Korean J Biol Sci 2: 259-267, 1998

Distribution of the 9-bp Deletion in COII/tRNA^{Lys} Intergenic Region of Mitochondrial DNA is Relatively Homogeneous in East Asian Populations

Sung-Soo Hong†, Satoshi Horai[1], and Chung-Choo Lee*

Department of Biology and [1]Deparment of Molecular Biology, Research Center for Cell Differentiation, Seoul National Uninversity, Seoul 151-742, Korea; [1]Department of Human Genetics, National Insititute of Genetics, Mishima, Shizuoka-Ken 411, Japan

Key Words:
Mitochondrial DNA
Intergenic COII/tRNA^{Lys} region
9-bp deletion
Korean population
Polymerase chain reaction
Geographic cline
Mongoloids

A deletion of one out of the two copies of 9-bp repeat sequence (CCCCCTCTA) between the cytochrome oxidase II and lysine tranfer RNA (COII/tRNA^{Lys}) genes in human mitochondrial DNA (mtDNA) has been used as a polymorphic anthropological marker for people of east Asian origin, and to lesser extent, Pacific and African populations. We searched for the 9-bp deletion of the intergenic COII/tRNA^{Lys} region in two Korean populations (175 from Seoul and 38 from Cheju) and examine the distibution of this deletion in world populations. The 9-bp deletion was detected directly by electrophoresis of the polymerase chain reaction (PCR)-amplified nucleotide (nt) 8211-8310 mtDNA fragment. The frequencies of the 9-bp deletion were significantly different between the Seoul (16%) and Cheju (8%) populations. Examination of data from the world populations suggests a geographic gradient. The frequency reaches its highest values in some Pacific island populations and decreases along the southeast Asia-Siberia transect. In spite of this geographic gradient, Mongoloid populations including Korean, Chinese, Japanese, and Mongolian populations were relatively homogeneous with regard to the 9-bp deletion type of the intergenic COII/tRNA^{Lys} region. These results indicate Koreans are genetically related to northeast Asian populations, and have a maternal Mongoloid ancestry. Therefore, the 9-bp deletion of the intergenic COII/tRNA^{Lys} region will provide significant information to elucidate the historical patterns of migration of the Mongoloids.

Recent, genetic studies on the Korean population at the DNA level give support to two viewpoints. Research on variation in nuclear DNA markers suggests a significant Korean contribution to the predominantly northeast Asian characteristics of the Mongoloid gene pool (Hong, 1993; Hong et al., 1993). In contrast, data from mitochondrial DNA (mtDNA) restriction fragment length polymorphisms (RFLP) in the Korean population support the fast train model, with little evidence of admixture between Korean and Mongoloid neighboring populations (Harihara et al., 1988; Ballinger et al., 1992; Hong, 1993). However, these studies have not resolved the question about the identity and origin of Koreans as the results are affected by founder events, genetic drift, admixture and migration.

Human mtDNA exhibits a substantial variation among individuals. A high correlation has been demonstrated between mtDNA variation and the ethnic origin of individuals (Johnson et al., 1983; Cann et al., 1984; Horai and Matsunaga, 1986; Torroni et al., 1992, 1993, 1994b). These features make mtDNA a valuable tool in the study of evolutionary genetics of modern populations. At present, the 9-bp deletion of cytochrome oxidase II and the lysine tRNA genes (COII/tRNA^{Lys} intergenic region) appears to be a genetic marker that is informative with regard to investigations of ancient migration process as in human populations. The intergenic COII/tRNA^{Lys} region of human mtDNA usually contains two tandemly arranged copies of a 9-bp sequence (Anderson et al., 1981). The region is a short noncoding region located between nucleotide (nt) 8272 and nt 8289 in the intergenic COII/tRNA^{Lys} region (or noncoding region V) of human mtDNA (Anderson et al., 1981). Length variation in this region was first inferred by Cann and Wilson (1983), using restrction fragment length polymorphism (RFLP), and subsequent sequence analysis demonstrated that one deletion involved the loss of one of two adjacent copies of a 9-bp sequence (CCCCCTCTA) in the intergenic COII/tRNA^{Lys} region (Wrischinik et al., 1987). This deletion has been found at varying frequencies in

* To whom correspondence should be addressed.
Tel: 82-2-872-1993, Fax: 82-2-880-6691
† Present address: Department of Anthropology, Pennsylvania State University, University Park, PA 16802, USA

populations from Asia (Horai et al., 1987; Stoneking and Wilson, 1989; Horai, 1991a, 1991b; Ballinger et al., 1992; Harihara et al., 1992; Passarino et al., 1993; Melton et al 1995), as well as in populations of Asian origin, including Polynesians (Hertzberg et al., 1989; Stoneking and Wislon, 1989; Lum et al., 1994; Redd et al., 1995; Sykes et al., 1995) and Native Americans (Schurr et al., 1990; Ward et al., 1991, 1993; Shields et al., 1992, 1993; Torroni et al., 1992, 1993a, 1994b; Horai et al., 1993; Lorenz and Smith, 1994; Merriwether et al., 1995), and is commonly referred to as an "Asian-specific" marker (Wrischinik et al., 1987; Hertzberg et al., 1989; Stoneking and Wilson, 1989).

However, some studies on the 9-bp deletion of the intergenic COII/tRNA^{Lys} region have suggested multiple origins of the deletion in Asia (Schurr et al., 1990; Ballinger et al., 1992; Torroni et al., 1993b; Redd et al., 1995); furthermore, this same 9-bp deletion was also found in Africa populations (Vigilant 1991; Chen et al., 1995; Soodyall et al., 1996). The 9-deletion of intergenic COII/tRNA^{Lys} region is thus not entirely exclusive to Asians and may even have multiple origins in Asia. It is considered to be an indicator of Asian affinities in these populations. In particular, the frequency of the 9-bp deletion across contemporary Asian populations has a unique distribution that is consistent with current ideas regarding prehistoric peopling of Asia (Horai et al., 1987; Hertzberg et al., 1989; Stoneking and Wilson 1989; Horai et al., 1991a, 1991b; Ballinger et al., 1992; Harihara et al., 1992; Lum et al., 1994). Therefore, analysis to date suggests that the 9-bp deletion of the mtDNA COII/tRNA^{Lys} region is an anthropological marker for peoples of Asian origin. Eariler, the 9-bp deletion was shown to be present in Korean population (Harihara et al., 1992; Ballinger et al., 1992), but samples analyzed were insufficient to be representative of Koreans.

To determine the frequency of the 9-bp deletion in two Korean populations (Seoul and Cheju) and to verify the previously reported data on the 9-bp deletion from the viewpoints of population affinities, we carried out a search for the 9-bp deletion of mtDNA intergenic COII/tRNA^{Lys} region.

Materials and Methods

Subjects

The blood samples used for the present study were randomly selected from Seoul and Cheju of Korea, respectively, and were not maternally related. The sample population sizes of Seoul and Cheju were 175 and 38, respectively. The second population surveyed in Korea was that in Cheju, a large island located in the southern part of Korea.

Isolation of total DNA

Total genomic DNA was extracted from the buffy coats isolated from whole blood (5 ml) using the procedure of Hong (1993). Buffy coats were incubated at 37℃ for 4-12 h in a solution of TEN buffer (10 mM Tris-HCl, 1 mM EDTA, 10 mM NaCl, pH 8.0), proteinase K (20 mg/ml), and 20% SDS. The mixture were extracted twice with an equal volume of phenol/chloroform and once with 24:1 chloroform/isoamyl alcohol. The DNA in the aqueous phase was precipitated by adding 1/10 volume of sodium acetate and 2 volumes of 100% ethanol. The DNA was then washed in 70% ethanol, dried, and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

PCR amplification

Amplification of the intergenic COII/tRNA^{Lys} region by PCR was carried out with the thermostable *Taq* DNA polymerase (Perkin Elmer Cetus) by the method described by Saiki et al., (1988). For amplification of the intergenic COII/tRNA^{Lys} region, the sequences of the two primers as described by Horai (1991a) were as follows:

primer A,
8211-5'-TCGTCCTAGAATTAATTCCC-3'-8230,
primer B,
8310-5'-AGTTAGCTTTACAGTGGGCT-3'-8281

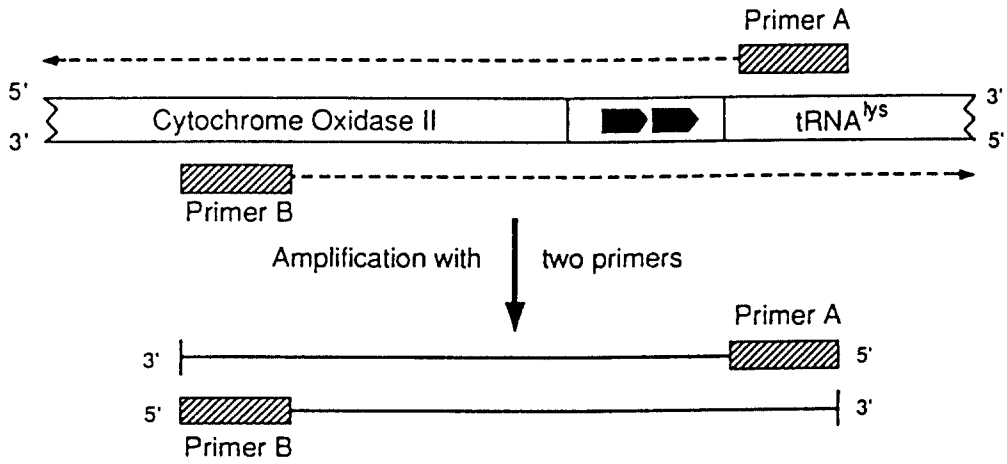
These oligonucleotide primers were synthesized using an Applied Biosystem model 380B DNA synthesizer. This set of primers amplifies a 100-bp segment of mtDNA as determined from the one complete sequence (Anderson et al., 1981).

Amplification reactions were performed in a final volume of 50 μl containing 200 ng total DNA, 1 g of each primer, 25 mM Tris-HCl (pH 8.8), 5 mM $MgCl_2$, 50 mM KCl, 200 μM of each dNTP mix, and 1 unit of *Taq* DNA polymerase. The reaction mixtures were overlaid with 1 drop of mineral oil (Sigma). The PCR was carried out for a total of 30 cycles with the use of a Thermal Cycler (Perkin Elmer Cetus). The cycle times were as follows: denaturation, 10 sec at 94℃; annealing, 10 sec at 45℃; primer extension, 15 sec at 72℃. At the end of each amplification, the incubation was prolonged for 10 min at 72℃. Ten microliters of each amplified mtDNA fragment were electrophoresed in 3% (w/v) NuSieve agarose gels (Sigma) containing 1 mg ethidium bromide (EtBr)/ml. The deletion pattern was determined by using a 123 ladder as a size marker and with UV fluorescence.

Results

According to the sequence of human mtDNA, the intergenic COII/tRNA^{Lys} region has two tandem copies of 9-bp sequence CCCCCTCTA (Fig. 1A). We observed an amplified fragment of 100-bp or 91-bp derived from the intergenic COII/tRNA^{Lys} intergenic region, which corresponded to the presence of two copies and one

A



B

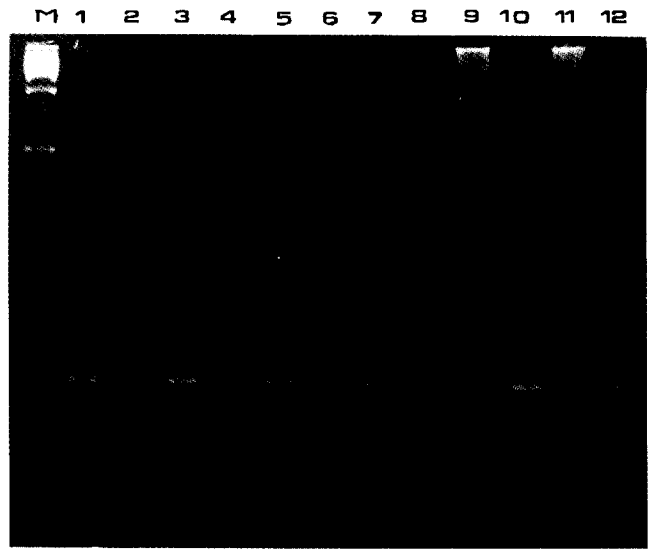


Fig. 1. A, The PCR method for amplifying the COII/tRNA^{Lys} intergenic region of human mtDNA. Two synthetic oligonucleotides of 20 bp in length (primers A and B) match invariant sequences flanking the COII/tRNA^{Lys} intergenic region, which contains two adjacent copies of a 9-bp sequence (normal type, shaded double arrows). B, PCR products amplified in the COII/tRNA^{Lys} intergenic region were separated by 3% NuSieve-agarose gel electrophoresis and stained with EtBr. The longer one, a 100 bp band, was a product of normal type (lanes 1, 3, 5, 7, 10, 12) and the shorter one, a 91 bp band (lanes 2, 4, 6, 8, 9, 11) , was a product that carries the 9-bp deletion in the COII/tRNA^{Lys} intergenic region. Lane M is 123 ladder as a size marker.

tandem copies of the 9-bp sequence, respectively (Fig. 1B). The 100-bp band (lanes 1, 3, 5, 7, 10, 12) was a product of normal intergenic COII/tRNA^{Lys} region, and the 91-bp band (lanes 2, 4, 6, 8, 9, 11) was a product of the intergenic COII/tRNA^{Lys} region that carries the 9-bp deletion.

The presence of the 9-bp deletion was examined in a total 213 individuals from two Korean populations (Seoul=175 and Cheju=38). The frequencies of the 9-bp deletion in two Korean populations observed in this study is are included in Table 1. The frequency of the 9-bp deletion was 16% for Seoul population, while for Cheju was 8% (Table 1). The overall frequency of the 9-bp deletion observed in the Koreans (n=213) represented by Seoul and Cheju populations was 14.6% and of the Koreans observed in this study combined with data from other studies (n=290) was 13.1% (Ballinger et al., 1992; Harihara et al., 1992) (Table 1). Table 1 lists world populations from this study and the previous studies, the number and percentage of 9-bp deleted individuals sampled from each population, and the references for data.

Discussion

The 9-bp deletion in the COII/tRNA^{Lys} intergenic region of mtDNA has been previously used as a polymorphic anthropological marker for people of east Asian origin, and to a lesser extent, Pacific and African populations (Wrischinik et al., 1987). It has been generally accepted that the 9-bp deletion occurred only once during the evolution of modern types of human mtDNA. We

Table 1. Geographic distribution and frequencies of the 9-bp deletion in the COII/tRNA^{Lys} intergenic region in world populations

Geographic region and population	Sample size	No. of individual	Freq. of deletion (%)	References
Pacific Region:				
Polynesians				
Niueans	30	30	100.0	Hertzberg et al., 1989
Tongans	88	82	93.2	Sykes et al., 1995
Cook Islander	224	204	91.1	Sykes et al., 1995
Samoans	24	24	100.0	Redd et al., 1995
Tahiti	114	110	96.5	Sykes et al., 1995
Autrales	68	65	95.6	Sykes et al., 1995
Marquesas	47	41	87.2	Sykes et al., 1995
Maoris (New Zealand)	30	30	100.0	Hertzberg et al., 1989
Tolais	40	38	95.0	Hertzberg et al., 1989
Melanesians				
Vanuatu	56	22	40.0	Sykes et al., 1995
Fijians	28	23	82.1	Hertzberg et al., 1989
Papua New Guinea (PNG)	114	50	43.9	Sykes et al., 1995
PNG Coastral	55	22	40.0	Stoneking and Wilson, 1989
PNG Highlander	30	0	0	Hertzberg et al., 1989
Micronesian				
Micronesia Marshall Islander	55	53	96.4	Sykes et al., 1995
Kapingamarangi	62	62	100.0	Sykes et al., 1995
Australians				
Aborigines	31	1	3.2	Hertzberg et al., 1989
Southeast Asia				
Malaysians				
Aborigines	31	1	3.2	Balliger et al., 1992
Malays	81	21	25.9	Melton et al., 1995
Orang Asli	30	11	36.7	Melton et al., 1995
Indonesians				
Nusa Tenggaras	96	23	24.0	Redd et al., 1995
Moluccas	50	8	16.0	Redd et al., 1995
Sabah Aborigines	32	6	18.8	Balliger et al., 1992
Borneo Sabah	74	30	40.5	Sykes et al., 1995
Java	98	25	25.5	Melton et al., 1995
Phillippine				
Filipino	74	20	27.0	Sykes et al., 1995
Miscellaneous	79	35	44.3	Melton et al., 1995
Ilocano	97	35	36.1	Melton et al., 1995
Negrito	37	34	91.9	Harihara et al., 1992
Vietnamese	28	5	17.9	Ballinger et al., 1992
Southern Chinese	103	23	22.3	Melton et al., 1995
Taiwanese native	88	32	36.4	Sykes et al., 1995
West Asia				
Sri Lanka (Vedda)	20	0	0	Harihara et al., 1992
Southern Napal (Tharus)	107	8	7.5	Passarino et al., 1993
Hindus	76	0	0	Passarino et al., 1993
Pakistani	76	0	0	Melton et al., 1995
South Indian	75	6	8.0	Melton et al., 1995
North Indian	47	0	0	Melton et al., 1995
Bangladeshi	31	0	0	Melton et al., 1995
East Asia				
Koreans				
Seoul 1	64	5	7.8	Harihara et al., 1992
Seoul 2	175	28	16.0	This study
Cheju	38	3	7.9	This study
Taejon	13	2	15.4	Ballinger et al., 1992
Japanese				
Aomori	61	8	13.1	Horai et al., 1991a
Shizuoka	116	19	16.4	Horai et al., 1991a
Fukuoka	77	12	15.6	Horai et al., 1991a
Okinawa	82	4	4.9	Horai et al., 1991a
Hokkaido	63	12	19.0	Harihara et al., 1992
Ainu	51	1	2.0	Harihara et al., 1992
Chinese Taiwanese	132	23	17.4	Horai et al., 1991a
East Asian	34	6	18.0	Cann et al., 1987
Central or north Asia				
Nothern Mongolian	292	23	7.9	Samuugiin et al., 1991
Southern Mongolian	278	23	8.1	Samuugiin et al., 1991
Yakuts	60	0	0	Petrishchev et al., 1993
Buryats	159	10	6.3	Ivanova et al., 1994
Mongolian	103	7	6.8	Kolman et al., 1996
Tibetans	54	3	5.6	Torroni et al., 1994c
West Evenks	70	0	0	Petrishchev et al., 1993
Siberia				
Siberian Eskimo	50	0	0	Torroni et al., 1993b
Yukagirs	27	3	11.0	Sukernik et al., 1996
Evens	43	0	0	Torroni et al., 1993b
Siberian Yup'ik	25	0	0	Shields et al., 1992
Koryaks	46	0	0	Torroni et al., 1993b
Chukchi	189	0	0	Ivanova et al., 1994
Evenks	82	0	0	Ivanova et al., 1994

Table 1. (Continued)

Geographic region and population	Sample size	No. of individual	Freq. of deletion (%)	References
Eastern Evenks	68	1	1.5	Petrishchev et al., 1993
Sel'kups	128	0	0	Ivanova et al., 1994
Northern Altaians	28	1	3.6	Sukernik et al., 1996
South Altaians	108	1	0.9	Petrishchev et al., 1993
Ust-Uiagan Altaians	13	0	0	Shields et al., 1992
Ust-Kan Altaians	25	3	12.0	Shields et al., 1992
Mansians	75	0	0	Petrishchev et al., 1993
Nivkhs	57	0	0	Torroni et al., 1993b
Udegeys	46	0	0	Torroni et al., 1993b
Nganasani	49	0	0	Sukernik et al., 1996
Kets	23	0	0	Sukernik et al., 1996
Siberia Russian	122	0	0	Petrishchev and Kutueva, 1994
Nananians	72	1	1.4	Petrishchev et al., 1993
Asian Eskimo	82	0	0	Ivanova et al., 1994
Alaska				
Dogorb	169	0	0	Merriwether et al., 1995
Ouzinkie Alaskan Eskimo	56	0	0	Merriwether et al., 1995
Old Harbor Alaskan Eskimo	156	4	2.6	Merriwether et al., 1995
St. Lawrence Alaskan Eskimo	72	0	0	Merriwether et al., 1995
SW Alaskan Yupik Eskimoi	177	0	0	Merriwether et al., 1995
St. Paul Alaskan Aleurs	78	0	0	Merriwether et al., 1995
Alaskan Athabascan	22	0	0	Shields et al., 1992
Inuit	35	0	0	Lorenz and Smith, 1994
Alaskan Inupiaq Eskimo	16	0	0	Shields et al., 1992
Canada Amerinds				
Chipewa	18	2	11.2	Lorenz and Smith, 1994
Ojibwa	43	3	3.6	Torroni et al., 1993a
Mohawk	208	19	9.1	Merriwether et al., 1995
Nuu-Chah-Nulth	63	2	3.2	Ward et al., 1993
Bella Coola	32	2	6.3	Ward et al., 1993
Haida	38	0	0	Ward et al., 1993
USA Amerinds				
Cochimi	13	6	46.1	Lorenz and Smith, 1994
Pima	31	14	45.2	Schurr et al., 1990
Kumial	16	10	62.5	Lorenz and Smith, 1994
Creek	18	2	11.1	Lorenz and Smith, 1994
Choctaw	20	3	15.0	Lorenz and Smith, 1994
Quechan	14	7	50.0	Lorenz and Smith, 1994
Apache	25	4	16.0	Torroni et al., 1993a
Zuni	20	11	55.0	Lorenz and Smith, 1994
Jemez	14	9	64.3	Lorenz and Smith, 1994
Navajo	48	18	37.5	Lorenz and Smith, 1994
Oklahoma Mvskoke	1	11	15.5	Merriwether et al., 1995
Cherokee	18	5	27.8	Lorenz and Smith, 1994
Washo	17	5	29.4	Lorenz and Smith, 1994
Oneota	50	5	10.0	Stone and Stoneking, 1994
Sioux	17	3	17.6	Lorenz and Smith, 1994
Cetral America Amerinds				
Guyami	16	5	31.2	Torroni et al., 1993a
Teribe	20	4	20.0	Torroni et al., 1994a
Guatuso	20	3	15.0	Torroni et al., 1994a
Boruca	14	10	71.4	Torroni et al., 1993a
Bribri/Cabecar	24	11	45.8	Torroni et al., 1993a
Mexican Maya	37	8	21.6	Schurr et al., 1990
Zapotec	15	5	33.3	Torroni et al., 1994b
Mixe	16	5	31.3	Torroni et al., 1994b
Baja Mixtec	14	1	7.1	Torroni et al., 1994b
Alta Mixtec	5	2	13.3	Torroni et al., 1994b
Nahua	16	3	18.8	Lorenz and Smith, 1994
South America Amerinds				
Amazons	20	2	10.0	Monsalve et al., 1994
Andes	13	5	38.5	Monsalve et al., 1994
Huilliche	91	23	25.3	Merriwether et al., 1995
Trapa Trapa Pehuenche	76	7	9.2	Merriwether et al., 1995
Butalelbum Pehuenche	28	1	3.6	Merriwether et al., 1995
Argentinian Maquche	39	15	38.5	Ginther et al., 1993
Chilean Maquche	45	9	20.0	Horai et al., 1993
San Pedro De Atacama	126	93	73.8	Merriwether et al., 1995
Parinacata Aymara	13	7	53.8	Merriwether et al., 1995
Codpa Aymara	11	7	63.6	Merriwether et al., 1995
Guanacagua Aymara	17	17	100.0	Merriwether et al., 1995
Illapata Aymara	13	12	92.3	Merriwether et al., 1995
Lluta Aymara	15	8	53.3	Merriwether et al., 1995
Caquena Aymara	25	14	56.0	Merriwether et al., 1995
Esquina Aymara	16	9	56.3	Merriwether et al., 1995
Visviri Aymara	89	53	60.0	Merriwether et al., 1995
Chilean Mummies	15	0	0	Merriwether et al., 1994
Quechua	23	7	30.4	Merriwether et al., 1995
Mataco	28	10	35.7	Torroni et al., 1993a
Kraho	14	8	57.1	Torroni et al., 1993a

Table 1. (Continued)

Geographic region and population	Sample size	No. of individual	Freq. of deletion (%)	References
Marubo	10	0	0	Torroni et al., 1993a
Ticuna	31	0	0	Schurr et al., 1990
Wapishana	12	3	25.0	Torroni et al., 1993a
Yanomama	24	4	16.7	Torroni et al., 1993a
Macushi	10	2	20.0	Torroni et al., 1993a
Markirtari	10	0	0	Torroni et al., 1993a
Piaroa	10	0	0	Torroni et al., 1993a
Columbians	20	4	20.0	Horai et al., 1993
Kuna	16	0	0	Torroni et al., 1993a
Western Africa:				
Yoruba	14	0	0	Soodyall et al., 1996
Gambians	48	0	0	Soodyall et al., 1996
Mandenkalu	60	0	0	Chen et al., 1995
Wolof	20	0	0	Chen et al., 1995
Senegalese	13	0	0	Chen et al., 1995
Cetral Africa				
Mbuti Pygmies	20	6	30.0	Soodyall et al., 1996
Efe Pygmies	33	10	30.3	Soodyall et al., 1996
Biaka Pygmies	17	4	23.5	Soodyall et al., 1996
Zaire Pygmies	22	6	27.3	Chen et al., 1995
Western Pygmies	17	4	23.5	Chen et al., 1995
Lese	22	3	13.6	Soodyall et al., 1996
Hadza	17	0	0	Soodyall et al., 1996
Luo	60	1	1.7	Soodyall et al., 1996
Southern Africa				
Kung	25	0	0	Soodyall et al., 1996
Sekele	48	0	0	Soodyall et al., 1996
Nama	48	0	0	Soodyall et al., 1996
Kwengo	50	0	0	Soodyall et al., 1996
Dama	96	0	0	Soodyall et al., 1996
Malawian	45	12	26.7	Soodyall et al., 1996
Venda	41	9	22.0	Soodyall et al., 1996
Lemba	26	7	26.9	Soodyall et al., 1996
Nguni	101	13	12.9	Soodyall et al., 1996
Tsonga	35	2	5.7	Soodyall et al., 1996
Sotho/Tswana	54	11	20.4	Soodyall et al., 1996
Herero	69	0	0	Soodyall et al., 1996
Himba	14	0	0	Soodyall et al., 1996
Ambo	36	3	8.3	Soodyall et al., 1996

chose the COII/tRNA^{Lys} intergenic region of mtDNA as an important marker to determine the genetic structure of Koreans. The present study is the largest survey (n=213) for the presence of the 9-bp deletion in Korean population.

Previous studies reported different frequencies of the 9-bp deletion in the Korean population (Ballinger et al., 1992; Harihara et al., 1992) (Table 1). We observed that the frequency (16.0%) of the 9-bp deletion in Seoul population (n=175) was similar to that (15.4%) of the Taejon population (n=13) of Korea reported by Ballinger et al. (1992), while the 9-bp deletion frequency (8.0%) of Cheju population (n=38) was similar to that (7.8%) of the Seoul population (n=64) reported by Harihara et al. (1992). We think that the reason for the difference is because the number of samples used in this study was more than that of the previous studies (Harihara et al., 1992; Ballinger et al., 1992). The overall frequency of the 9-bp deletion in the Korean population (n=213) represented by Seoul and Cheju populations was 14.6%. The overall frequency in Korean population based on this study combined with data from other studies (n=290) was 13.1%. From data reported by Ballinger et al. (1992) and Harihara et al. (1992), however, the frequency of the 9-bp deletion observed in Korean population (n=77) was 9.1%. Therefore, we think that the low or high frequency from Seoul and Taejon populations reported by Harihara et al., (1992) and Ballinger et al., (1992) may be due to sample number.

The difference of distribution of the 9-bp deletion between two geographic regions (Seoul and Cheju) of Korea was statistically significant. This observation is also found in mainland and island populations of Japan (Horai et al., 1987, Horai, 1991a; Harihara et al., 1992) (Table 1). The distribution of the 9-bp deletion for island populations (2.0% for Ainu and 4.9% for Okinawa) in Japan showed lower frequencies than that (16.1%) of mainland populations. Cheju Island has had a long isolation from various mainland subtribes of Korea. Therefore, the low frequency in the Cheju population might be a result of random genetic drift combined with low admixture with mainland subtribes and might reflect an influx of the deletion type from northern Mongoloids (historical document). Also, the 9-bp frequency of Cheju population was similar to that of the Mongolian population (Sambuugiin et al., 1991; Kolman et al., 1996) Although the biological diversity of the present-day Cheju people is thought to be derived from a number of immigrant mainland populations, we suggest that the contributions are not easily distinguished, partly because of the linguistic unity evident in the people.

Present and published information on the frequencies

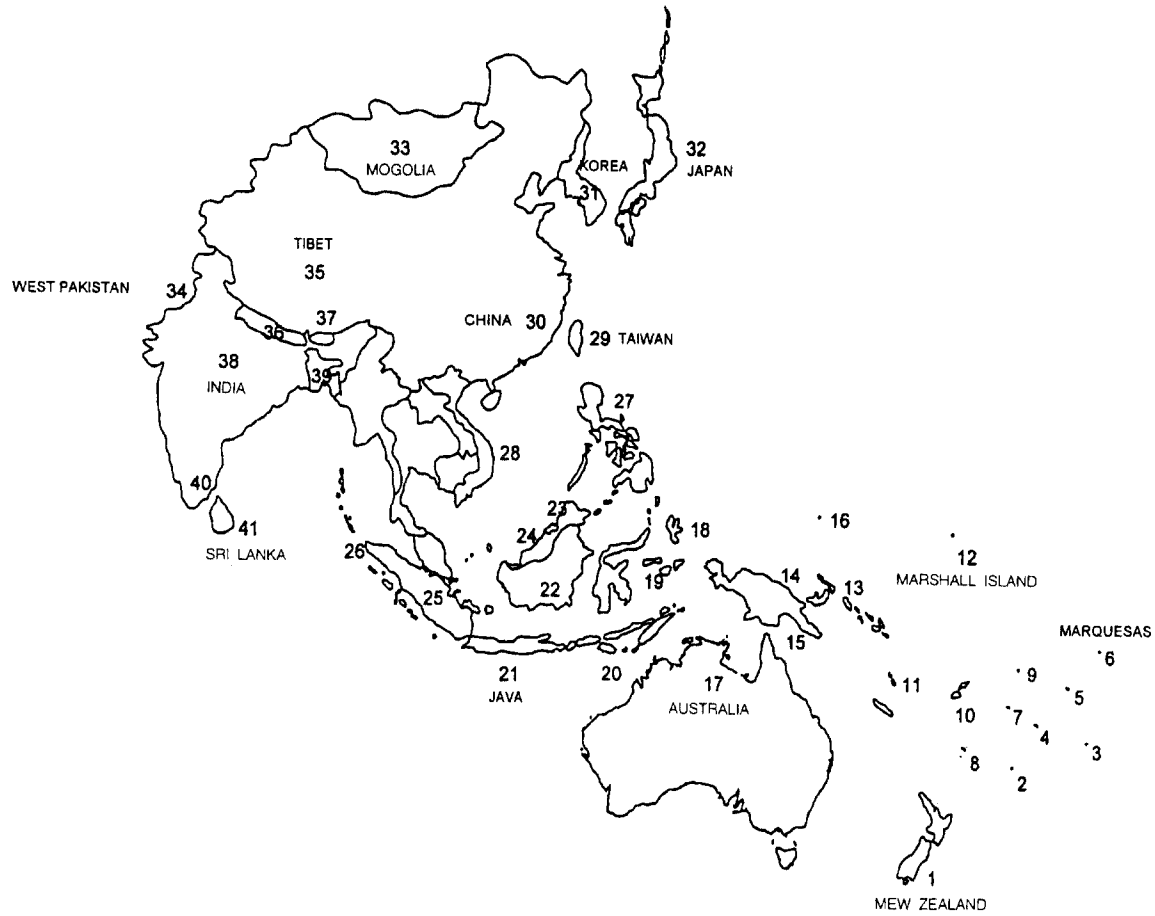


Fig. 2. Map showing sampling area. Numbers indicate the location of population. (1) New Zealand, (2) Niue, (3) Autrales, (4) Cook Island, (5) Tahiti, (6) Marquesas, (7) Tonga, (8) Maoris, (9) Samoa, (10) Fiji, (11) Vanuatu, (12) Marshall Island, (13) Tolais, (14) PNG Coastral, (15) PNG Highland, (16) Kapingamarangi, (17) Autrailia, (18) Moluccas, (19) east Indonesia, (20) Nusa, (21) Java, (22) Borneo, (23) Malay Sabah, (24) Malay, (25) Sumatura, (26) Orang Asli, (27) Philippine, (28) Vietnam, (29) Taiwan, (30) China, (31) Korea, (32) Japan, (33) Mongol, (34) west Pakistan, (35) Tibet, (36) Nepal, (37) Hindu, (38) North India, (39) Bangladeshi, (40) South India, and (41) Sri Lanka.

of the 9-bp deletion in Pacfic, several Asian, Siberia, Alaska, America Amerind, and African populations is summarized in Table 1. It is of considerable interest that the 9-bp deletion decreases in frequency from south to north in Asia as shown in Fig. 2. The frequency of the 9-bp deletion was highly variable in southeast Asian and Pacific populations, reaching 100% in certain isolated islander populations of Oceania (Hertzberg et al., 1989). According to a currently held hypothesis, the 9-bp deletion is thought to originate in populations of eastern Asia (Horai, 1991a), where this 9-bp deletion occurs at the highest frequencies. Its appearance in Polynesian and Melanesian populations is explained by migration from east Asia, and the abrupt increase in the 9-bp deletion frequency in certain Polynesian populations is associated with the founder effect (Horai and Matsunaga 1986; Horai, 1991a). An Increase in the populations of southeast Asia finally led to their contact with Pacific and other Asian populations, at which point the 9-bp deletion began to spread to the north. This 9-bp deletion is assumed to spread in Asian and American Indian populations via migration of ancient peoples, which, at present, is evidenced by a geographic gradient of its frequency, decreasing northward in Asia (Torroni et al., 1992, 1993a, b, 1994b; Shields et al., 1993; Lonenz and Smith, 1994; Kolman et al., 1996). In spite of geographic separation of their populations, however, Mongoloid populations including the Koreans, Japanese, Mongolians, and so on are relatively homogeneous with regard to the 9-bp deletion type of the mtDNA COII/ $tRNA^{Lys}$ intergenic region. This mean that the 9-bp deletion frequencies in the Koreans are essentially indistinguishable from those found in the northeast Asian populations (Table 1): considering that Koreans and other Asian populations (Japanese and Mongolians) are closely related peoples of the same language group, this result is not surprising.

In conclusion, present data and previous studies suggest that a distinct geographic cline in the frequency of the 9-bp deletion exists among Asian and Pacific populations. Mongoloid descendants are now

distributed over a wide area of east Asia, southeast Asia, and Pacific region, having adapted to a wide variety of environments. Therefore, data about the 9-bp deletion of the intergenic COII/tRNA^{Lys} region will provide significant information to elucidate the historical patterns of migration and phylogenetic relationships of Mongoloids. As future studies involve even more through geographic sampling, especially from the northern Chinese, north Koreans, and Mongolians, we expect to obtain a deeper understanding of the dispersal of the Mongoloids.

Acknowledgements

This study was supported in part by a Post-Doctoral Fellowship of Korea Sciences and Engineering Foundation to SSH and by Korea Sciences and Engineering Foundation through Research Center for Cell Differentiation at Seoul National University, SNU Daewoo Research Fund, and research funds (to Dr. Satoshi Horai) from National Institute of Genetics (Japan).

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[Received April 9, 1998; accepted May 1, 1998]