Brief Communication: Patterns of Linkage Disequilibrium and Haplotype Diversity at Xq13 in Six Native American Populations

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ABSTRACT Comparative studies of linkage disequilibrium (LD) can provide insights into human demographic history. Here, we characterize LD in six Native American populations using seven microsatellite markers in Xq13, a region of the genome extensively studied in populations around the world. Native Americans show relatively low diversity and high LD, in agreement with recent genome-wide survey and a scenario of sequential founder effects accompanying human population dispersal around the globe. LD in Native Americans is similar to that observed in some recently described small population isolates and higher than in large European isolates (e.g., Finns), which have been extensively analyzed in medical genetics studies. Haplotype analyses are consistent with a colonization of the New World by a differentiated East Asian population, followed by extensive genetic drift in the Americas. Am J Phys Anthropol 142:476–480, 2010. © 2009 Wiley-Liss, Inc.

Patterns of linkage disequilibrium (LD) across the genome are influenced by a range of factors, including variable mutation and recombination rates, natural selection, and population demography (Ardlie et al., 2002). Genome-wide comparisons of LD in different human populations have been carried out in the CEPH-HGDP panel and the HapMap reference set. Other extensive population surveys have been performed for a few regions of the genome, including a ~13 Mb segment on Xq13, which has been examined in a range of populations across the world (Laan and Paabo, 1997; Zavattari et al., 2000; Angius et al., 2001; Kaessmann et al., 2002; Katoh et al., 2002; Latini et al., 2004; Laan et al., 2005; Marroni et al., 2006; Branco et al., 2008; Bellis et al., 2008, Leite et al., 2009). So far, comparative studies of LD including Native Americans are fairly scant, often limited to the five populations of the CEPH-HGDP panel (Sawyer et al., 2005; Conrad et al., 2006; Jakobsson et al., 2008; Li et al., 2008; Bosch et al., 2009). To further the analysis of LD in Native Americans here we examine the Xq13 region, previously studied around the world, in six Native American populations.

MATERIALS AND METHODS

Samples

DNA samples (isolated from peripheral blood) were obtained from consenting individuals representing six Native American populations: Wayuu (n = 48 chromosones), Ingano (n = 48), Kogi (n = 44), Zenu (n = 46), and Ticuna (n = 30), from Colombia, and Cree (n = 25) from Saskatchewan, Canada. Following the linguistic classification of Ruhlen (1991), Wayuu and Ticuna both belong to the Equatorial-Tucanoan linguistic stock. Wayuu is one of the largest Native American groups in Colombia, with an estimated population size of 135,000, whereas the Ticuna have a population size of ~8,000. The Ingano is an Andean population, with a population size of ~18,000. Kogi and Zenu are Chibchan-Paezan populations, with estimated population sizes of 3,000 and 34,000, respectively. See Mesa et al. (2000) for more information of the Native Americans in Colombia. Cree belongs to a large population (~200,000 in Canada) organized into many smaller groups. The Cree in Saskatchewan have a census of roughly 73,500.

Additional supporting information may be found in the online version of this article.

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Genotyping

We studied seven microsatellite markers at Xq13: DXS983, DXS8037, DXS8092, DXS1225, DXS8082, DXS986, and DXS995, spanning more than 13 Mb, from physical map position 69.36–82.64 Mb (GenBank Build 36.2) and about 3.4 cM, from genetic map position 83.93–87.29 cM (Kong et al., 2002). Microsatellites were typed on ABI PRISM 377 DNA analyzer using PCR products obtained as described by Laan and Paabo (1997) and data processing by GENESCAN version 3.1 and GENOTYPER version 2.5. The missing data rate is 2%.

Statistical analyses

Gene diversities were computed using Arlequin 2.0 (Schneider et al., 2000). Extracting full information of the phased male samples and unphased female samples, GENECONTEING (Zhao, 2004) was used to obtain maximum-likelihood estimate of haplotype frequencies. Pair-wise LD was assessed using a Monte Carlo approximation to Fisher's exact test with the POWERMARKER 3.0 program (Liu and Muse, 2005). A randomized sampling correction was used to avoid a bias due to differences in sample size. Multilocus LD was estimated with the $r_d$ statistic using the MULTILOCUS program (Agapow and Burt, 2001). A matrix of Nei's $D_A$ distances (Nei et al., 1983) between populations was obtained from multilocus (DXS1225-DXS8082) haplotype frequencies using PowerMarker 3.0, and the results displayed by multidimensional scaling (MDS) using the SPSS package 12.0.1.

RESULTS

Gene diversity and LD

Native Americans show Xq13 microsatellite gene diversities that are mostly lower than in Eurasian populations, ranging between 0.325–0.620 and 0.594–0.755, respectively (Supporting Information Table 2). Considering each region as a single group, gene diversity is lower in Native Americans (0.638) than in East Asians (0.682), Volga-Ural populations (0.754), and Europeans (0.729).

On average, 57.3% marker pairs (12 of 21) show significant LD across Native American populations (Fig. 1 and Supporting Information Table 3), a considerably higher proportion than observed in non-isolated Eurasian populations, where 14.3% marker pairs (3 of 21) are in significant LD. The increased pair-wise LD in Native Americans is comparable to that reported for some small isolated Eurasian populations, such as the Saami and Khoton. A similar pattern is observed for multilocus LD (see Fig. 1), Native Americans averaging an $r_d$ of 0.172 compared to an average of 0.025 in European and Asian populations. Again, only the Saami and Khoton have values of $r_d$ comparable to those observed in Native Americans (0.14 and 0.12, respectively). There is a significant negative correlation between the logarithm transformation of population size and LD, measured by multilocus $r_d$ ($r = -0.616, P < 0.01$), or by proportion of significant LD pairs ($r = -0.664, P < 0.01$). There is no significant difference in the proportion of LD pairs between Cree from Canada and the other five populations from Colombia (two-tailed t-test: $P = 0.205$).

Haplotype diversity at DXS1225-DXS8082

Very strong LD has been observed between markers DXS1225 and DXS8082 (located 162 kb apart), in populations from around the world (Supporting Information Table 3). The haplotype frequency distribution for these two markers in Native Americans and other continental groups is shown in Table 1. The three most common haplotypes in Native Americans (defined using allele sizes) are 198–225, 198–227, and 202–221. These three haplotypes are found at elevated frequencies in the Kogi. Two of them predominate in the Wayuu (198–225 at 33% and 202–221 at 17%), Ingano (198–227 at 37% and 202–221 at 32%), and Zenu (198–225 at 39% and 198–227 at 22%). One haplotype is markedly prevalent in the Cree (198–227 at 60%) and the Ticuna (202–221 at 80%). An important differentiation in haplotype frequency is seen between continental groups. Haplotype 198–225 is relatively common in East Asians (~12%) but, of the other two common Native American haplotypes, 198–227 is rare (<6%) outside of the Americas, and 202–221 has very low frequency in East Asians and is absent from Volga-Urals and Europeans. Conversely, the most common haplotype in East Asia (202–217 with a frequency of 25%) and two prominent Volga-Ural and European haplotypes (202–211 and 210–219 with frequencies of 10–28%) are rare or absent in Amerindian populations. These two most common European haplotypes are present at low frequencies in the Wayuu, Ingano, and Zenu. This likely reflects a low level of non-native admixture in these populations, as observed in a larger dataset (Wang et al., 2007).

MDS of a distance matrix calculated from the DXS1225-DXS8082 haplotype frequencies (see Fig. 2) shows three main clusters—Europeans, East Asians, and Native Americans—corresponding to continental populations examined, with Volga-Urals occupying an intermediate position between Europeans and East Asians. Europeans cluster together, separately from Volga-Ural populations, with the exception of the Mari. Russians and Saami are closer to the remaining Volga-Urals than...
to other European populations. Native American populations display the highest within-group distances, whereas Europeans and Volga-Ural populations form tighter clusters. This reflects the considerable variation in haplotype frequencies across native populations, resulting in substantially a higher $F_{ST}$ amongst Native Americans than amongst populations from other regions (0.17 vs. 0.02–0.04, respectively).

### DISCUSSION

A low-genetic diversity and high LD at Xq13 was observed in all the Native American populations examined here, with increased LD being apparent both in two-locus and multilocus analyses. It is worth noting though that results from using different genetic markers could lead to different conclusions (Sawyer et al., 2005).

Studies with genome-wide coverage are therefore needed to verify the findings. Our observations are consistent with previous genome-wide surveys, indicating that Native Americans have lower diversity and higher LD relative to other continental regions (Conrad et al., 2006; Jakobsson et al., 2008; Li et al., 2008). These patterns have been interpreted as resulting from sequential bottleneck effects during the dispersal of human populations around the world with entry into the Americas representing the last of these founder events (Prugnolle et al., 2005; Ramachandran et al., 2005; Wang et al., 2007). The population contraction at the colonization of the American continent appears to have been quite substantial, with recent estimates, suggesting that as few as 100 individuals could have been the initial colonizers (Ray et al., 2009). Our results suggests that the increased LD in Native American populations is comparable to that seen in some small population isolates described in other parts of the world, such as the Saami, and considerably higher than in larger isolates, such as the Finns, which have been extensively examined in medical genetics studies. Interestingly, gene diversity in Native Americans is often considerably lower than in those isolates, suggesting that Native American populations could provide further advantages for trait gene identification (Terwilliger et al., 1998; Peltonen et al., 2000).

### TABLE 1. Common haplotypes of DXS1225-DXS8082 marker pair

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Haplotypes with frequency >0.1 are in bold. The most common haplotype in each population or group is underlined. Haplotype frequency between 0.005 and 0.05 is indicated as *. NA, Native American; EA, East Asian; VU, Volga-Ural; EU, European.

Fig. 2. Multidimensional scaling on Nei’s $D_A$ distance matrix derived from frequency of haplotypes at markers DXS1225 and DXS8082. Native American populations are shown in blue, East Asian in green, Volga-Ural in yellow, and European in red. RSQ = 0.930.
LITERATURE CITED


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