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 heterozygosities and patterns of population differentiation (FST) of 14 polymorphisms related to the innate immune response in five Native American populations (Ach, Guarani-Kaiowá, Guarani-Nandeva, 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 (Ach) 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 autoimmune 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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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; Schubelenburg 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: Nandeve, Mbyá (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-Nandeve and 72 Guarani-Kaiwá 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...
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-mediated removal and phagocytosis of particles coated by these proteins (Ghiran et al., 2000). Cluster differentiation 14 (CD14) is a monocyctic 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 polymorphism and infectious diseases (Anas et al., 2010; Areeshi et al., 2013). Complement factor H (CFH) mediates the alternative pathway C3b/C4b deposits on the complement receptor type 1 (CR1) (Brownlee et al., 1996). CFH inhibits the action of factor D and enhances the decay of the C3 and C5 convertases (Brownlee et al., 1996). CFH polymorphisms are associated with susceptibility to glomerular diseases (Nias et al., 2015; Fall et al., 2016). CFH variants are also associated with 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 Edwards-Watterson neutrality test (infinite allele model) (Edwards, 1972; Watterson, 1975). Mean heterozygosities and their standard errors 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 Edwards-Watterson neutrality test (infinite allele model) (Edwards, 1972; Watterson, 1975). Mean heterozygosities and their standard errors (Nei, 1987) were calculated with the DISPAN software (Otto, 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...
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).

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

<table>
<thead>
<tr>
<th>SNP</th>
<th>Aché</th>
<th>Nandeva</th>
<th>Kaiowá</th>
<th>Kaingang</th>
<th>Xavante</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4833095</td>
<td>0.431</td>
<td>0.497</td>
<td>0.503</td>
<td>0.501</td>
<td>0.450</td>
</tr>
<tr>
<td>rs2066842</td>
<td>0.010</td>
<td>0.000</td>
<td>0.000</td>
<td>0.188</td>
<td>0.000</td>
</tr>
<tr>
<td>rs2107538</td>
<td>0.437</td>
<td>0.138</td>
<td>0.223</td>
<td>0.251</td>
<td>0.053</td>
</tr>
<tr>
<td>rs352143</td>
<td>0.022</td>
<td>0.081</td>
<td>0.041</td>
<td>0.141</td>
<td>0.422</td>
</tr>
<tr>
<td>rs8078340</td>
<td>0.299</td>
<td>0.271</td>
<td>0.352</td>
<td>0.289</td>
<td>0.225</td>
</tr>
<tr>
<td>rs1024611</td>
<td>0.010</td>
<td>0.234</td>
<td>0.242</td>
<td>0.437</td>
<td>0.012</td>
</tr>
<tr>
<td>rs2287886</td>
<td>0.108</td>
<td>0.439</td>
<td>0.223</td>
<td>0.455</td>
<td>0.406</td>
</tr>
<tr>
<td>rs1927911</td>
<td>0.502</td>
<td>0.355</td>
<td>0.499</td>
<td>0.366</td>
<td>0.296</td>
</tr>
<tr>
<td>rs2274567</td>
<td>0.218</td>
<td>0.411</td>
<td>0.501</td>
<td>0.527</td>
<td>0.429</td>
</tr>
<tr>
<td>rs3764880</td>
<td>0.466</td>
<td>0.502</td>
<td>0.491</td>
<td>0.481</td>
<td>0.419</td>
</tr>
<tr>
<td>rs179008</td>
<td>0.000</td>
<td>0.319</td>
<td>0.153</td>
<td>0.315</td>
<td>0.447</td>
</tr>
<tr>
<td>rs352140</td>
<td>0.290</td>
<td>0.460</td>
<td>0.348</td>
<td>0.363</td>
<td>0.502</td>
</tr>
</tbody>
</table>

Mean ± SD 0.241 ± 0.057 0.309 ± 0.048 0.298 ± 0.052 0.343 ± 0.033 0.305 ± 0.054

*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

<table>
<thead>
<tr>
<th>SNP</th>
<th>AME</th>
<th>CEU</th>
<th>CHB</th>
<th>YRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4833095</td>
<td>0.500</td>
<td>0.330</td>
<td>0.437</td>
<td>0.169</td>
</tr>
<tr>
<td>rs2066842</td>
<td>0.041</td>
<td>0.436</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>rs2107538</td>
<td>0.254</td>
<td>0.251</td>
<td>0.473</td>
<td>0.495</td>
</tr>
<tr>
<td>rs352143</td>
<td>0.161</td>
<td>0.286</td>
<td>0.066</td>
<td>0.477</td>
</tr>
<tr>
<td>rs8078340</td>
<td>0.288</td>
<td>0.229</td>
<td>0.019</td>
<td>0.357</td>
</tr>
<tr>
<td>rs1024611</td>
<td>0.201</td>
<td>0.439</td>
<td>0.465</td>
<td>0.331</td>
</tr>
<tr>
<td>rs2287886</td>
<td>0.493</td>
<td>0.446</td>
<td>0.431</td>
<td>0.236</td>
</tr>
<tr>
<td>rs1927911</td>
<td>0.443</td>
<td>0.408</td>
<td>0.488</td>
<td>0.423</td>
</tr>
<tr>
<td>rs2274567</td>
<td>0.493</td>
<td>0.243</td>
<td>0.290</td>
<td>0.382</td>
</tr>
<tr>
<td>rs3764880</td>
<td>0.501</td>
<td>0.399</td>
<td>0.314</td>
<td>0.390</td>
</tr>
<tr>
<td>rs179008</td>
<td>0.258</td>
<td>0.318</td>
<td>0.000</td>
<td>0.266</td>
</tr>
<tr>
<td>rs352140</td>
<td>0.495</td>
<td>0.500</td>
<td>0.490</td>
<td>0.403</td>
</tr>
</tbody>
</table>

Mean ± SD 0.344 ± 0.047 0.357 ± 0.027 0.290 ± 0.060 0.335 ± 0.040

*Comparison among heterozygosities: Friedman; P = 0.506. Heterozygosities do not differ significantly.

TABLE 5. Pairwise FST among Amerindian populations with 95% CI

<table>
<thead>
<tr>
<th>Population</th>
<th>Aché</th>
<th>Guarani-Kaiowá</th>
<th>Guarani-Nandeva</th>
<th>Kaingang</th>
<th>Xavante</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guarani-Kaiowá</td>
<td>0.263</td>
<td>–</td>
<td>(0.233–0.292)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Guarani-Nandeva</td>
<td>0.271</td>
<td>0.050</td>
<td>(0.227–0.317)</td>
<td>(0.034–0.077)</td>
<td>–</td>
</tr>
<tr>
<td>Kaingang</td>
<td>0.264</td>
<td>0.068</td>
<td>0.042</td>
<td>(0.230–0.299)</td>
<td>(0.035–0.087)</td>
</tr>
<tr>
<td>Xavante</td>
<td>0.306</td>
<td>0.117</td>
<td>0.049</td>
<td>0.075</td>
<td>(0.265–0.344)</td>
</tr>
</tbody>
</table>

*There are no significant differences among the pairwise FST between Aché and Kaingang, Aché and Xavante, as well as Aché and Guarani, since the confidence intervals overlap.
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 in the host genome. Therefore, innate immunity genes would be perfect targets for natural selection.

Several studies have demonstrated that Native American populations have differentiated patterns 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 can be seen in Table 6, where we can observe the pairwise FST values among the different populations. The values 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 is 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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Thanks are due to the Fundação Nacional do Indio for authorizing this study and for logistic assistance. The Amerindian leaders and the subjects of investigation were adequately informed about the aims of the study and gave their approval, which is gratefully acknowledged. Our research program was approved by the Brazilian Ethics National Committee (Resolution 123/98). J.D.L. conceived study design, conducted genetic determination, analyses and data interpretation and wrote the manuscript. F.M.S. conducted sample recruitment and critical revision of the article. A.M.H. conducted sample recruitment. K.H. conducted sample recruitment. M.L.P.E. conducted sample recruitment. L.T.T. conducted sample recruitment. M.H.H. conducted sample recruitment, conceived study design, assisted the data interpretation, and edited the manuscript.

LITERATURE CITED


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