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Address for correspondence and reprints: Dr. David C. Page, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142.
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mtDNA and Y Chromosome–Specific Polymorphisms in Modern Ojibwa: Implications about the Origin of Their Gene Pool

To the Editor:

The Ojibwa, the second-largest Native American group in Canada, are an Algonkian-speaking people, who have the greatest concentration in the province of Ontario (Szathmary et al. 1974). Their geographic distribution has been established in the last two centuries. During this period, the population has been divided into two main branches that have accumulated some cultural (Dunning 1959) and genetic differentiation (Szathmary et al. 1974). The northern Ojibwa appear to have been least influenced by Europeans, whereas the southeastern Ojibwa of the Lake Huron region appear to have been exposed to a great European influence. On the basis of genetic analysis of some serum and red-cell protein polymorphisms, Szathmary and Reed (1972) and Szathmary et al. (1974) were able to reveal the presence of "Caucasian" alleles in the southeastern Ojibwa and to give an estimate of Caucasian admixture of ~30%; however, more recent data on other autosomal locus polymorphisms indicate that the genetic admixture may be as great as 50% (D. E. C. Cole and L. A. Rubin, unpublished data).

Previous studies of mtDNA variation have shown that the Ojibwa are the Native American group with the highest proportion (~25%) of mtDNAs not belonging to haplogroups A, B, C, and D (Torroni et al. 1993). These additional mtDNAs, which are all defined by the

absence of the *Dde*I sites at nucleotide positions (nps) 1715 and 10394, were initially attributed to recent genetic admixture with Europeans (Torroni et al. 1993). However, a recent extensive reanalysis of Native American control region and haplotype data (Forster et al. 1996) has shown that these additional Ojibwa mtDNAs belong to a well-defined haplogroup that corresponds to the haplogroup X uncommonly observed in Europeans (Torroni et. al., in press). The data from the study by Forster et al. (1996) indicate that haplogroup X in the Ojibwa is not due to post-Columbian genetic admixture with Europeans, but it represents a fifth Native American founding haplogroup of Asian ancestry, which is currently limited to the Algonkian-speaking populations.

To gather new data on mtDNA variation in the Ojibwa, we have screened the mtDNAs of 35 subjects from Manitoulin Island in Lake Huron (southeastern Ojibwa) for the marker mutations that define the five Native American haplogroups (table 1). This Ojibwa sample showed haplogroup frequencies not significantly different (two-tailed Fisher's exact test: $P \cong .06$) from those of the Ojibwa from northern Ontario (Torroni et al. 1993), and only two mtDNAs were found to lack the marker mutations that characterize haplogroups A, B, C, D, and X. A detailed screening for the European mtDNA markers (Torroni et al. 1994 and in press) showed that both of these mtDNAs were defined by the *Alu*I site loss at np 7025 and were members of haplogroup H, the most-common European-specific haplogroup. Therefore, mtDNA analysis suggests that only 5.7% of the Ojibwa gene pool can be attributed to Europeans. This finding does not support a strong European genetic influence in modern Ojibwa, unless gene flow had occurred almost exclusively through European males.

To determine the origin of Ojibwa Y chromosomes, we have analyzed all males encompassed in our sample for the recently described DYS199 polymorphism (Underhill et al. 1996). This polymorphism consists of a C-to-T transition at np 181 and is apparently confined to Native American populations. The T allele was reported in the Karitiana (100%), the Surui (94.1%), the Mayan (63.5%), the Eskimos (66.7%), and the Navajo (50.0%) but in none of 119 subjects from Old World populations. To define Y chromosome haplotypes, our Ojibwa males were also analyzed for the DYS287 Y *Alu* polymorphism (YAP; Hammer and Horai 1995) and the variation at the microsatellite locus DYS19 (Roewer et al. 1992). The Ojibwa Y chromosome haplotype frequencies are provided in table 2, along with the data obtained on four additional population groups (Europeans, western Africans, southeastern Asians, and the Guahibos from the Estados Amazonas in Venezuela) that were examined for comparison.

The data provided in table 2 confirm that the T allele at the DYS199 locus appears to be confined to the American continent and is in strong linkage disequilibrium with a particular microsatellite allele (186 bp) at the DYS19 locus. This combination could well represent a specific haplotype associated with the process of human colonization of the New World, as proposed by Underhill et al. (1996). However, in contrast to the South American Guahibos in whom the T allele reaches a frequency of 87.1%, the Ojibwa were found to harbor only the C allele at the DYS199 locus. This observation makes the Ojibwa the only Native American population in which, at least for the moment, the DYS199 polymorphism has not been reported.

Genetic drift and founder events may have eliminated the T alleles from the population. However, the Ojibwa retain all of the Amerind mtDNA haplogroups (table 1), thus making unlikely a dramatic founder event affecting only Y chromosomes. Therefore, we can envision only two possible explanations for the absence of the T allele at the locus DYS199 in the Ojibwa from Manitoulin Island. First, a massive intrusion of Y chromosomes from non–Native American populations may have extremely diluted the T alleles in modern Ojibwa. This scenario would indicate that the European gene flow

Table 1

mtDNA Haplogroups in the Ojibwa

		Haplogroup[b]					
Population	No.[a]	A (+663 *Hae*III)	B (9-bp deletion)	C (+13262 *Alu*I)	D (−5176 *Alu*I)	X (−1715 *Dde*I)	H (−7025 *Alu*I)
Ojibwa (Manitoulin Island)	35	31.4	8.6	25.7	2.9	25.7	5.7
Ojibwa (northern Ontario)[c]	28	64.2	3.6	7.1	...	25.0	...

[a] Number of subjects analyzed.

[b] The marker mutations that define each haplogroup are indicated in parentheses. Haplogroup frequencies are in percent. Haplogroup H is European specific (Torroni et al. 1994 and in press).

[c] The data for the Ojibwa from northern Ontario are from Torroni et al. (1993).

Table 2

Y-Chromosome Haplotypes in the Ojibwa and Other Human Populations

		Haplotype[b]									
		C/*Alu*+[c]				C/*Alu*−					T/*Alu*−
Population	No.[a]	186	190	194	198	186	190	194	198	202	186
Ojibwa	16	...	...	...	...	4	7	3	2	...	...
Guahibos	31	...	...	1	...	...	2	1	...	...	27
Europeans	23	1	...	...	...	...	17	5	...	...	...
Western Africans	24	...	4	8	4	...	...	7	1	...	...
Southeastern Asians	28	...	...	...	...	...	...	20	6	2	...

[a] Number of subjects analyzed.

[b] The alleles at the DYS199 locus are indicated by a C or a T; the presence/absence of the *Alu* insertion is indicated by *Alu*+/*Alu*−; the alleles at the DYS19 microsatellite locus are indicated by the length of the corresponding PCR fragment.

[c] The presence of the *Alu* insertion could be indicative of African ancestry (Hammer 1994; Spurdle et al. 1994). The analysis of the two *Alu*+ Y chromosomes observed in one Guahibo and one European for the presence of the "African" G allele at the DYS271 locus (Seielstad et al. 1994) failed to provide any evidence of African origin for the European Y chromosome but revealed the presence of the G allele on the Guahibo Y chromosome. On the basis of a frequency of the G/*Alu*+ haplotype among Africans of 65.1% (231/355; R. Scozzari unpublished data) and of 0.0% for the same haplotype among Native Americans (0/30; Seielstad et al. 1994), this gave a rough estimate of African admixture in the Guahibos of about 5%, similar to that previously obtained with nine autosomal markers (Salzano and Callegari-Jacques 1988).

in modern Ojibwa was not only extensive but almost exclusively male-mediated, and it is supported by the high value (~30%) of European genetic admixture for the autosomal genes (Szathmary et al. 1974), the high frequency of the T allele (~50%) among Native Americans of North America (Underhill et al. 1996), and the limited presence of European mtDNAs in modern Ojibwa. Second, the founding gene pool of the Ojibwa, and in general of the Algonkian-speakers, was different from that of the other Amerind populations. That is, the Algonkians may have resulted from a distinct migrational event from Asia, and therefore we may not expect to find the T allele in the Ojibwa. This hypothesis is supported by the high incidence of haplogroup X mtDNAs in the Algonkians, their absence in the Na-Dene, the Eskimos, and other Amerind populations (Torroni et al. 1993; Forster et al. 1996), and the geographical location of the Algonkians who represent the northernmost of the Amerind population groups.

Data currently available do not permit us to choose between these two hypotheses. However, we believe that additional data on the frequency and distribution of the DYS199 polymorphism in the northern Ojibwa and other Algonkian populations may resolve this issue.

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Rosaria Scozzari,[1,2] Fulvio Cruciani,[1] Piero Santolamazza,[1] Daniele Sellitto,[2] David E. C. Cole,[3] Laurence A. Rubin,[3] Damian Labuda,[4] Elisabetta Marini,[5] Valeria Succa,[5] Giuseppe Vona,[5] and Antonio Torroni[1]

[1]*Dipartimento di Genetica e Biologia Molecolare, Università La Sapienza, and* [2]*Centro di Studio sulla Genetica Evoluzionistica, CNR, Rome;* [3]*Departments of Clinical Biochemistry, Medicine, and Genetics, University of Toronto, Toronto;* [4]*Centre de Cancérologie Charles Bruneau, Centre de Recherche de l'Hopital Sainte- Justine, Montreal; and* [5]*Dipartimento di Biologia Sperimentale, Sezione di Scienze Antropologiche, Università di Cagliari, Cagliari*

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Address for correspondence and reprints: Dr. Rosaria Scozzari, Dipartimento di Genetica e Biologia Molecolare, Università "La Sapienza," P. le Aldo Moro 5, 00185 Roma, Italy. E-mail: rscozzar@axcasp.caspur.it
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Diagnostic Testing: A Cost Analysis for Prader-Willi and Angelman Syndromes

To the Editor:

Recently, two different approaches were described by the American Society of Human Genetics/American College of Medical Genetics Test and Technology Transfer Committee (ASHG/ACMG) (1996) regarding the molecular diagnosis of Prader-Willi syndrome (PWS) and Angelman syndrome (AS). It was recommended that individual laboratories use an approach based on a number of factors, including the local availability of testing, previous results for specific patients, and the level of diagnostic expertise of the referring physician. With the rising cost of medical care and the need to manage cost in all areas of health care, we propose that cost also be considered a factor.

We considered three approaches for molecular diagnostic testing of these syndromes. Approach IA is the same as approach I described by the ASHG/ACMG (1996) and begins with methylation studies. Normal methylation results essentially rule out PWS and most AS. If methylation results are positive, FISH and PCR can be used to determine whether deletion, uniparental disomy (UPD), or imprinting mutations are present. If a 15q11-q13 deletion is present, a parental karyotype is obtained (paternal for PWS, maternal for AS) to identify any parental chromosome abnormality. The second approach, IB, is an alternative to approach IA and begins with FISH studies. A 15q11-q13 deletion by FISH confirms PWS or AS. Parental karyotypes can then be obtained as described above. Negative FISH results are followed by methylation studies. Positive methylation results confirm a diagnosis of PWS or AS, depending on which parental allele is missing, and should be followed by PCR to identify UPD and imprinting mutations. The third strategy is approach II as described by the ASHG/ACMG. The order of tests to be performed in this approach, as described in the ASHG/ACMG report, is not intrinsically clear to the reader. For the sake of this discussion, we assumed that FISH and methylation studies are performed simultaneously in approach II. Normal methylation and FISH results rule out PWS and most AS. Abnormal methylation with normal FISH results should be followed by PCR, to distinguish UPD and imprinting mutations. Positive FISH results should be followed by a parental karyotype.

Smith et al. (1995) suggested that FISH replace the high-resolution karyotype in the diagnosis of PWS and AS because a high-resolution karyotype is less reliable than FISH in detecting deletions in PWS and AS. In addition, they recommended a routine karyotype for all patients, to identify other chromosome abnormalities. DNA studies in those with negative FISH studies were recommended only if the clinical diagnosis was reconfirmed. This approach is similar to approach IB.

Consensus of diagnostic criteria for PWS (Holm et al. 1993) and AS (Williams et al. 1995) have been proposed. When Holm's criteria (Holm et al. 1993) for PWS are met, a molecular mutation (deletion, UPD, or imprinting) can be identified in 96%–97% of patients (Robinson et al. 1991; Gillessen-Kaesbach et al. 1995). In the study done by Gillessen-Kaesbach (1995), the patients who did not meet Holm's criteria had negative molecular studies. While a score of ≥ 8 points, according to Holm's criteria, is considered "typical" PWS, Erdel et al. (1996) found that 30% of patients with 5 or 6 points ("suspected" PWS) had positive molecular stud-