Brief Communication: Haplogroup X Confirmed in Prehistoric North America

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ABSTRACT Haplogroup X represents approximately 3% of all modern Native North American mitochondrial lineages. Using RFLP and hypervariable segment I (HVSI) sequence analyses, we identified a prehistoric individual radiocarbon dated to 1,340 ± 40 years BP that is a member of haplogroup X, found near the Columbia River in Vantage, Washington. The presence of haplogroup X in prehistoric North America, along with recent findings of haplogroup X in southern Siberians, confirms the hypothesis that haplogroup X is a founding lineage. Am J Phys Anthropol 119:84–86, 2002. © 2002 Wiley-Liss, Inc.

Using RFLP and hypervariable segment I (HVSI) sequence analyses, a mitochondrial DNA haplogroup that represents approximately 3% of all modern Native North American samples (Forster et al., 1996; Brown et al., 1998; Smith et al., 1999) and whose known worldwide distribution also includes West Asia and Europe (Torroni et al., 1996) has been identified. Due to the absence of haplogroup X in East Asia, presumed to be the homeland of the founders of Native America, diverse hypotheses were proposed to explain the presence of this haplogroup in North America. Brown et al. (1998) suggested the possibility of an ancient European migration to North America. Stanford (1997) suggested that early Holocene Europeans used a trans-Atlantic route to colonize the Americas and that Clovis technology was derived from the Solutrean tradition of the Iberian Peninsula. The presence of haplogroup X in North America might also be due to recent European admixture, since this haplogroup is found in high frequency in the Ojibwa (Brown et al., 1998; Malhi et al., 2001), among whom nuclear markers exhibit evidence of extensive European admixture (Szathmary and Auger, 1983).

Most of these hypotheses have been refuted by more recent research. Derenko et al. (2001) reported the presence of haplogroup X in Altaian populations from southern Siberia, where the other four Native American founding haplogroups are also present. Therefore, the Altai are the only known modern ethnic group whose membership represents all five Native American haplogroups and, assuming the New World was colonized by a single migration, constitute a possible origin of the founders of Native America. Despite unsuccessful attempts to extract ancient mtDNA from the Kennewick specimen (Kaestle, 2000; Merriwether and Cabana, 2000; Smith et al., 2000b), other early Holocene skeletons in North America with cranial features unlike modern Native Americans (e.g., from Wizard's Beach and Hourglass Cave) exhibit haplogroups that are found in Native Americans but not in Europeans (Stone and Stoneking, 1996; Kaestle and Smith, 2001). These lines of evidence, together with recent criticism of similarities between the Clovis and Solutrean cultures (Straus, 2000) that were cited by Stanford (1997), strongly suggest that haplogroup X did not reach the Americas via an ancient European migration.

The wide geographic and linguistic distribution of haplogroup X in modern Native North American groups (Forster et al., 1996; Brown et al., 1998; Smith et al., 1999) and the predominance among those of a characteristic mutation in HVSI of the control region not found in European or Asian members of haplogroup X (the G → A transition at np 16,213), imply that it is a founding Native American lineage. In addition, the presence of three (allegedly full-blood) Algonquian-speaking Native Americans (Smith et al., 2000a; Malhi et al., 2001) who exhibit both the haplogroup X with the transition

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at np 16,213 and the rare Albumin marker, Albumin*Naskapi, also only found in North America, suggests that haplogroup X is not found in North America due to recent European admixture.

The most convincing evidence that haplogroup X is not the result of Viking or even more recent European admixture would be its presence in ancient Native Americans. Ancient samples from the Norris Farms site (Stone and Stoneking, 1998), the Windover site (Hauswirth et al., 1994), and the Amazon Basin (Ribeiro-Dos-Santos et al., 1996) exhibit the characteristic HVSI control region markers found in individuals assigned to haplogroup X, but they could not be confidently assigned that haplogroup because they were not tested for the *Acc*I restriction site at np 14,465. We confirmed the presence of haplogroup X in one prehistoric sample excavated at a site on the Columbia River near Vantage, Washington and radiocarbon dated to 1,340 ± 40 years BP. Extensive precautions were taken to limit contamination of this sample and to detect any such contamination when it did occur. To eliminate surface contamination, the tooth was soaked in 10% bleach for 10 min, followed by ultraviolet light (254 nm) irradiation. Extraction and amplification setups were performed in a dedicated ancient DNA laboratory that is routinely bleach-sterilized. Negative controls were included at various stages of the extraction and amplification setup processes. Multiple extractions were performed on the tooth to confirm haplogroup assignment. For a complete review of the extraction method and precautions used to prevent contamina-

TABLE 1.
Nucleotide positions of the mutations for the prehistoric sample and lab researcher

	16189	16213	16223	16234	16240	16242	16278	16311	14465 AccI
CRS	T	G	C	C	A	C	C	T	–
Vantage	C	A	T	.	.	.	T	.	+
RSM	.	.	.	T	C	G	.	C	–

CRS is the Cambridge Reference (Anderson et al., 1981). A dot (.) denotes identity with the Cambridge Reference sequence; a plus sign (+) denotes a site gain; minus sign (−) denotes a site loss.

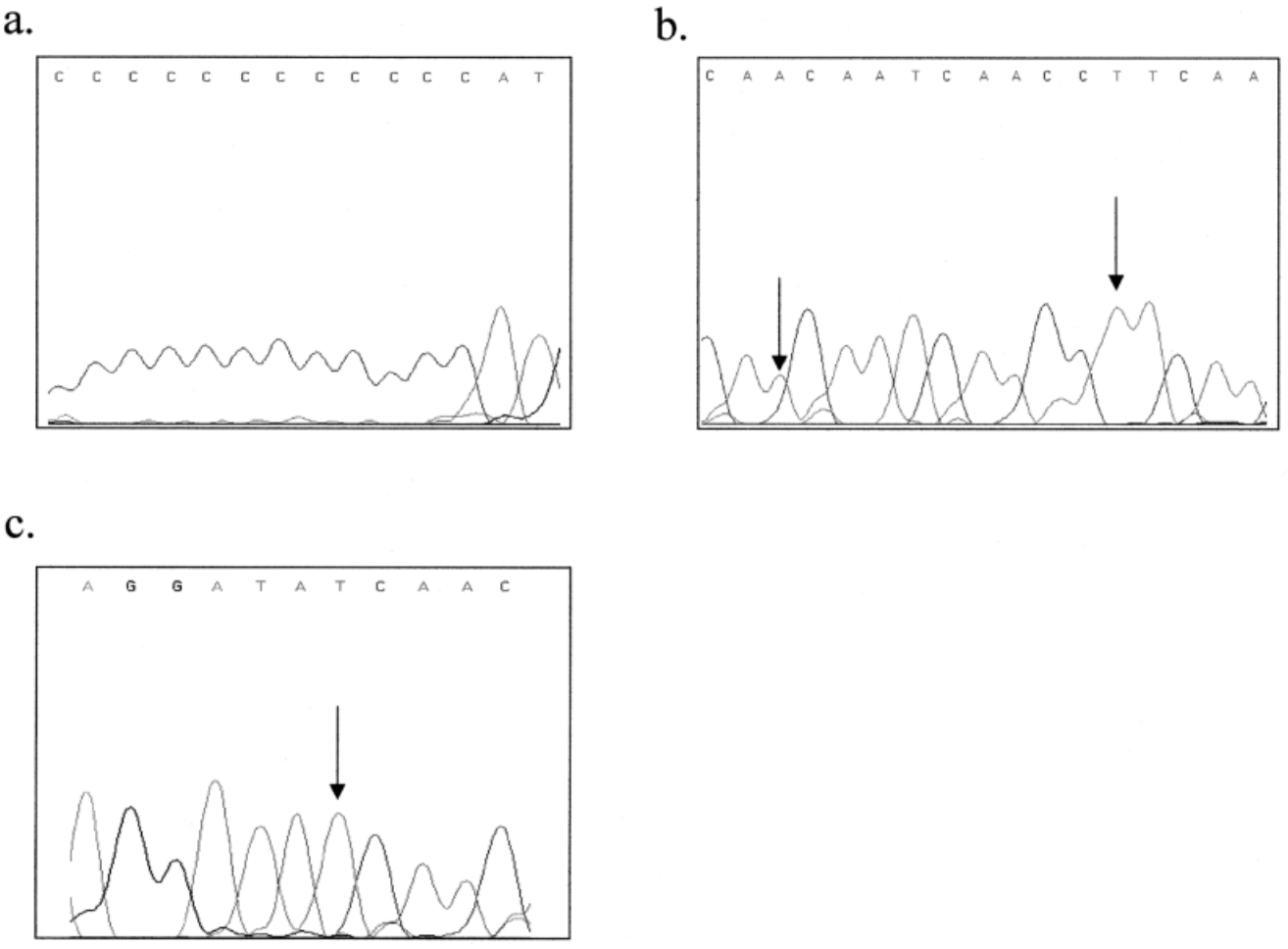

Fig. 1. Electropherogram of mutational sites for the prehistoric sample. **a:** Reverse complement electropherogram, displaying the Poly C region created by a T → C transition at np 16,189. **b:** Reverse complement electropherogram, displaying the G → A transition and C → T transition at np 16,213 and np 16,223, respectively. **c:** Electropherogram displaying the C → T transition at np 16,278.

tion of the sample, see Smith et al. (2000b). Extraction and most amplification setup negative controls exhibited no evidence of contamination. When negative controls did exhibit contamination, the data was excluded from the analysis. Samples from the Vantage site (500–2,000 years BP) were well-preserved, and a high percentage (88%) could confidently be assigned to a haplogroup (Malhi, 2001). This sample exhibited the HVSI control region markers (transitions at np 16,189, 16,223, and 16,278) and the *Acc*I restriction site at np 14,465 found in individuals assigned to haplogroup X as well as the G → A transition at np 16,213 that is specific to the Native American subclade of haplogroup X (Table 1; Fig. 1). To the best of our knowledge, this is the first evidence of haplogroup X in prehistoric America to be confirmed using both control region markers and the diagnostic restriction site gain in the coding region. This verifies a prehistoric presence of haplogroup X in North America that is probably derived from a Siberian source population.

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