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Brief Communication: Variability of Innate Immune System Genes in Native American Populations—Relationship With History and Epidemiology

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KEY WORDS Amerindians; innate immune genes; pathogens; Amerindian demography; natural selection

ABSTRACT

Objectives: The immune system of a host, defending him/her against invading pathogens, has two main subsystems: innate immunity and acquired immunity. There are several evidences showing that Native American populations are immunologically different from non-Native populations. Our aim was to describe the variability of innate immune system genes in Native American populations.
Materials and Methods: We investigated heterozygozities and patterns of population differentiation (F_{ST}) of 14 polymorphisms related to the innate immune response in five Native American populations (Aché, Guarani-Kaiowá, Guarani-Ñandeva, Kaingang, and Xavante) and the results were compared with the three major world population data (YRI, CEU, and CHB) available at the 1,000 genomes database.
Results: Mean heterozygosities ranged between 0.241 ± 0.057 (Aché) and 0.343 ± 0.033 (Kaingang), but no significant differences were observed (Friedman test, $P = 0.197$). Mean heterozygosities were also not significantly different when Amerindians were pooled and compared with the 1000 genomes populations (Friedman test, $P = 0.506$). When the Native American populations were grouped as Amerindians, a significantly higher F_{ST} value (0.194) was observed between the Amerindian and African populations. The Ewens-Watterson neutrality test showed that these markers are not under strong selective pressure.
Discussion: Native American populations present similar levels of heterozygosity as those of other continents, but are different from Africans in the frequency of polymorphisms of innate immune genes. This higher differentiation is probably due to demographic processes that occurred during the out-of-Africa event. Am J Phys Anthropol 159:722–728, 2016. © 2015 Wiley Periodicals, Inc.

Individuals display variable ability to fight infections, as well as variable susceptibility to inflammatory and auto immune diseases (reviewed in Quintana-Murci and Clark, 2013). Immune function is likely to be a critical determinant of an organism's fitness, yet most natural populations exhibit tremendous genetic variation for immune traits. In vertebrates this system is composed by two main subsystems: innate and acquired immunity (Pancer and Cooper, 2006). The primary characteristic of the innate immune system is speed, since the protective inflammatory response will start immediately after pathogen exposure. After this first response, innate immunity will play a central role in activating the subsequent adaptive immune response.

Population genetics is an approach that can provide invaluable genetic and statistical information about immune targets and involves the analysis of allele distributions in human populations at loci known or presumed to be involved in host defense and/or self-tolerance. Immunological heterogeneity, both among individuals and among populations, can help to understand the way in which natural selection has acted on host genes over time, by determining the current patterns of variability in the general population (Casanova et al., 2013).

South American Indians present a remarkable number of populations spread over a vast territorial area. The long history of genetic isolation and the great interpopulation diversity make Amerindians very unique. The populations are small, they still follow kinship rules, and mortality is caused mostly by infectious diseases. Many studies had been undertaken among them that

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TABLE 1. *Characteristics of the populations investigated*

Populations and number of individuals investigated	Aché 98	Guarani; Kaiowá 72; Ñandeva 72	Kaingang 72	Xavante 78
Localities	Arroyo Bandera Chupa-pou	Amambai Limão Verde Porto Lindo	Nonoai	Pimentel Barbosa
Geographic location	55°W, 23°S 56°W, 24°S	55°W, 23°S 55°W, 23°S 54°W, 23°S	52°W, 27°S	51°W, 13°S
Country and region	South Paraguay	Central Brazil	South Brazil	Central Brazil
Linguistic group	Tupi	Tupi	Jê	Jê
Non-Indian admixture (%)[a]	0.0	3.0	6.6	0.0
Sampling period	1998	1992–1993	2000	1990

[a] Estimated by Callegari-Jacques and Salzano (1999).

involved not only genetics, but other areas that are essential for evolutionary interpretations, such as demography, epidemiology and social anthropology. Examination of genetically isolated populations permits analysis of the evolutionary basis for immune gene variation, allowing insight into the role of these genes in health and disease.

There are several evidences showing that Native American populations are immunologically different from non-Native populations (Hurtado et al., 2004; Lindenau et al., 2013). Several investigations reported that Native American populations have a lower variability in several immune system genes such as KM, GM, Kell, HLA, and KIR (Bhatia et al., 1995; Black and Pandey, 1997; Black, 2004; Prugnolle et al., 2005; Augusto et al., 2013, 2015). Recently it has been reported that the pattern of adaptive immune system variability in Amerindians populations differs from that observed in the HapMap CEU population as evaluated by F_{ST} analyses (Lindenau et al., 2013). It is well established that environmental exposure to parasites, helminths, and physical injuries were very different during the evolutionary histories of world populations, leading to differences in immune response patterns observed among these groups (Finkelman and Urban, 2001; Hurtado et al., 2003; Lazzaro and Little, 2009; Schulenburg et al., 2009; Cagliani and Sironi, 2013). Nevertheless, knowledge about the Native American gene population variability of the immune system is still scarce, especially in relation to innate immunity. The present study describes the frequencies of 14 SNPs in innate immune system genes of five Native South American populations, discusses their evolutionary relationships, and the possible implications for immune response when pathogen exposure happens.

MATERIALS AND METHODS

Study subjects

A total of 392 individuals from five Amerindian populations were investigated. Their names and the characteristics of the samples investigated are described in Table 1.

The Aché (or Guayaki) are Tupi-speakers that live in eastern Paraguay. They remained isolated from non-Amerindians, subsisting basically in a hunter-gatherer way of living, until the 1970s. After that, more permanent contact was established. Extensive studies of this population including historical, demographic, social, and medical data were reported by Hill and Hurtado (1996, 1999). Currently there are about 1,000 Aché living in several rural settlements. A total of 98 subjects were sampled for this study.

Kaingang's territory has always been southern Brazil, and they presently live in four Brazilian states (São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul). They are the third most frequent Native American population in this country. Genetic, medical, and demographic information about them have been obtained since the 1960s and a selected bibliography can be found in Marrero et al. (2007). Farming is their main subsistence activity, although hunt and gather has also been important (Marrero et al, 2007). We analyzed 72 individuals from their Nonoai settlement.

The Guarani are the Brazilian most populous Native American population. Contact with non-Amerindians date to the beginning of the Spanish and Portuguese Conquests, in the 16th century (Monteiro, 2009). They are agriculturalists and fishermen. Three main cultural-linguistic subdivisions can be discerned among them: Ñandeva, M'byá (Kaiwá), and Kaiowá. Extensive genetic comparisons between Guarani and Kaingang have been undertaken, and a review can be found in Marrero et al. (2007). A total of 72 Guarani-Ñandeva and 72 Guarani-Kaiowá were included in this study. At present both populations (Kaingang and Guarani) are in advanced stage of acculturation.

The Xavante live in over 100 villages in seven reserves in the state of Mato Grosso, Central Brazil. Data collection for this study was conducted at the Pimentel Barbosa village in 1990. It is the largest village in the reserve of the same name. We analyzed 78 individuals from this settlement. Permanent contact of the Xavante with outsiders took place in the late 1940s (Coimbra et al., 2002). Until recently they were predominantly hunters and gatherers with incipient agriculture (Salzano and Callegari-Jacques, 1988).

Selected genes

We have chosen 13 genes involved in different stages of the innate immune response (Table 2). The polymorphisms investigated were selected based on association studies with infectious diseases in different populations, considering diverse pathogen exposition of continental populations, pattern recognition receptor, chemokine, and nitric oxide systems. Brief information about them follows.

Ten of them are pattern recognition receptors: *TLR1*, *TLR2*, *TLR4*, *TLR7*, *TLR8*, *TLR9*, CD209 (*DC-SIGN)*, *CR1*, *NOD2*, and *CD14*. Toll like receptors (TLRs) are expressed on many cell types, being immune response

TABLE 2. Minor allele frequencies for 14 SNPs in 13 innate immune system genes in five Amerindian populations, compared with corresponding data of 1,000 genomes CEU, YRI, and CHB samples

						Aché	Kaingang	Guarani Kaiowá	Guarani Ñandeva	Xavante	Amerindians
Gene	dbSNP ID	Allele	CEU 99	YRI 108	CHB 103	98	72	72	72	78	392
TLR2	rs111200466	Del	0.172	0.218	0.383	0.000	0.129	0.024	0.026	[a]	0.040
CD14	rs2569190	C	0.470	0.685	0.383	0.386	0.556	0.639	0.849	[a]	0.604
TLR1	rs4833095	T	0.793	0.093	0.320	0.311	0.465	0.479	0.557	0.662	0.483
NOD2	rs2066842	T	0.318	0.000	0.000	0.005	0.104	0.000	0.000	0.000	0.021
CCL5	rs2107538	T	0.146	0.440	0.379	0.319	0.146	0.127	0.074	0.027	0.149
NOS2	rs8078340	A	0.131	0.231	0.010	0.182	0.174	0.226	0.160	0.128	0.174
CCL2	rs1024611	A	0.677	0.792	0.364	0.005	0.319	0.140	0.134	0.006	0.113
CD209	rs2287886	G	0.667	0.796	0.311	0.057	0.655	0.873	0.678	0.720	0.563
TLR4	rs1927911	G	0.717	0.301	0.583	0.495	0.761	0.543	0.771	0.821	0.670
CR1	rs2274567	A	0.859	0.745	0.825	0.197	0.796	0.536	0.715	0.691	0.563
TLR8	rs3764880	A	0.727	0.736	0.194	0.366	0.606	0.421	0.470	0.704	0.507
TLR7	rs179008	T	0.197	0.157	0.000	0.000	0.194	0.083	0.197	0.333	0.152
TLR9	rs352140	C	0.535	0.722	0.578	0.175	0.764	0.778	0.646	0.526	0.551
	rs352143	C	0.172	0.389	0.034	0.011	0.076	0.021	0.042	0.299	0.088

[a] Not determined due to genotyping problems.

mediators to a variety of pathogens (reviewed in Kawai and Akira, 2010). They are either expressed on cell surface (as TLR1, TLR2, and TLR4) or intracellularly (as TLR7, TLR8, and TLR9). Several common polymorphisms associated with infectious diseases have been reported for different TLRs (Turvey and Broide, 2010). CD209 is a type II transmembrane protein predominantly expressed on dendritic cells (the antigen-presenting cells). Its presence in macrophages depends on tissue type and state of activation (Geijtenbeek et al., 2000). Nucleotide-binding oligomerization domain-containing protein 2 (NOD2), also known as caspase recruitment domain-containing protein 15 (CARD15), is a cytoplasmic sensor protein that is implicated in a variety of inflammatory and infectious diseases (Inohara and Nunez, 2003). Complement receptor (CR1) is a complement regulator that has three binding sites for C4b and two for C3b. It is found on the red cell surface, but mostly on white cells, and on glomerular podocytes (Gelfand et al., 1975; Fearon, 1985). It can also bind C1q and MBL (mannose binding lectin) and, thus, might play a role in the complement-independent removal and phagocytosis of particles coated by these proteins (Ghiran et al., 2000). Cluster of differentiation 14 (CD14) is a monocytic differentiation antigen that regulates innate immune responses to pathogens, acting as a co-receptor for TLR4 (Liu et al., 2012). It is expressed mainly by monocyte/macrophage lineage cells and it is required for the recognition of extracellular lipopolysaccharides (LPS) and lipoteichoic acid (LTA). Recent research findings revealed associations between the CD14 gene promoter polymorphism and infectious diseases (Anas et al., 2010; Areeshi et al., 2013).

Another set is composed by molecules that act as chemokines, namely CCL2 and CCL5. Monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) is a β-chemokine produced by monocytes and macrophages (Gu et al., 2000). Regulated on Activation, Normal T-Cell Expressed and Secreted (RANTES, also known as CCL5) is a member of the C-C chemokine subfamily. These chemokines are very important for granuloma formation in tuberculosis. CCL2 and CCL5 gene variants were associated with higher susceptibility to infectious diseases (Azad et al., 2012).

Finally, NOS2 (nitric oxide synthase), also known as iNOS2, does not belong to the two previously described categories, but nitric oxide is a pleiotropic regulator of neurotransmission, inflammation, and autoimmunity (Foster et al., 2013).

Laboratory and statistical methods

Genomic DNA was extracted from blood samples and genotyping was carried out by TaqMan® SNP Genotyping Assay methods (Applied Biosystems, Foster City, USA), except for the TLR2 and CD14 variants. The TLR2 deletion (rs111200466) was detected by 7% polyacrylamide gel electrophoresis after PCR amplification; while the CD14 polymorphism (rs2569190) was genotyped by PCR-RFLP as previously described (Greene et al., 2009; Ayaslioglu et al., 2013). Allele frequencies were directly obtained by gene counting and compared with those of the Yoruba of Ibadan, Nigeria (YRI), Utah residents with northern and Western Europe ancestry (CEU), and Han Chinese of Beijing, China (CHB) obtained from the 1,000 genomes database (McVean, 2012). The number of individuals considered was 99 for the CEU population; 108 for YRI; and 103 for CHB. Hardy-Weinberg equilibrium was tested for each locus within each population using the Markov chain as implemented in Arlequin v.3.5 with Bonferroni correction (Excoffier and Lischer, 2010). Arlequin was also employed to perform the Ewens–Watterson neutrality test (infinite allele model) (Ewens, 1972; Watterson, 1975). Mean heterozygosities and their standard errors (Nei, 1987) were calculated with the DISPAN software (Ota, 1993). Since these estimates do not follow a normal distribution, they were compared across populations with the Friedman test using the SPSS v.18 software. Interpopulation variability was determined by F_{ST} and their 95% confidence intervals were estimated with the R software using the diveRsity package (Keenan et al., 2013).

RESULTS

Table 2 shows minor allele frequencies (MAF) of the investigated polymorphisms in Native Americans, CEU, CHB, and YRI populations. The first point to consider is the difference observed among Amerindians. The Aché showed the lowest allele frequencies in 10 out of the 14 variants investigated (71%). In contrast, the Xavante

TABLE 3. Mean heterozygosities for 12 SNPs in five Amerindian populations[a]

	Aché	Ñandeva	Kaiowá	Kaingang	Xavante
rs4833095	0.431	0.497	0.503	0.501	0.450
rs2066842	0.010	0.000	0.000	0.188	0.000
rs2107538	0.437	0.138	0.223	0.251	0.053
rs352143	0.022	0.081	0.041	0.141	0.422
rs8078340	0.299	0.271	0.352	0.289	0.225
rs1024611	0.010	0.234	0.242	0.437	0.012
rs2287886	0.108	0.439	0.223	0.455	0.406
rs1927911	0.502	0.355	0.499	0.366	0.296
rs2274567	0.318	0.411	0.501	0.327	0.429
rs3764880	0.466	0.502	0.491	0.481	0.419
rs179008	0.000	0.319	0.153	0.315	0.447
rs352140	0.290	0.460	0.348	0.363	0.502
Mean ± SD	0.241 ± 0.057	0.309 ± 0.048	0.298 ± 0.052	0.343 ± 0.033	0.305 ± 0.054

[a] Comparison among heterozygosities: Friedman; $P = 0.197$. Heterozygosities do not differ significantly.

TABLE 4. Mean heterozygosities for 12 SNPs in Amerindian, CEU, CHB and YRI populations[a]

	AME	CEU	CHB	YRI
rs4833095	0.500	0.330	0.437	0.169
rs2066842	0.041	0.436	0.000	0.000
rs2107538	0.254	0.251	0.473	0.495
rs352143	0.161	0.286	0.066	0.477
rs8078340	0.288	0.229	0.019	0.357
rs1024611	0.201	0.439	0.465	0.331
rs2287886	0.493	0.446	0.431	0.326
rs1927911	0.443	0.408	0.488	0.423
rs2274567	0.493	0.243	0.290	0.382
rs3764880	0.501	0.399	0.314	0.390
rs179008	0.258	0.318	0.000	0.266
rs352140	0.495	0.500	0.490	0.403
Mean ± SD	0.344 ± 0.047	0.357 ± 0.027	0.290 ± 0.060	0.335 ± 0.040

[a] Comparison among heterozygosities: Friedman; $P = 0.506$. Heterozygosities do not differ significantly.

TABLE 5. Pairwise F_{ST} among Amerindian populations with 95% CI[a]

	Aché	Guarani-Kaiowá	Guarani-Ñandeva	Kaingang	Xavante
Guarani-Kaiowá	0.263 (0.233–0.292)	–			
Guarani-Ñandeva	0.271 (0.227–0.317)	0.050 (0.034–0.077)	–		
Kaingang	0.264 (0.230–0.299)	0.058 (0.035–0.087)	0.042 (0.022–0.069)	–	
Xavate	0.306 (0.265–0.344)	0.117 (0.089–0.151)	0.049 (0.031–0.071)	0.075 (0.050–0.102)	–

[a] There are no significant differences among the pairwise F_{ST} between Aché and Kaingang, Aché and Xavante, as well as Aché and Guarani, since the confidence intervals overlap.

showed the highest frequency in 5 (42%) of the 12 variants studied among them. These two populations showed very contrasting allele frequencies for nine (75%) of the 12 allele distributions. As for the interethnic comparisons, Amerindians showed the lowest or second lowest frequencies in nine (64%) of the 14 comparisons.

The genotype frequencies for all SNPs tested in this study are presented in Supporting Information Table 1. The observed genotype distributions were in agreement with Hardy-Weinberg equilibrium (HWE) for most SNPs after Bonferroni correction. The exceptions were rs3764880 in Aché, Kaingang and Xavante, and rs179008 in Kaingang and Xavante. The small sample sizes used for these comparisons, and eventual deviations from random mating that may occur in these relatively small populations, could contribute to these findings.

Mean heterozygosities ranged between 0.241 ± 0.057 (Aché) and 0.343 ± 0.033 (Kaingang), but no significant differences were observed (Table 3; Friedman test, $P = 0.197$). Mean heterozygosities were also not significantly different when Amerindians were pooled and compared with the 1,000 genomes populations (Friedman test, $P = 0.506$; Table 4).

Pairwise F_{ST} among Amerindian populations are shown in Table 5. Since the confidence intervals for all comparisons overlap, there are no significant differences among populations. When the Native American populations were grouped as Amerindians, a significantly higher value (0.194) was observed between the Amerindian and YRI populations as compared with the Amerindian versus CEU (0.127) and Amerindian versus CHB (0.113) comparisons (Table 6).

The Ewens-Watterson neutrality test showed that the probability of observing random samples with F values identical or smaller than the original sample could be accepted, suggesting that these markers are not under strong selective pressure (Table 7).

DISCUSSION

Infectious diseases and epidemics have always accompanied and characterized human history, representing one of the main causes of death. Even today, despite progress in sanitation and medical research, infectious diseases still are a major killer. Individuals vary in their resistance to infectious disease. Much of this variation is genetic and, in natural populations, considerable attention has been focused on the potential for pathogens to act as a selective force on genetic diversity (Cagliani and Sironi, 2013).

Modern humans encountered changeable environments during the colonization of the world. A significant phenotype variation, involving different behaviors, lifestyles and cultures, was then generated among modern human populations. Wu and Zhang (2011) analyzed the level of population differentiation among different sets of human genes. They concluded that few genes involved with the immune system showed high levels of population differentiation. Quintana-Murci and Clark (2013) argued that the innate immune system position, as first host defense line against pathogens, makes it an excellent model to evaluate the selective pressures that pathogens have exerted in the host genome. Therefore, innate immunity genes would be perfect targets for natural selection.

TABLE 6. Pairwise F_{ST} among Amerindians and others populations with 95% CI[a]

	AME	CEU	CHB	YRI
AME	–			
CEU	0.127 (0.107–0.147)	–		
CHB	0.113 (0.094–0.133)	0.179 (0.153–0.205)	–	
YRI	0.194 (0.174–0.214)	0.173 (0.150–0.197)	0.205 (0.177–0.234)	–

[a] There are significantly higher differences in the pairwise F_{ST} between Amerindians and YRI populations as compared with CEU and CHB, since the confidence intervals do not overlap.

Several studies have demonstrated that Native American populations have a differentiated pattern of variability in the immune system, either in HLA-KIR diversity or in the adaptive profile (Tsuneto et al., 2003; Augusto et al., 2013, 2015; Lindenau et al., 2013). They showed a reduced number of HLA and KIR alleles in relation to non-Native populations, that was considered as one of the explanations for their differentiated susceptibility to introduced diseases (Augusto et al., 2013, 2015; Lindenau et al., 2014). The pattern of adaptive immune system variability in Amerindian populations also differs from that observed in the HapMap CEU population (Lindenau et al., 2013).

This same trend seems not to happen with the innate immune markers studied in the present investigation. Our results show that average heterozygosities do not differ among world populations. On the other hand, Amerindians show, as expected, a higher genetic distance considering these alleles from Africans, as compared with European and East Asian samples. This agreement with historical data, at face value, would indicate the absence of differential selection.

Wang et al. (2007) found that Native Americans are strongly differentiated from the rest of the world. Considering that these populations are the youngest in the world, they discussed that it is difficult to infer selection as the main responsible for this differentiation. The little time allowed for selection to operate and small population sizes raised the possibility that demographic factors would be the better explanation for these results. Hofer et al. (2009) also suggested that demographic factors are probably the best explanation for the differentiation observed between Africa and Americas. Taking into account human evolutionary history, we need to consider, for instance, the spatial and demographic bottlenecks that occurred during the out-of Africa to Eurasia and the Americas. As discussed by Travis et al. (2007), these bottlenecks could be responsible for allelic surfing during subsequent spatial expansions.

CONCLUSION

Our results suggest that Native American populations present similar levels of heterozygosity as those of other continents, but are different from Africans in the frequency of polymorphisms of innate immune genes. This

TABLE 7. Ewens-Watterson neutrality test for 14 SNPs in five Amerindian populations[a]

	Aché	Ñandeva	Kaiowá	Kaingang	Xavante
rs111200466	–	0.691 (>0.999)	0.610 (>0.999)	0.363 (>0.999)	–
rs2569190	0.083 (0.996)	0.294 (>0.999)	0.108 (>0.999)	0.039 (0.546)	–
rs4833095	0.150 (>0.999)	0.044 (0.572)	0.014 (0.182)	0.026 (0.364)	0.119 (>0.999)
rs2066842	>0.999 (>0.999)	–	–	0.373 (>0.999)	–
rs2107538	0.135 (>0.999)	0.500 (>0.999)	0.342 (>0.999)	0.323 (>0.999)	0.679 (>0.999)
rs352143	0.800 (>0.999)	0.565 (>0.999)	0.729 (>0.999)	0.474 (>0.999)	0.156 (>0.999)
rs8078340	0.276 (>0.999)	0.301 (>0.999)	0.212 (>0.999)	0.306 (>0.999)	0.341 (>0.999)
rs1024611	>0.999 (>0.999)	0.368 (>0.999)	0.345 (>0.999)	0.137 (>0.999)	>0.999 (>0.999)
rs2287886	0.473 (>0.999)	0.135 (>0.999)	0.338 (>0.999)	0.128 (>0.999)	0.176 (>0.999)
rs1927911	0.004 (0.048)	0.206 (>0.999)	0.031 (0.403)	0.214 (>0.999)	0.272 (>0.999)
rs2274567	0.249 (>0.999)	0.183 (>0.999)	0.023 (0.299)	0.230 (>0.999)	0.138 (>0.999)
rs3764880	0.093 (>0.999)	0.022 (0.286)	0.048 (0.624)	0.085 (>0.999)	0.169 (>0.999)
rs179008	–	0.250 (>0.999)	0.426 (>0.999)	0.269 (>0.999)	0.134 (>0.999)
rs352140	0.265 (>0.999)	0.102 (>0.999)	0.238 (>0.999)	0.227 (>0.999)	0.030 (0.330)

[a] Values in parentheses are adjusted for multiple comparisons by the Bonferroni test. The dashes indicate the impossibility of performing the test due to either absence of variation in the indicated population, or absence of study for the given polymorphism.

difference is probably due to demographic processes. Native Americans provide good models to elucidate human evolutionary history, but interpretation of the findings are difficult due to decimation of many groups as a consequence of diseases introduced by non-Natives. Further investigations on carefully selected samples using a genomic approach would be welcome.

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