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An Ancient DNA Test of a Founder Effect in Native American ABO Blood Group Frequencies

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ABSTRACT Anthropologists have assumed that reduced genetic diversity in extant Native Americans is due to a founder effect that occurred during the initial peopling of the Americas. However, low diversity could also be the result of subsequent historical events, such as the population decline following European contact. In this study, we show that autosomal DNA from ancient Native American skeletal remains can be used to investigate the low level of ABO blood group diversity in the Americas. Extant Native Americans exhibit a high frequency of blood type O, which may reflect a founder effect, genetic drift associated with the historical population decline, or natural selection in response to the smallpox epidemics that occurred following European contact. To help distinguish between these possibilities, we determined the ABO genotypes of 15 precontact individuals from eastern North America. The precontact ABO frequencies were not significantly different from those observed in extant Native Americans from the same region, but they did differ significantly from the ABO frequencies in extant Siberian populations. Studies of other precontact populations are needed to better test the three hypotheses for low ABO blood group diversity in the Americas, but our findings are most consistent with the hypothesis of a founder effect during the initial settlement of this continent. Am J Phys Anthropol 137:342–347, 2008.

The ABO blood group system was first described by Karl Landsteiner in 1901. Subsequent research showed that this system is defined by three major alleles (A, B, and O) at the ABO locus on chromosome 9 (Yip, 2002; Daniels, 2005), and recent studies of the underlying sequence variation at this locus have identified more than 100 (mostly rare) alleles (Ferri et al., 2004). The ABO alleles code for three antigens (A, B, and H) and four phenotypes (blood types A, B, AB, and O), and their frequencies vary worldwide as a result of natural selection, gene flow, and genetic drift (Mourant, 1954; Thompson, 1972). In the Americas, indigenous populations generally exhibit a high frequency of blood type O (Mourant et al., 1976; Daniels, 2002), and three hypotheses have been proposed to explain this pattern.

First, the high frequency of blood type O may reflect a founder effect that occurred during the initial colonization of the Americas. Mitochondrial DNA (mtDNA), Y chromosome, and autosomal data all suggest that present-day Native Americans are descended from a single founding population that migrated to the Americas from Beringia (Merriwether et al., 1995; Zegura et al., 2004; Schroeder et al., 2007; Tamm et al., 2007; Wang et al., 2007; Fagundes et al., 2008). There is some evidence that this ancestral population may have been derived from two different areas of Siberia (Lell et al., 2002; Starikovskaya et al., 2005), but it seems that a relatively small number of founders gave rise to all Native American populations. Only a few founding mtDNA and Y chromosome haplotypes have been identified (Malhi et al., 2002; Schurr, 2004; Zegura et al., 2004; Tamm et al., 2007; Achilli et al., 2008), and all Native American populations that have been genotyped for autosomal locus D9S1120 share a unique allele at that locus (Schroeder et al., 2007; Wang et al., 2007). Native Americans also exhibit lower genetic diversity than populations from other continental regions (Wang et al., 2007), and estimates of the effective size of the founding population range from 70 to 1,000 individuals (Hey, 2005; Fagundes et al., 2008; Kitchen et al., 2008). Because Siberian populations exhibit greater ABO diversity (Mourant et al., 1976), it is possible that low ABO diversity in the Americas stems from an initial founder effect.

Alternatively, the high frequency of blood type O might reflect genetic drift associated with the historical population decline. Both archaeological and historical evidence show that European contact and colonialism led to a significant reduction in indigenous population sizes (Swanton, 1946; Smith, 1987; Ubelaker, 1988; Livi-Bacci, 2006). Scholars have debated the magnitude of this decline (Dobyns, 1983; Ubelaker, 1988; Daniels, 1992), but all agree that substantial depopulation occurred as a result of warfare, slavery, epidemics of European diseases, removals and relocations, and the destruction of traditional lifestyles (Crosby, 1972; Dobyns, 1983, 1993; Thornton, 1984; Ramenofsky, 1987; Thornton et al., 1992; Livi-Bacci, 2006). Since depopulation can cause genetic bottlenecks and reduce genetic diversity (Nei et al., 1975; Hoelzel, 1999), these historical events may have influenced the pattern of ABO diversity in the Americas. Some previous studies have found evidence of a European-induced bottleneck, including

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TABLE 1. ABO frequencies in extant Native Americans from eastern North America

Population	*N*	A	B	O	Reference
Caddo, Oklahoma	47	0.162	0.000	0.838	Gray and Laughlin (1960)
Catawba, South Carolina	104	0.218	0.081	0.702	Pollitzer et al. (1967)
Cherokee, North Carolina	166	0.018	0.009	0.973	Pollitzer et al. (1962)
Choctaw/Chickasaw, Oklahoma	81	0.044	0.000	0.956	Landsteiner and Levine (1929)
Chippewa, Minnesota	161	0.064	0.000	0.936	Matson et al. (1954)
Ojibwa, Manitoulin Island	105	0.172	0.044	0.784	Szathmary et al. (1975)
Ojibwa, Pikangikum	95	0.225	0.000	0.775	Szathmary et al. (1975)
Omaha, Nebraska	168	0.056	0.003	0.938	Matson (1941)
Pawnee, Oklahoma	80	0.226	0.012	0.762	Gray and Laughlin (1960)
Penobscot, Maine	249	0.270	0.002	0.729	Allen and Corcoran (1960)
Seminole, Florida	381	0.019	0.004	0.978	Pollitzer et al. (1970a)
Seminole, Oklahoma	224	0.039	0.039	0.923	Pollitzer et al. (1970b)
Sioux, South Dakota	260	0.159	0.015	0.825	Matson (1941)
Wichita, Oklahoma	49	0.243	0.020	0.736	Gray and Laughlin (1960)
Winnebago, Nebraska	86	0.238	0.000	0.747	Matson (1941)

Bolnick and Smith's (2003) analysis of mtDNA variation in southeastern North America and Wang et al.'s (2004) analysis of 175 genome-wide markers in the Oklahoma Choctaw. Salzano and Callegari-Jacques's (2006) study of 421 nuclear loci also found that recent population history had likely influenced Native American genetic variability.

Finally, the high frequency of blood type O in extant Native Americans might be the result of selection in response to disease epidemics following European contact. Some pathogens resemble ABO antigens (i.e., molecular mimicry), which makes it more difficult for the immune system to detect and destroy them (Mourant, 1954; Berger et al., 1989; Henry, 2001; Seymour et al., 2004). Individuals with certain ABO blood types may therefore be more susceptible to certain diseases (Berger et al., 1989; Marionneau et al., 2001; Seymour et al., 2004). Blood type A may be most susceptible to smallpox, for example, because a substance resembling the A antigen is present in the *Variola* virus (Pettenkofer and Bickerich, 1960; Vogel et al., 1960; but also see Springer and Wiener, 1962). Individuals with blood type A do appear to suffer higher infection rates, greater disease severity, and increased mortality from smallpox (Vogel et al., 1960; Chakravartti et al., 1966; Vogel and Chakravartti, 1966; Adalsteinsson, 1985), so the repeated and widespread smallpox epidemics of the 16th-19th centuries may have selected against Native Americans with A alleles and increased the frequency of O and B alleles in the surviving population.

In this study, we show that ancient DNA (aDNA) from pre-European contact skeletal remains can be used to investigate the three hypotheses for the high frequency of blood type O in extant Native Americans. If ABO frequencies are similar in precontact and extant populations, it would suggest that events following European contact had little impact on ABO frequencies in the Americas. In that case, the high frequency of blood type O would likely reflect a founder effect during the initial settlement of the Americas. If, however, ABO frequencies differ between precontact and extant Native Americans, it would suggest that historical events have had a significant impact on present-day patterns. In addition, if precontact Native Americans exhibit a greater frequency of A alleles, it would be consistent with the hypothesis that smallpox epidemics selected against individuals with A alleles in the Americas.

SUBJECTS AND METHODS

ABO frequencies were compiled from the published literature for 45 extant Siberian populations (Mourant et al., 1976) and 15 extant Native American populations from eastern North America (Table 1). All individuals with known non-native admixture were excluded. Skeletal samples were also obtained from three archaeological sites: the Pete Klunk mound group (a Middle Woodland mortuary site dated to 1,825 ± 75 ybp and associated with the Havana Hopewell archaeological tradition) in Calhoun County, Illinois (Perino, 1968), the Wright mound (an Early Woodland Adena burial site dated to 1,950–1,790 ybp) in Montgomery County, Kentucky (Webb, 1940), and the Hardin Village cemetery (a Fort Ancient site dated to 500–325 ybp) in Greenup County, Kentucky (Hanson, 1966). The extraction and analysis of DNA from these samples was approved by the Office of Human Research Protection at the University of California, Davis, and the present study was reviewed by the Institutional Review Board at the University of Texas at Austin.

DNA was extracted from the skeletal samples using a standard phenol–chloroform protocol (Bolnick and Smith, 2007), and endogenous ancient mtDNA was successfully amplified in previous studies (Bolnick and Smith, 2007; Bolnick, unpublished data). In this study, we amplified a 103/104 base-pair (bp) fragment of exon 6 and a 64 bp fragment of exon 7 of the ABO locus using the primers and polymerase chain reaction (PCR) conditions described in Hummel et al. (2002). PCR amplifications were performed in 25 μl volumes containing 1–5 μl of DNA template, 5 mM Tris–HCl (pH 8.4), 12.5 mM KCl, 0.8 mM dNTPs, 0.26 mM bovine serum albumin, 0.375 mM $MgCl_2$, 0.15 mM of each primer, and 0.75 units of Platinum Taq (Invitrogen). Four microliters of each amplification product were visualized with ethidium bromide on 6% polyacrylamide gels to confirm amplification. The presence or absence of specific restriction sites allows the identification of five common ABO alleles (A, B, O_1, O_{1v}, and O_2), so PCR products were digested with the appropriate restriction enzymes following Hummel et al. (2002). Eight microliters of the digested products were then visualized as described above to determine whether each restriction site was present or absent, and ABO genotypes were determined following Hummel et al. (2002).

TABLE 2. ABO and STR data for the precontact samples

Sample	Site	Genotype	Phenotype	Amel	D3S1358	D8S1179	D5S818	vWA	D21S11	D13S317	FGA	D7S820
C30-24	Klunk	O_1O_{1v}	O	XX	15	12,15	11	16,17				
C30-25	Klunk	AO_1	A	XY	15,16	13,15	9,11	17	31.2	12,13	24,26	12
C30-71B	Klunk	$O_{1v}O_{1v}$	O	XX								
C31-11(A)	Klunk	$O_{1v}O_{1v}$	O	XY	15	12,13	7,13	17				
C34-1	Klunk	O_1O_1	O	XY								
C34-14	Klunk	$O_{1v}O_{1v}$	O	XX	15		16					
C34-20	Klunk	$O_{1v}O_{1v}$	O	XX	15	13	12	16,17		13		
C34-24	Klunk	$O_{1v}O_{1v}$	O	XY		10						
C34-30	Klunk	$O_{1v}O_{1v}$	O	XX	15,16	14	11	16,17				
C35-13	Klunk	O_1O_1	O									
C35-17	Klunk	$O_{1v}O_{1v}$	O	XX	15,17	10						
C35-23	Klunk	$O_{1v}O_{1v}$	O	XX	16	14	11	16				
C35-27	Klunk	$O_{1v}O_{1v}$	O	XX		14	12					
C40-59	Klunk	$O_{1v}O_{1v}$	O	XY	15,18	15	7,11	16,18		9		
15MM6 burial 14	Wright	O_1O_1	O	XY	15,16	12						

Ancient DNA is typically degraded and subject to contamination from exogenous sources, so we followed accepted procedures to avoid contamination of the aDNA and to detect any contamination that did occur (Kelman and Kelman, 1999; Kaestle and Horsburgh, 2002). All pre-PCR work was performed in a laboratory dedicated to pre-PCR aDNA research. This laboratory is equipped with dedicated equipment, overhead ultraviolet lights, positive air pressure, and HEPA-filtered ventilation. The post-PCR laboratory is located on a different floor of the building, and personnel movement between facilities was unidirectional (from pre-PCR aDNA lab to post-PCR lab) on any given day. Lab coats, hair covers, face masks, shoe covers, and gloves were worn at all times in the aDNA lab. Sterile, disposable supplies were used whenever possible. All reagents were certified as being DNA-free or molecular grade, and PCR tubes were irradiated with UV light (254 nm) for 15 min before use. PCR setup was performed in a laminar flow hood. All results were confirmed through multiple amplifications of two independent extractions of each sample, and at least three negative (blank) controls were included in each PCR amplification to identify the presence of any contamination. Finally, following Hummel et al. (2002), we also used the AmpF*l*STR Profiler Plus Amplification Kit (Applied Biosystems) to genotype the aDNA samples for autosomal short tandem repeats (STRs). All reported STR alleles were confirmed through at least two independent amplifications. This last analysis was performed to verify that the aDNA extracts did not match the STR profiles of the authors.

Precontact frequencies of the ABO alleles, genotypes, and phenotypes (ABO blood type) were calculated, and 95% confidence intervals were computed using the Wilson procedure with a continuity correction (Wilson, 1927; Newcombe, 1998). Arlequin 2.000 (Schneider et al., 2000) was used to test for significant departures from Hardy–Weinberg equilibrium. ABO allele frequencies in the precontact population were compared to those in the extant Native American and Siberian populations using pairwise exact tests in Arlequin (Schneider et al., 2000) to test the null hypothesis that each set of frequencies was drawn from the same distribution. Since most data from the extant populations were collected before technology allowed the identification of O_1, O_{1v}, and O_2 alleles, all O alleles were grouped together in the precontact population for this comparison.

*TABLE 3. Allele, genotype, and phenotype frequencies in the precontact sample (*N = *15)*

	Frequency	95% Confidence interval
Alleles		
A	0.033	0.002–0.191
B	0.000	0.000–0.141
O_1	0.267	0.130–0.462
O_{1v}	0.700	0.504–0.846
O_2	0.000	0.000–0.141
Genotypes		
AA	0.000	0.000–0.254
AO_1	0.067	0.004–0.340
AO_{1v}	0.000	0.000–0.254
AO_2	0.000	0.000–0.254
AB	0.000	0.000–0.254
BB	0.000	0.000–0.254
BO_1	0.000	0.000–0.254
BO_{1v}	0.000	0.000–0.254
BO_2	0.000	0.000–0.254
O_1O_1	0.200	0.053–0.486
O_1O_{1v}	0.067	0.004–0.340
O_1O_2	0.000	0.000–0.254
$O_{1v}O_{1v}$	0.667	0.387–0.870
$O_{1v}O_2$	0.000	0.000–0.254
O_2O_2	0.000	0.000–0.254
Phenotypes		
A	0.067	0.004–0.340
B	0.000	0.000–0.254
O	0.933	0.660–0.997
AB	0.000	0.000–0.254

RESULTS

We attempted to determine the ABO genotypes of 39 precontact individuals (26 Klunk, 9 Wright, and 4 Hardin Village individuals) whose mtDNA haplogroups and haplotypes had been previously identified (Bolnick and Smith, 2007; Bolnick, unpublished data). Fifteen samples were successfully genotyped (Table 2), comprising a 38.5% success rate. Allele, genotype, and phenotype frequencies (and 95% confidence intervals) are given in Table 3. The O_{1v} and O_1 alleles, as well as the $O_{1v}O_{1v}$ and O_1O_1 genotypes, were most common in the precontact sample. Because this sample exhibited excess homozygosity, tests of Hardy–Weinberg equilibrium found that the population was not in equilibrium ($P = 0.003$). When ABO allele frequencies in the precontact and extant populations were compared (Table 4), the precon-

TABLE 4. ABO allele frequencies in the precontact and extant populations

Population	*N*	A	B	O	Reference
Precontact Native Americans	15	0.033	0.000	0.967	This study
Extant Native Americans (Eastern North America)	2,256	0.120	0.014	0.865	See Table 1
Extant Siberians	6,826	0.190	0.188	0.623	Mourant et al. (1976)

tact population was significantly different from the extant Siberians ($P < 0.001$) but not the extant Native Americans ($P = 0.341$).

Fourteen of the fifteen precontact samples yielded data for at least one locus with the AmpF*l*STR Profiler Plus Amplification Kit (Table 2). In two cases, only the amelogenin locus amplified. The other 12 samples yielded PCR products for the amelogenin locus and 1–8 STR loci. In all cases, the amelogenin data were consistent with the sex determination based on skeletal morphology (from Perino, 1968). The STR data provided confirmation that autosomal DNA was preserved in these samples and showed that the STR profiles of the precontact samples differed from those of the authors.

DISCUSSION

This study is the first to use aDNA to ascertain the ABO blood types of precontact Native Americans. The aDNA results reported here are thought to be authentic because (1) the same genotypes were repeatedly obtained from multiple amplifications and multiple independent extracts of a particular sample, (2) stringent procedures were followed during all stages of the analysis to prevent and detect contamination, (3) clean negative controls were observed at all stages of the analysis, (4) the ABO genotypes of the aDNA samples differed from those of the authors (AA and O_1O_1) in most (12/15) cases, (5) the STR profiles of the aDNA samples differed from those of the authors, and (6) the STR alleles observed in the aDNA samples have been previously observed in extant Native Americans, with those alleles that were most common here also being common in the extant populations studied (Sun et al., 2003; Gross and Budowle, 2006). Although five aDNA samples exhibited an A or O_1 allele, as do the authors, the amelogenin and/or STR genotypes differed from those of the authors in four of the five samples. The fifth sample (C35-13) did not yield any PCR products with the AmpF*l*STR Profiler Plus Amplification Kit, which is consistent with the presence of highly degraded aDNA. Because the ABO genotype of this sample was obtained from multiple amplifications of two independent extracts, we believe it to be endogenous, but note that our general findings do not change if this sample is excluded from the analysis.

Although we tested samples from archaeological sites spanning more than 1,500 years, the samples that yielded autosomal DNA likely spanned less than 125 years and all but one came from a single site (the Klunk mound group). This site is thought to have served as the cemetery for most members of the local community (Buikstra, 1976), and the sampled individuals were drawn from a limited number of generations (Bolnick and Smith, 2007). Consequently, it is probable that they represent a single biological population. Our sample of this population was fairly small, because many of the remains did not yield autosomal DNA, and the 95% confidence intervals for allele, genotype, and phenotype frequencies were relatively wide as a result. Thus, although this dataset suggests that blood type O was present at a high frequency in this population, additional data are needed from this and other precontact populations to confirm the frequencies reported here.

It should also be noted that this population did not appear to be in Hardy–Weinberg equilibrium due to an excess of homozygotes and a paucity of heterozygotes. Allelic dropout (the stochastic failure of one allele to amplify) is common in studies of degraded nuclear DNA (Broquet and Petit, 2004) and might be responsible for the lack of heterozygous ABO genotypes. However, allelic dropout occurs randomly and it should not always affect the same allele in independent amplifications of a sample. Because all ABO genotypes were confirmed through multiple amplifications of multiple extracts, it is unlikely that allelic dropout was a major problem in this study. Other possible explanations for the paucity of heterozygotes in this sample include inbreeding and sampling error, and data from additional samples would help to distinguish between these possibilities.

Although studies of other ancient populations are needed to better characterize the ABO frequencies present in the Americas before European contact, the precontact sample reported here did not differ significantly from extant Native Americans from the same region. This study therefore provides no evidence that historical events greatly altered the pattern of indigenous ABO diversity. The precontact sample was significantly different from the extant Siberians, indicating that it is statistically unlikely that it was drawn from a population with ABO frequencies similar to those seen in Siberia. These results are most consistent with the hypothesis of a founder effect during the initial peopling of the Americas. It is possible that the high frequency of O stems from other events in Native American prehistory, such as selection in response to infectious diarrhea or other gastrointestinal ailments in a new environment (Vogul and Motulsky, 1986). However, a founder effect is more compatible with the evidence from other genetic loci (Zegura et al., 2004; Hey, 2005; Schroeder et al., 2007; Tamm et al., 2007; Wang et al., 2007; Fagundes et al., 2008). In addition, we note that the high frequency of O in this one precontact population could have been due to isolation and localized genetic drift, but such a scenario is inconsistent with both archaeological and mtDNA evidence (Bolnick and Smith, 2007). Thus, while the results from this study are suggestive, ABO genotyping should be performed in other ancient and extant populations to better test the founder effect hypothesis.

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