, *TNFa* -238GA, *TNFa* -308GA, *LTa* +80AC, and *FASL* -844TC were found at higher frequencies in the Pardo group. However, the variants *LTa* +252AG and *FAS* -670AG were more frequent in the black population (Table 4).

Discussion

The genetic differences among the three Brazilian ethnicities were detected in this study. As expected, the white and black groups presented statistically significant genotypic differences. In addition, significant differences were observed between Pardos and blacks, but not between Pardos and whites. Our initial hypothesis was that the Pardo group was a link between the other two groups, basically an intermixed group composed of white and black background. However, our results suggest that the Pardo ethnicity, in addition, is most probably also derived from native Indian.

Our results showed a greater similarity between the Pardo and white groups than between Pardo and black groups, thus again suggesting the participation of the Indian population in the formation of the Pardo group. Another hypothesis to explain this observation would be that the first Brazilian native Indians were descendants of the Asian hunters, a group with genetic similarities with white Europeans (Ministério da Justiça, 2012). Cavalli-Sforza (1998) described an unexpected high amount of miscegenation in the Brazilian population and determined that physical traits are superficial and imprecise in the characterization of ethnical groups and genetic origin. The F_{st} values demonstrate that the genetic distance between the three populations is not statistically significant, with values <0.05 , suggesting a high admixture of the three populations in question.

Other evidence suggested that 27% of Brazilian blacks did not descend from Africans as previously thought. The same study showed that 87% of the current Brazilian population has 10% genetic similarity with the African population (Pena and Bortolini, 2004). Moreover, the fact that *IκBL* -62AT, *IκBL* -262TC, *TNFa* -238GA, *TNFa* -308GA, *LTa* +80AC e *FASL* -844TC polymorphisms were found at greater rates in the Pardo group than in blacks and whites is also an evidence for an Indian contribution in the formation of the Pardo ethnicity.

HWE results showed that the population as a whole is not at equilibrium. HWE equilibrium depends directly on the lack of selection, random mating, and the large population size (Ridley, 2004). Our results suggest that the Brazilian population is under a miscegenation process at the current time, since some genotypes are not equally distributed. In addition, we conclude that the Pardo ethnicity derived from a complex mixture of ethnicities, including the native Indian population.

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TABLE 4. ALLELIC FREQUENCIES ACCORDING TO ETHNICITY

SNP allele	White	Pardo	Black
<i>IκBL</i> -62AT			
A	0.648	0.612	0.738
T	0.352	0.388	0.262
<i>IκBL</i> -262TC			
T	0.871	0.813	0.900
C	0.129	0.188	0.100
<i>TNFa</i> -238GA			
G	0.934	0.931	0.938
A	0.066	0.069	0.063
<i>TNFa</i> -308GA			
G	0.909	0.896	0.938
A	0.091	0.104	0.063
<i>LTa</i> +80AC			
A	0.459	0.400	0.417
C	0.541	0.600	0.583
<i>LTa</i> +252AG			
A	0.758	0.690	0.560
G	0.242	0.310	0.440
<i>FAS</i> -670AG			
A	0.504	0.452	0.346
G	0.496	0.548	0.654
<i>FASL</i> -844TC			
T	0.467	0.429	0.635
C	0.533	0.571	0.365

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:

Adriana Madeira Álvares Silva-Conforti, PhD

Departamento de Biologia

Universidade Federal do Espírito Santo

Alto Universitário s/n

Alto Universitário, caixa postal 16

Alegre, Espírito Santo 29500-000

Brazil

E-mail: adriana.biomol@gmail.com