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Absence of the Asian-specific Region V Mitochondrial Marker in Native Beringians

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Summary

The Asian-specific 9-bp deletion between the genes for mitochondrial cytochrome oxidase II and lysine transfer RNA has been used to trace aboriginal human movements out of Southeast Asia and into portions of the South Pacific. Although it has been used to estimate the number of independent lineages that occur in the New World, it has not been studied in native peoples of the Beringian region. Thus, we have used PCR to amplify and compare the lengths of DNA segments surrounding this deletion in native peoples of Beringia and the adjacent regions, as well as natives of the Altai Mountains of Southwestern Siberia. Of the 176 individuals analyzed here, the deletion was found in only 3 of 25 individuals from the Ust-Kan region on the Altai Mountains. We comment on the distribution of this marker and on potential relationships between Beringians and other Native American groups in which this marker has been surveyed. One Chukchi possessed three copies of the 9-bp sequence, which suggests (1) that the number of copies of this sequence in humans may be more variable than had been believed and (2) that a mechanism of replication based on tandem duplication may be a potential explanation for the origin of this length mutation in humans.

Introduction

There is no argument among serious researchers that a Mongoloid stock first colonized the New World from northern Asia. Nor is there controversy about the fact that these continental pioneers used the exposed Bering Landbridge that then connected the Asian Far East with Alaska (Laughlin and Harper 1979; Szathmary 1985). However, there are still questions concerning the number of migrations into the New World, when they may have occurred, their composition, and the ultimate fate of the descendants of these early Americans (Owen 1984; Irving 1985; Greenberg et al. 1986; Szathmary, in press).

Stanford (1983) and Bada et al. (1984) propose a recent migration of all New World groups within the past 15,000 yrs. Turner (1984), Williams et al. (1985), and Greenberg et al. (1986) support a three-migration process, which implies that Paleo-Indians arrived 12,000–14,000 years before the present (BP), Na Dene about 10,000–11,000 BP, and Eskaleuts about 4,000 BP. On the basis of artifacts in caves, Adovasio and Carlisle (1984) support a deeper time depth (20,000–40,000 BP) for human occupation in the New World.

More recently, Schurr et al. (1990) and Torroni et al. (1992) have assessed variation among RFLPs in mtDNA of Native Americans and have proposed a minimum of four migrations accompanied by significant founder effects. However, Ward et al. (1991) used sequence analysis of the highly variable control region of mtDNA of the Nuu-Chah-Nulth (Wakashan) Indians of Vancouver Island, Canada, and described levels of genetic diversity inconsistent with founder effects.

Cann and Wilson (1983) inferred at least 10 length mutations from restriction-fragment analysis of whole mtDNA of humans, and, of these, the 9-bp deletion (9 bpd) between the genes for the second subunit of

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cytochrome oxidase and lysine transfer RNA in region V has been used as a marker for populations of Asian origin (Wrischnik et al. 1987; Hertzberg et al. 1989; Stoneking and Wilson 1989). Phylogenetic analysis suggests that this Asian-specific genetic marker has apparently arisen only once and occurs in about 15% of Southeast Asians (Wrischnik et al. 1987). It is fixed in some Polynesian groups (Samoans, Maoris, and Niueans), reaches very high frequencies in others (Cook Islanders, Tongans, and Fijians), but is absent from Aboriginal Australians and the Highlanders of Papua New Guinea (Hertzberg et al. 1989; Stoneking and Wilson 1989).

In the New World, the 9 bpd has been observed in Pimans and Navajos of Arizona and in Mayans of the Yucatan Peninsula (Schurr et al. 1990; Torroni et al. 1992). It has also been observed in low frequency in the Nuu-Chah-Nulth of Vancouver Island (Ward et al. 1991). It has not been observed in the Ticuna of the western Amazon region of Brazil (Schurr et al. 1990), in the Dogrib Indians of the central Canadian Arctic (Torroni et al. 1992), or in the mtDNA of the 7,000–8,000-year-old archaic Indian brain from Little Salt Spring, FL (Pääbo et al. 1988). None of these studies has included DNA of the extant native peoples of the Beringian region, and thus we have analyzed groups indigenous to this area, in the hope that a better understanding of the process of human entry into the New World might be obtained. Our use of the term "Beringian" refers to those native groups which presently live on either side of the Bering Strait or in the adjoining areas and who have been described, on the basis of genetic inference, as descendants of populations that inhabited Beringia.

Material and Methods

Sample Collection

The mtDNA of 176 individuals was analyzed in this study (table 1 and fig. 1). Whole mtDNA was purified from Athabascans and some Inupiaq Eskimos by means of cesium chloride density gradients (Carr and Griffith 1987), and the area surrounding the region V deletion was subsequently amplified and scored for the presence or absence of the deletion. DNA of the remaining sample was purified from whole blood via the phenol extraction procedure of Maniatis et al. (1982) and was amplified in the same way as the other samples. Forty-five individuals from the Altai region of southwestern Siberia (Ust-Kan, Chibit, and Ust Ulagan) were also included, since they are believed by

Table 1

Distribution of Asian-specific 9-bpd: Present Study

Population	Sample Size	No. Detected
Extant Beringians:		
Alaskan Athabascan	22[a]	0
Alaskan Inupiaq Eskimo	16[b]	0
Alaskan Yup'ik	9	0
Alaskan Aleut	4	0
Siberian Yup'ik	25	0
Chukchi	46	0
Soviet Far East:		
Yukagir	4	0
Nanaika	1	0
Lamut	1	0
Chuvanka	2	0
Koryak	1	0
South central Asia:		
Altai:		
Ust-Kan	25	3
Chibit	7	0
Ust-Ulagan	13	0
Total	176	3

[a] mtDNA isolated from placenta.

[b] Five of the 16 mtDNAs were isolated from placenta.

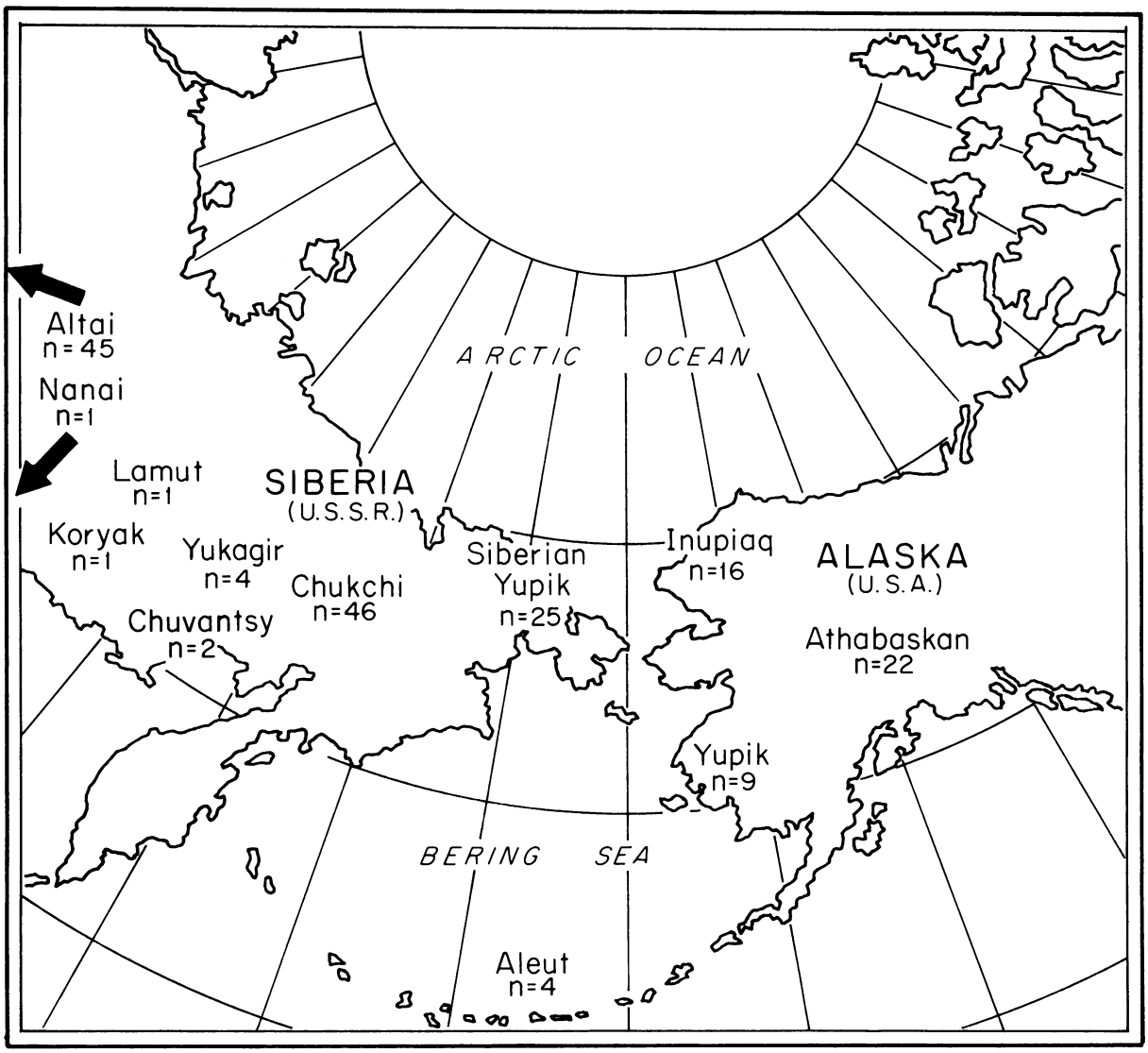

Figure 1 Map of Beringian region showing general locations and sample sizes of tribal groups from which mtDNA was analyzed

some Soviet scientists to be related to Asian Beringians (Schanfield et al. 1980).

Amplifications

The region of mtDNA surrounding the 9 bpd was PCR amplified in a Perkin Elmer Cetus thermal cycler. Samples were run as a 25-μl reaction mix containing 2.5 μl (10 μM) of each primer (A = 8196-5′-ACAGTTTCATGCCCATCGTC-3′-8215; B = 8316-5′-ATGCTAAGTTAGCTTTACAG-3′-8297 of Wrischnik et al. [1987]), 2.5 μl 10 × dNTP (10 mM) mix, 2.5 μl 10 × *Taq* buffer (Hertzberg et al. 1989), 1.5 units *Taq* polymerase, and 1 μl of DNA. The reaction mix was overlaid with a drop of mineral oil to prevent evaporation. DNA samples were denatured for 1 min at 94°C; primers were annealed to the template for 30 s at 56°C and were elongated for 30 s at 65°C. This process was repeated for a total of 40 cycles. Eight microliters of each amplified product were electrophoresed through 6% NuSieve agarose containing ethidium bromide in Tris-borate-EDTA buffer for 3½ h at 75 V. *Msp*I-digested pBR322 DNA was used as a size standard.

Sequencing of DNA

Single-strand DNA was obtained for sequencing through asymmetric PCR with the limiting primer diluted 1:50. The product was subjected to three successive rinses with water in Millipore centrifuge tubes and was resuspended in 40 μl water. DNA was sequenced via the dideoxy chain-termination procedure of Sanger et al. (1977) by using Sequenase. To anneal the DNA template and the primer, 7 μl DNA, 1 μl primer (A = 8196-5′-ACAGTTTCATGCCCATCGTC-3′-8215), and 2 μl 5 × reaction buffer (USB) were mixed in 1.5-ml microfuge tubes and were allowed to react for 4 min at 65°C. The samples were then cooled at room temperature for 45 min. A labeling mix containing 1 μl DTT, 1.8 μl sterile, double-distilled H_2O, 0.2 μl labeling mix, 0.5 μl dATP (35S), 1.7 μl enzyme dilution buffer, 1.0 μl Mn buffer, and 0.3 μl Sequenase was then added to each annealed sample and was allowed to react at room temperature for 5 min. A 3.5-μl portion of the sample was added to 2.5 μl of each deoxy/dideoxy termination mix in microtiter plate wells (Nunc) and reacted for 2 min at 37°C. Four microliters stop solution were added to each well to end the reaction. Samples were then electrophoresed through a 6% polyacrylamide gel which had been preconditioned for 2 h in 1 × TBE running buffer. Electrophoresis was carried out for 1 h at 50 W, and the gel was rinsed in a mixture of 10% acetic acid and 10% ethanol for 20 min. After drying, the gel was exposed to Kodak autoradiographic film overnight.

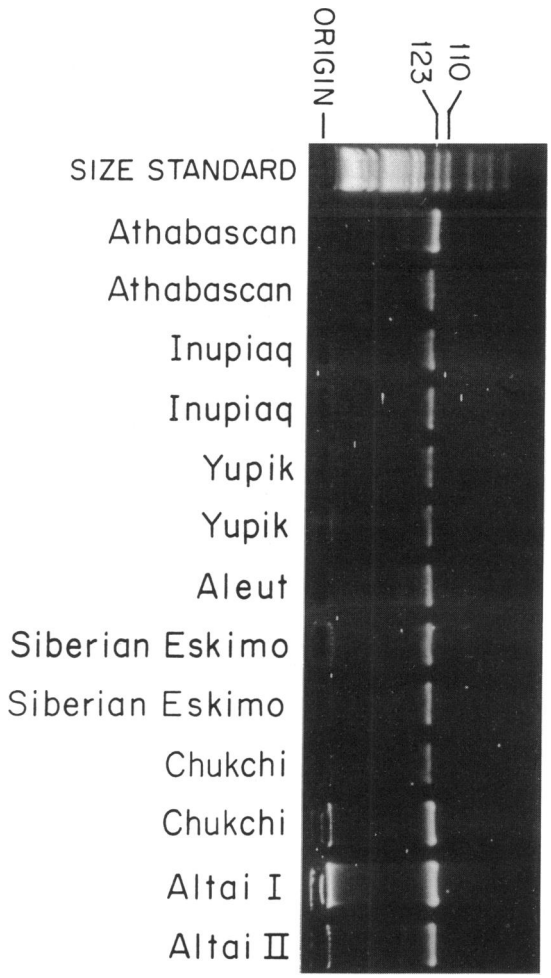

Figure 2 mtDNA of region V electrophoresed in 6% NuSieve agarose. *Msp*I-digested pBR322 DNA is used as a size standard at the left. All individuals shown have two copies of 9-bp sequence, and thus their DNAs are 121-bp in length.

Results

None of the 122 Beringians studied here possessed the 9 bpd (fig. 2 and table 1), nor did any of the nine indigenous peoples of the Soviet Far East (table 1). Of the 45 Altai surveyed, only 3 of 25 individuals from Ust-Kan possessed the deletion (fig. 3 and table 1). One of the 46 Chukchi possessed three copies of the CCCCCTCTA sequence (fig. 3). All of these observations have been verified at the level of the DNA sequence (fig. 4).

Discussion

That the deletion was not found in Alaskan Athabascans, Inupiaq Eskimos, Yup'ik Eskimos, Aleuts, Siberian Eskimos, or Chukchi suggests the possibility of a common ancestry for these groups and that their common ancestor in Asia may have lacked the deletion. The Yukagir, Nanai, Lamut, Chuvantsy, and Koryak of the Soviet Far East do not possess the deletion and thus may share a common ancestor with the Beringians studied here.

Absence of the deletion in all Beringians may also suggest that these groups are closely related. Schmiechen et al. (in press) could not separate Athabascans and Inupiaq Eskimos of Alaska even when 300 restriction fragments of mtDNA per individual were analyzed. Furthermore, sequence analysis of the highly variable control region of Alaskan Athabascans, Inupiaq Eskimos, Siberian Eskimos, and Chukchi also suggests that they are closely related (authors' unpublished data).

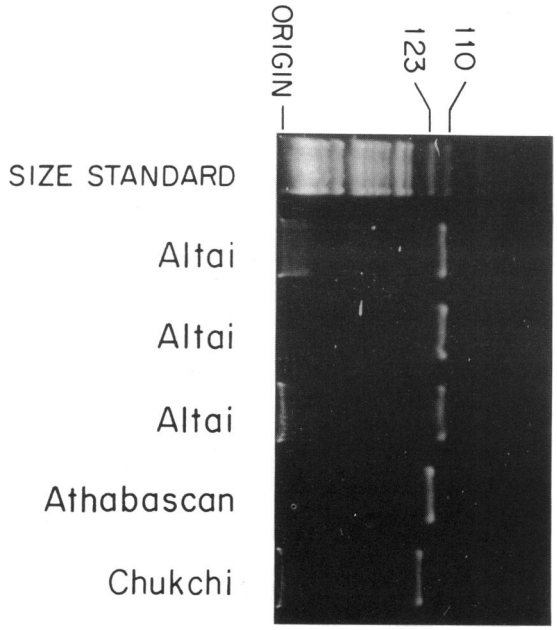

Figure 3 mtDNA as in fig. 2, except that lanes 1–3 contain DNA from three Altai (deleted form, 112 bp), lane 4 contains DNA from nondeleted individual (typical of Beringians, 121 bp), and lane 5 contains DNA from Chukchi who has three copies of the 9-bp sequence. Its amplified DNA is thus 130-bp in length.

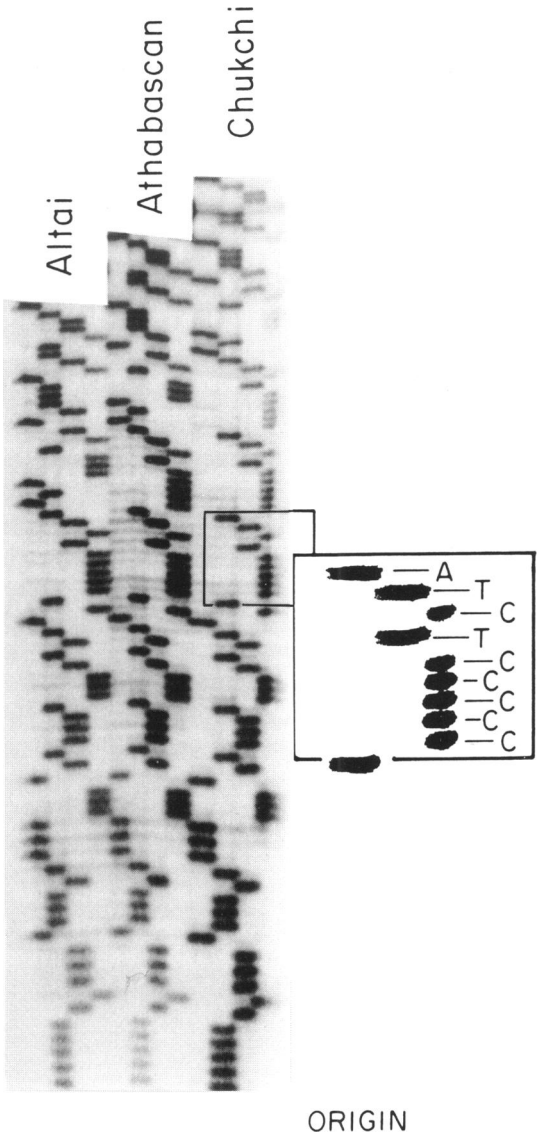

Figure 4 Sequences of mtDNA from region V. The individual on the left has one copy of the 5′-CCCCCTCTA-3′ sequence in the sense strand. The middle individual has two copies (as do all Beringians), while the third individual from the left, a Chukchi, has three copies of the 9-bp sequence.

The deletion has not been found either in the Haida Indians of Queen Charlotte Island, Canada, or in the Inuit Eskimos of West Greenland (R. H. Ward, personal communication). Moreover, it has not been found in the Dogrib Indians (Torroni et al. 1992) of the Northwest Territories of Canada (table 2). Thus, two major groups of northerly distributed indigenous peoples of Alaska, Canada, and Greenland—the Eskaleuts and the Na Dene Indians—appear to lack the deletion. This observation supports Szathmary's (1981, 1985) contention, based on allozyme data, that Eskimos and Athabascans are closely related.

The Navajo of Arizona and the Nuu-Chah-Nulth of Vancouver Island, Canada, possess the deletion (Ward et al. 1991; Torroni et al. 1992). It is possible that the 3.2% of the Nuu-Chah-Nulth who possess the deletion acquired it by admixture. The deletion is present in 37.5% of the Navajo studied (Torroni et al. 1992), but there is substantial evidence that admixture has occurred between these people and other Native American tribes that occupied regions through which the Navajo presumably migrated during their southern radiation. The Navajo are believed to have existed in northwest Canada, but by 1500 A.D. their descendants had established themselves far to the south in Arizona (Hester 1962). These migrants could have acquired the deletion during their movements south, particularly since they most certainly had associations with Native Americans whose descendants possess the deletion in high frequency (Schurr et al. 1990). It is also known that the present-day population of approximately 150,000 Navajo is descended from as few

Table 2

Distribution of Asian-specific 9-bpd: Other Studies

Population	Sample Size	mtDNA Deletion	
		No. Detected	% Positive
Far East and Pacific:			
East Asian[a]	34	6	18
Japan[b]	116	19	16
Samoans[c]	30	30	100
Maoris[c]	30	30	100
Niueans[c]	30	30	100
Cook Islanders[c]	30	26	87
Tongans[c]	30	23	77
Fijians[c]	28	23	82
Coastal Papua New Guinea[a,c]	123	30	24
Highland Papua New Guinea[a,c]	94	0	0
Australia[a,c]	51	0	0
North America:			
Navajo[d]	48	18	37.5
Pima[e]	31	14	45.2
Maya[e]	37	8	21.6
Nuu-Chah-Nulth[f] (Vancouver Island, Canada)	63	2	3.2
Dogrib Athabascan[d] (Northwest Territories, Canada)	30	0	0
Ticuna[e]	31	0	0

[a] Stoneking and Wilson (1989).
[b] Horai and Matsunaga (1986).
[c] Hertzberg et al. (1989).
[d] Torroni et al. (1992).
[e] Schurr et al. (1990).
[f] Ward et al. (1991).

as several thousand individuals (Hester 1962). Thus, the 37.5% frequency of the deletion types in Navajo is explainable if admixture and accompanying population growth are invoked.

Schurr et al. (1990) identified at least four separate human lineages in the Americas by analyzing polymorphic sites and the region V 9 bpd. Three of these four lineages lack the deletion. The native American haplotypes AM 1 and AM 6 that have been reported by Schurr et al. (1990) lack the deletion but possess the number 2 *Hin*cII restriction-fragment pattern (Wallace et al. 1985). These Native Americans have these markers in common with Athabascans and Inupiaq Eskimos (Schmiechen 1991) and therefore may be part of the same lineage.

Loss of an *Rsa*I restriction site at position 16329 occurs in all Na Dene populations (Tlingit, Dogrib, and Navajo) examined by Torroni et al. (1992). Consequently, they have suggested that this site loss may be a specific marker for all Na Dene. On the contrary, the *Rsa*I restriction site at 16329 is present in 21 of 22 Alaskan Athabascans whose mitochondrial control regions we have sequenced (Schmiechen 1991). Moreover, this specific *Rsa*I restriction site is present in all Alaskan and Siberian Eskimos, Aleuts, and Chukchi whom we have analyzed (authors' unpublished data). This raises the possibility that these groups may have constituted a lineage separate from other Na Dene groups.

It appears that the region V 9 bpd has not accompanied Aleuts, Eskimos, and Athabascans into the New World. It may have arrived in Nuu-Chah-Nulth and Navajo through admixture.

The deletion is common in Pimas and Mayans, who are members of the Native American group. This pattern could be interpreted as indicating that the deletion lineages arrived in the Americas before the lineages that are commonly found in the Northwest and Beringia. Absence of the deletion in the Ticuna of the Amazon rainforest of Brazil has been interpreted to

have occurred through bottlenecking, which possibly occurred at the Isthmus of Panama prior to the establishment of the Ticuna in Brazil (Schurr et al. 1990).

Three of the 25 individuals from the Ust-Kan region of the Altai Mountains possess the deletion and, therefore, may not be a direct link to the Beringians. This is supported by sequence data of the mitochondrial control region which suggest that the Altai are very distantly related to Beringians and that they are a diverse group whose intrapopulation variation exceeds that among all of the Beringians for whom sequence data are known (authors' unpublished data). These observations are supported by classic blood group analysis of Altai populations (Crawford and Enciso 1982).

The occurrence of three tandem repeats of the CCCCCTCTA sequence in one Chukchi in this study presumably arose as a novel mutation, since this triplicate sequence has never been observed until now. Wrischnik et al. (1987) identified in individual 101 of their study an additional sequence which presumably arose via two mutation events, a T-to-C transition followed by a slipped mispairing insertion of four more cytosines. A slipped mispairing insertion of the entire 9-bp sequence (Levinson and Gutman 1987) may have given rise to the triplicate individual observed here.

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