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Saami Mitochondrial DNA Reveals Deep Maternal Lineage Clusters

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Abstract
The mitochondrial DNA of 62 Saami from the north of Norway was analyzed in the D loop hypervariable region I and II and sequences were compared to other gene pools. Two major (lineage 1 and 2) and two minor (lineage 3 and 4) maternal lineage clusters were found. Lineage 1 (56.9% of all hitherto analyzed Saami samples) contains a substantial number of branching haplotypes which are unknown in European gene pools. Lineage 2 (31.5%) and lineage 4 (3.6%) have few branching points and are present at a low rate throughout European gene pools. Lineage 3 (4.7%) has polymorphisms characteristic of circumpolar lineages.

Introduction

Human mitochondrial DNA (mtDNA) sequence analysis has become a standard tool for studying the genetic history of human populations, the genetic variation within ethnic groups, their genetic relations to other populations and the past occurrence of population bottlenecks. Several characteristics of mtDNA give insight into human population history: mtDNA is inherited maternally and it is predominantly uniclonal within cells [1]. Furthermore, due to their hemizygous nature, lineages are in general not perturbed by recombination even though rare events of recombination have been reported [2]. mtDNA polymorphism is mostly studied in the two rapidly evolving hypervariable regions within the noncoding displacement (D) loop [3–5]. Different estimates for the substitution rate in the D loop have been derived from phylogenetic analyses [6–11] ranging from 0.025 to 0.26/site/million years and studies of individual family trees suggest even higher substitution rates [1].

The Saamis are a northern Scandinavian population with a genetic history that is different from other European populations [12].

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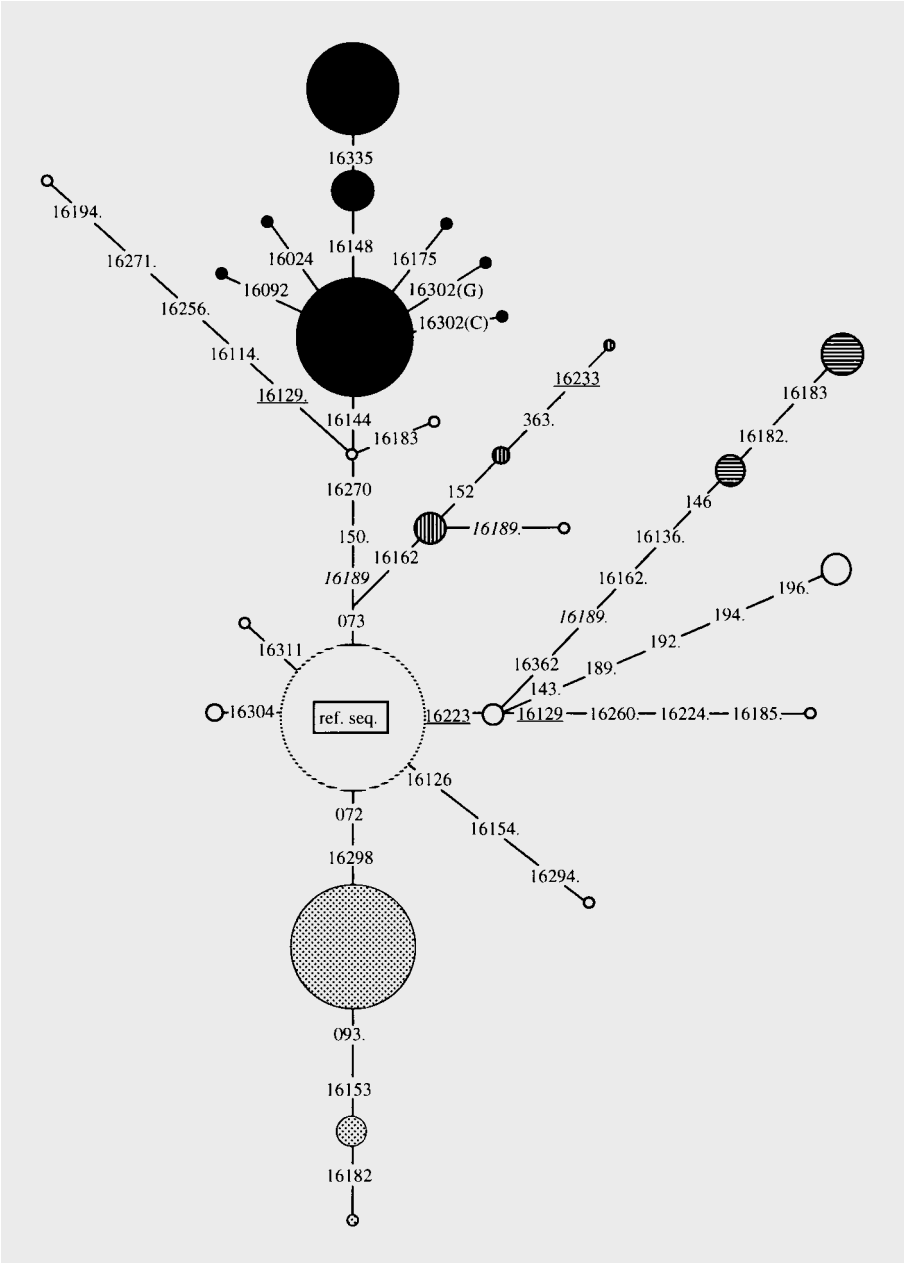

Fig. 1. Skeleton network constructed from Saami mtDNA haplotypes using our own data and data from Lahermo et al. [18], Dupuy and Olaisen et al. [17] and Richards et al. [21]. The size of the circles is proportional to the frequency of a given haplotype. Black circles mark lineage 1, gray circles mark lineage 2, horizontally striped circles mark lineage 3 and vertically striped circles mark lineage 4. The sequence of branching substitutions could not always be established, in particular in rare lineages without known intermediate haplotypes and are marked with a dot. Positions with possible parallel substitutions, e.g. 16233, are underlined. The hypervariable position 16189 is in italics.

Language studies have revealed that the 9 (other authors 7–8) different Saami languages (Kildin, Skolt, Ter, Inari, Northern (coast, inland), Lule, Pite, Southern and Ume) are a part of the Finno-Ugric language family [13–15] but it was recently suggested that the Finnish population, which is genetically close to the European gene pool, has adopted their language from Saami-speaking people while giving up a presumed original Indo-European language [16]. This hypothesis would make Saami people important players in North European language development. Genetically, our and other studies suggest an extensive admixture between various Saami subpopulations [12, 17, 18] (fig. 1). Previous studies of Saami mtDNA using hypervariable region I revealed two major lineages [12, 17, 18]. Our analysis which includes both mtDNA hypervariable regions confirms the predominance of two deep maternal lineages and the presence of two further minor lineages. The given data allow the construction of a phylogenetic tree that links Saami haplotypes to known Eurasian lineages.

Materials and Methods

DNA

Blood samples were collected from unrelated donors with a Saami family history in the inland area of Kautekeino and Karasjok, and in the costal area of Tana, Porsanger and Nesseby. Leukocytes were isolated by a modification of the procedure described by Blin and Stafford [19]. DNA from leukocytes was extracted by incubation overnight at 37°C in an extraction buffer containing 1 mg/ml proteinase K, 1% DTT, 400 m*M* NaCl, 2 m*M* EDTA, 10 m*M* Tris, pH 8.2 and 0.5% SDS and DNA was extracted with phenol-chloroform [20].

PCR

Genomic DNA was amplified by PCR using 200 n*M* of the primers described by Vigilant et al. [6]. Amplification was performed using 10–50 ng genomic DNA, 200 μ*M* of each dNTPs and 1.25 U DNA polymerase (Dynazyme, Heidenreich) in the buffer provided by the supplier. After an initial denaturation for 4 min at 95°C, 35 amplification cycles followed by an annealing for 1 min at 55 and 58°C for hypervariable region I and II, respectively, an extension for 1 min at 72°C, a denaturation for 1 min at 95°C and a final extension at 72°C for 10 min.

Sequencing

5 μl from each PCR product were incubated for 30 min at 37°C with 10 U exonuclease I and 1 U shrimp alkaline phosphatase (USB). Enzymes were inactivated for 5 min at 80°C. 1.5 μl of the so treated PCR fragments were sequenced manually using an AmpliCycle sequencing kit (Applied Biosystems). Sequences were analyzed from position 16024 to 16374 in hypervariable region I and from position 64 to 378 in hypervariable region II. Where possible, further flanking sequences were included.

Results and Discussion

In the present study we have sequenced the major region encompassing hypervariable region I and II from 62 unrelated Saami people from northern Norway and related it to known human mtDNA sequence polymorphisms in other Saami populations in circumpolar regions of Europe and Asia.

Lineage 1 is characterized by the transitions A-G 263, A-G 073, C-T 150, C-T 16270, hypervariable T-C 16189 and T-C 16144 [3], and a single or two substitutions at the common T-C 16144 branching point (fig. 1, 2) indicating a recent diversification of a common ancestral line. Both, C-T 16270 and T-C 16189 transitions (even though not in combination) are known in European populations. The A-G 073 transition is believed to be an early branching event being present in about 50% of British haplotypes and absent in the remaining haplotypes with a minimum age for the branching event of betweeen 15,000 and 25,000 years before present [21]. The initial C-T 16270 transition in lineage 1 is found for example in 8% of the white English Cauca-

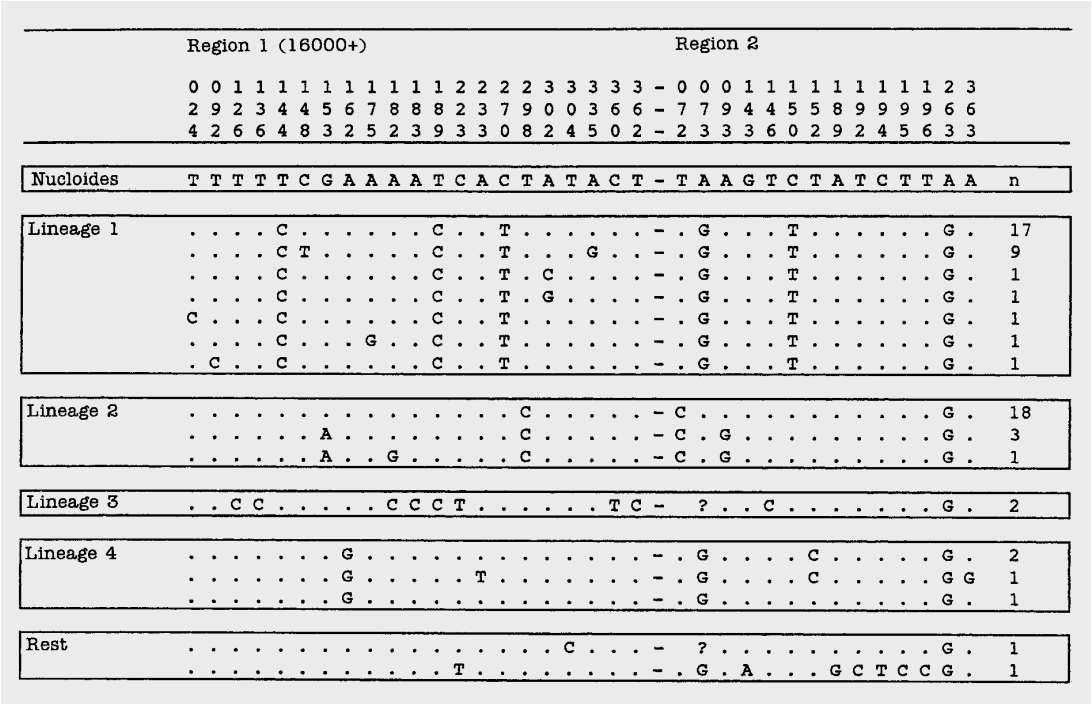

Fig. 2. Distribution of Norwegian Saami mtDNA sequences. Nucleotides according the Anderson et al. [3].

sians [22] and in 6.5% of Sardinians [23]. However, the subsequent T-C 16144 transition in lineage 1 and its further branches are unique to Saami and have hitherto not been found in other populations [9, 21, 23, 24–27]. It remains to be seen whether it can be found in Uralic-speaking people, for example Samoyeds [25].

Different estimates have been given for the substitution rate in the D loop depending on the inclusion of variable nucleotide positions that may have experienced multiple mutations and the size of the population in the calculations [1, 2, 6–11]. If a substitution rate between 1/5,000 and 1/10,500 years in the hypervariable region I is assumed, an ancestry of the root of lineage 1 of more then 5,000–10,500 years may be suggested. However, dating of branching events in mtDNA is controversial and since the mutation rate of mtDNA is not completely understood it is not possible to draw firm conclusions about the genetic history of lineage 1.

Lineage 2 is characterized by the T-C 16298 and 072 T-C transitions and the one side branch (G-A 16153; A-G 093, A-G 16182 [3]; fig. 1, 2, table 1) combined with the lack of the A-G 073 transition. Lineage 2 is found in 35.5% of the analyzed Norwegian Saami Sequences. The T-C 16298 transition is also seen in 12% Finns and 6% Karelians, and rather homogeneously in 2–11% of other European populations, in a few cases with branching haplotypes [12, 18, 21–24]. However, lineage 2 has not been found in Far East Asia and in eastern circumpolar areas [9, 11,

Table 1. Distribution of haplotype lineages in Saami populations

	Kautek[a, b]		Karasjok[a, b]		Coast Northern[b]		Finland[c]		Total[a, b, c]	
	n	%	n	%	n	%	n	%	n	%
Lineage 1	57	76	80	51.3	9	39.1	11	50[d]	157	56.9
Lineage 2	17	22.6	62	39.7	6	26.1	2	9.1	87	31.5
Lineage 3	–	–	5	3.2	2	8.7	6	27.3	13	4.7
Lineage 4	–	–	6	3.9	4	17.4	–	–	10	3.6
Others	1	1.4	3	1.9	2	8.7	3	13.6	9	3.3
Total	75		156		23		22		276	

[a] Dupuy and Olaisen [17].
[b] This report.
[c] Lahermo et al. [18].
[d] 38.4% lineage 1 if Sajantila et al. [12] is included.

27]. The occurrence of the T-C 16298 mutation in other populations has been interpreted as a possible parallel substitution [12]. However, the presence of the two coupled transitions, T-C 16298 and 072 T-C, in Saami and in other European mtDNAs makes a parallel evolution in Europe highly unlikely.

The predominance of two rather divergent major mtDNA lineages in the Saami gene pool would be most easily explained by two independent migration waves of small founding populations into Northern Scandinavia, one being the founders of the more diverse lineage 1 and the other the founders of the less diverse lineage 2. The different roots for both lineages are supported by the suggested ancestry of the A-G 073 substitution that separates lineage 2 from lineage 1, and the widespread distribution of lineage 2 in Europe contrasting with an almost complete absence of lineage 1 in Europe (with the exception of Finland) [18, 21]. Alternatively, a simultaneous admixture of small, isolated and stationary populations may account for the observed haplotype dichotomy.

Due to the low degree of genetic diversification of lineage 2 and its uneven geographic distribution (showing a frequency of 9.1% in the lake Inarii area in Finland [12] but a frequency of 60% in Norbotten, Sweden) it is tempting to speculate that lineage 2 contributed to the Saami gene pool more recently than lineage 1. This would be in accordance with Richards et al. [21], who classified lineage 2 as a European lineage with more recent roots than lineage 1. The northwards spread of tool types in the neolithic and early Bronze Age has been documented both along the Norwegian coast into Finnmark (Norway) and along the Swedish coast into Norbotten (Sweden) and may coincide with the introduction of mtDNA haplotypes into the Saami gene pool. From 600 AD, a more massive northwards expansion of the Indo-European-speaking 'Norrøn' population is documented, however lineage 2 is found at only a low frequency in random Norwegian mtDNA (3%) [17] and is not present in the hitherto analyzed Icelandic mtDNA [12, 21] making those migrants a possible but unlikely source for the introduction of lineage 1 into the Saami gene pool.

Two more lineages have been found that occur at low frequency. Lineage 3 (4.7%) includes a 16223 C-T and 16362 T-C transition (fig. 1, 2) that is frequent in eastern circumpolar areas (found in 36 of 37 circumpolar lineages [9]), but absent in Europe. The origin of this lineage is thus likely to be, directly or indirectly, in the east. The low frequency of this lineage in the analyzed Norwegian Saami samples (Kautekeino 0%, Karasjok 3.2%, coast 17.4%) is interesting, as the Saami culture is seen, by archaeological evidence, as a part of a larger circumpolar culture [26]. It is thus striking that these circumpolar contacts did not leave more genetic traces in the Norwegian Saami mtDNA. One explanation would be an introduction of lineage 3 into the Saami gene pool by for example trade contacts between circumpolar populations. Another explanation would be an early presence of lineage 3 in Northern Scandinavia after an initial expansion of circumpolar populations that is documented in the final stages of the last glaciation, followed by a reduction in predominance by subsequent Uralic and European immigrants. A third scenario would be a simultaneous admixture of various immigrants at the end of the last glaciation. Only the analysis of ancient DNA will be able to settle the timing of the introduction of different lineages into the current Saami mtDNA gene pool.

Lineage 4 is well within the European gene pool [21] and is found at a frequency of 3.6% in Saami samples (fig. 1, 2).

In summary, we have analyzed the genetic composition of Saami mtDNA in northern Norway and correlated our findings with other European and circumpolar gene pools in an attempt to reconstruct aspects of the genetic history of Saami. Our findings are in accordance with the recent reports on Saami mtDNA and with the known archaeological records of the region [12, 17, 18, 21, 26]. More studies to trace genetically related populations to lineage 1 are currently under way.

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References

1 Parsons T, Muniec D, Sullivan K, Woodyatt N, Alliston-Greiner R, Wilson M, Berry D, Holland K, Weedn V, Gill P, Holland M: A high observed substitution rate in the human mitochondrial DNA control region. Nat Genet 1997;15:363–368.
2 Howell N, Kubacka I, Mackey DA: How rapidly does the human mitochondrial genome evolve? Am J Hum Genet 1996;59:501–509.
3 Anderson S, Bankler AT, Barrell BG, deBruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger S, Schreier PH, Smith AJH, Staden R, Yound IG: Sequence and organization of the human mitochondrial genome. Nature 1981;290:457–465.
4 Cann RL, Stoneking M, Wilson AC: Mitochondrial DNA and human evolution. Nature 1987;325:31–36.
5 Wallace DC: Mitochondrial DNA sequence variation in human evolution and disease. Proc Natl Acad Sci USA 1994;91:8739–8746.
6 Vigilant L, Pennington R, Harpending H, Kocher T, Wilson AC: Mitochondrial DNA sequences in single hairs from a southern African population. Proc Natl Acad Sci USA 1989;86:9350–9354.
7 Lundstrom R, Tavare S, Ward RH: Estimating substitution rates from molecular data using the coalescent. Proc Natl Acad Sci USA 1992;89:5961–5965.
8 Pesole G, Sbisa E, Preparata G, Saccone C: The evolution of the mitochondrial D-loop region and the origin of modern man. Mol Biol Evol 1992;9:587–598.
9 Shields G, Schmiechen AM, Franzier BL, Redd A, Voevoda MI, Reed JK, Ward RH: mtDNA sequences suggest a recent evolutionary divergence for Beringian and northern American populations. Am J Hum Genet 1993;53:549–562.
10 Ward RH, Redd A, Valencia D, Franzier BL, Pääbo S: Genetic and linguistic differentiation in the Americas. Proc Natl Acad Sci USA 1993;90:10663–10667.

11 Horai S, Hayasaka K, Kondo R, Tsugane K, Takahata N: Recent African origin of modern humans revealed by complete sequences of hominoid mitochondrial DNAs. Proc Natl Acad Sci USA 1995;92: 532–536.
12 Sajantila A, Lahermo P, Anttinen T, Lukka M, Sistonen P, Savontaus M-L, Aula P, Beckman L, Tranebjaerg L, Gedde-Dahl T, Issel-Tarver L, DiRienzo A, Pääbo S: Genes and languages in Europe: An analysis of mitochondrial lineages. Genome Res 1995;5:42–52.
13 Qvigstad J: Die lappischen Dialekte in Norwegen. Oslo, Brøggers Boktrykkeri, 1925.
14 Sinor D: Uralic languages: Description, history and foreign influences. Leiden, Brill, 1988.
15 http://www.sil.org/ethnologue/families/Uralic.html.
16 Sajantila A, Pääbo S: Language replacement in Scandinavia. Nat Genet 1995;11:359–360.
17 Dupuy BM, Olaisen B: mtDNA sequences in Norwegian Saami and main populations. Adv Forensic Homogen 1996;6:23–25.
18 Lahermo P, Sajantila A, Sistonen P, Lukka M, Aula P, Peltonen L, Savontaus M-L: The genetic relationship between the Finns and Finnish Saami (Lapps): Analysis of nuclear DNA and mtDNA. Am J Hum Genet 1996;58:1309–1322.
19 Blin N, Stafford DW: A general method for isolation of heigh molecular weight DNA from eukaryotes. Nucleic Acids Res 1976;3:2303–2308.
20 Kanter E, Baird M, Shaler R, Balazs I: Analysis of restriction fragment length polymorphisms in DNA recovered from dried bloodstains. J Forensic Sci 1986;32:403–408.
21 Richards M, Corte-Real H, Forster P, Macaulay V, Wilkinson-Herbots H, Demaine A, Papiha S, Hedges R, Bandelt H-J, Sykes B: Paleolithic and neolithic lineages in European mitochondrial gene pool. Am J Hum Genet 1996;59:185–203.
22 Bertranpetit J, Sala J, Calafell F, Underhill P, Moral P, Comas D: Human mitochondrial DNA variation and the origin of the Basques. Ann Hum Genet 1995;59:63–81.
23 Di Rienzo A, Wilson AC: Branching pattern in the evolutionary tree for human mitochondrial DNA. Proc Natl Acad Sci USA 1991;88:1597–1601.
24 Piercy R, Sullivan KM, Benson N, Gill P: The application of mitochondrial DNA typing to the study of white Caucasian genetic identification. Int J Legal Med 1993;106:85–90.
25 Piazza A, Rendine S, Minch E, Menozzi P, Mountain J, Cavalli-Sforza L: Genetics and the origin of European languages. Proc Natl Acad Sci USA 1995;92:5836–5840.
26 Haetta AM: Fra steinalder til samisk jernalder. Emneheft nr II Høgskolen i Finnmark. Vadsø, Helfjords Boktrykkeri, 1980.
27 Torroni A, Sukernik R, Schurr T, Starikorskaya Y, Cabell M, Crawford M, Comuzzie A, Wallace DC: mtDNA variation of aboriginal Siberians reveals distinct genetic affinities with native Americans. Am J Hum Genet 1993;53:591–608.