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Mitochondrial DNA diversity among five tribal populations of southern India

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DNA samples from 160 unrelated individuals belonging to five Dravidian tribal populations of southern India were analysed for ten mitochondrial DNA (mtDNA) restriction site polymorphisms (RSPs) and one insertion/deletion polymorphism. There is extensive sharing of mtDNA haplotypes among all the tribal populations studied, indicating that there was a small female founding population in India. The 9-bp deletion analysed was observed only in the Kadar population with a low frequency. The Asian-specific haplogroup M is found at a higher frequency in all the populations, thus supporting the hypothesis that this haplogroup arose in India and was carried to Africa from India. Haplogroup U is also found in all the populations and it is consistent with the theory that Dravidian-speaking populations were more widespread in India and that the Aryan-speakers pushed them to their present habitat.

CONTEMPORARY ethnic populations of India manifest a great deal of biological and cultural variability[1]. Based on cultural patterns, the tribal populations of India are clearly distinguished from the non-tribal groups, such as the populations belonging to the Hindu caste fold. The tribals constitute about 8.08% (2001 census) of the total Indian population. They are considered to be the original inhabitants of India. Tribals may represent relic populations of unknown origin, but potentially of great genetic interest[2]. The origins and migrational histories of the tribal populations of the Indian subcontinent are not clearly understood. Some tribal populations of southern India bear many Negroid physical characteristics[3,4], and may therefore be the representatives arriving in India on an ancient wave of out-of-Africa migration. Most ethnic populations of southern India are linguistically Dravidian. Some researchers have proposed that the contemporary Dravidian-speaking tribes of southern and central India may be descendants of the original inhabitants of the Indian subcontinent[5], although we have recently provided genomic evidence that the Dravidian-speaking tribals may have arrived in India after the Austro-Asiatic-speaking tribals[6].

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Considerable insight into the peopling of India has been derived from past studies based on blood group, serum protein and red-cell enzyme polymorphisms. Studies on genomic polymorphisms from defined ethnic groups are still scanty. The emerging molecular genetic data will be useful to reconstruct the human evolution in India. In this communication, we report the findings of our study on mitochondrial DNA (mtDNA) markers among five tribal populations of India. The study was undertaken with a view to partially reconstruct the population histories of the Dravidian-speaking tribal populations of southern India. The mtDNA molecule is maternally inherited as a single locus because of lack of recombination, yet it is extremely polymorphic and therefore highly informative for discriminating among populations. The mutation rate of human mtDNA is ten times higher than that of human nuclear DNA[7] and the lack of recombination allows for mtDNA types to be related as matrilines[8].

A 9-bp deletion in the mitochondrial genome has been useful for examining genetic relationships among human populations[9,10]. The 9-bp deletion is a length variant in the intergenic region between the cytochrome-c oxidase subunit II and the mitochondrial tRNA for lysine (COII/tRNA^{Lys}). The deletion motif results from the loss of one of two CCCCCTCTA tandem repeats[11] and it was originally identified as an 'Asian-specific' polymorphism[12].

Blood samples (5–10 ml by venipuncture) were collected with prior informed consent from individuals of five tribal groups belonging to the Dravidian linguistic family. The tribal groups were: Kadar ($n = 40$), Paniya ($n = 30$), Irula ($n = 30$), Kota ($n = 30$) and Kurumba ($n = 30$). These tribal communities inhabit the southern regions of India. Further details about these populations are provided in Table 1.

DNA was isolated from each individual using a standard protocol[13]. Each DNA sample was screened for ten mtDNA restriction site polymorphisms (RSPs) and one insertion/deletion polymorphism (IDP). The RSPs screened were *Hae*III gain at nt 663, *Hpa*I gain at nt 3592, *Alu*I loss at nt 5176, *Alu*I loss at nt 7025, *Dde*I and *Alu*I gains at nt 10394 and 10397 respectively, *Hinf*I gain at nt 12308, *Hinc*II loss at nt 13259, *Alu*I gain at nt 13262, *Hae*III gain at nt 16517 and the IDP screened was the COII/tRNA^{Lys} intergenic 9-bp deletion that occurs between nt 8272 and 8289, which has been used as a marker for populations of Asian and Asian-derived origins such as Polynesians and Native Americans. All the sites were chosen such that individuals could be classified into different haplogroups that are most relevant for Indian populations. mtDNA RSP analysis were performed using standard primers and protocols[14].

In Table 2, the allele frequencies are presented separately for each of the five populations. A subset of these data has already been published by us[6]. While *Dde*I

Table 1. Study population, sample size and geographical information

Population	Sample size	Geographical	Occupation	Remarks
Kadar	40	Annamalai hills of Tamil Nadu; Palghat and Trichur districts of Kerala	Hunting and food gathering	Autochthons of Annamalai and Trichur forests of South India. Possess several Negrito traits
Paniyan	30	Nilgiri hills of Tamil Nadu	Agricultural labour	Possess Negrito morphological features
Irula	30	Nilgiri hills of Tamil Nadu	Shifting cultivation	Possess several Negrito morphological features
Kota	30	Nilgiri hills of Tamil Nadu	Artisans, musicians and agriculturists	One of the original inhabitants of Nilgiri hills, highly inbred group
Kurumba	30	Nilgiri hills of Tamil Nadu	Hunting and food gathering	Possess several Negrito features

Table 2. Allele frequencies at 10 mtDNA RFLP loci in five tribal populations

Population	*Hae*III (663) gain	*Hpa*I (3592) gain	*Alu*I (5176) loss	*Alu* (7025) loss	*Dde*I (10394) gain	*Alu*I (10397) gain	*Hinf*I (12308) gain	*Hinc*II (13259) loss	*Alu*I (13262) gain	*Hae*III (16517) gain	9-bp gain
Kadar	0.000	0.000	0.000	0.000	0.900	0.900	0.075	0.125	0.125	0.700	0.05
Paniyan	0.000	0.000	0.000	0.000	0.600	0.600	0.067	0.000	0.000	1.000	0.000
Irula	0.000	0.000	0.000	0.000	0.533	0.533	0.233	0.033	0.033	0.766	0.000
Kota	0.000	0.000	0.000	0.000	0.967	0.967	0.000	0.000	0.000	1.000	0.000
Kurumba	0.033	0.000	0.000	0.000	0.767	0.761	0.067	0.000	0.000	1.000	0.000

(10394) and *Alu*I (10397) sites were polymorphic in all the populations, the *Hpa*I (3592), *Alu*I (5176) and *Alu*I (7025) were monomorphic in all the populations analysed. The 9-bp COII/tRNA^{Lys} intergenic length mutation was observed only in the Kadar population with a frequency of about 0.05%.

Eleven-locus haplotypes were constructed and their frequencies estimated in each population. A total of ten distinct haplotypes were observed among 160 individuals screened. The maximum number of haplotypes (7 of 10) was observed among the Kadar, while the Kotas harboured only 2 haplotypes. Frequencies of haplotypes in each study population, as also in the pooled sample are presented in Table 3. In the pooled data set, only one haplotype (00111101010) accounted for about 66% of all mtDNA molecules. It may therefore be inferred that this is the most ancient haplotype. It was found that only three of the ten distinct haplotypes were present at high frequencies, the remaining 7 haplotypes were present in small frequencies. One haplotype (00110001010) was found to be present in the Paniyas, Irulas, Kotas and the Kurumbas, while it was completely absent among the Kadars.

Based on the occurrence of mutations, mtDNA molecules have been classified into several haplogroups. The RFLP sites examined permit the classification of our data into the following haplogroups: Haplogroup A defined by the presence of *Hae*III (663) site, B defined by the presence of COII/tRNA^{Lys} intergenic 9-bp deletion and the presence of *Hae*III (16517) site, C defined by the presence of *Dde*I (10394) and *Alu*I (10397) site and the simultaneous absence of *Hinc*II (13259), D defined by the absence of *Alu*I (5176) site and the presence of *Dde*I (10394) and *Alu*I (10397) sites, M defined by the presence of *Dde*I (10394) and *Alu*I (10397) sites, and U defined by the absence of the *Dde*I (10394) site and the presence of *Hinf*I (12308) site, H defined by the absence of *Dde*I (10394) site and the presence of *Alu*I site (10397), and L defined by the presence of *Dde*I (10394) and the presence of *Hpa*I (3592) sites.

The frequencies of the haplogroups in the study populations are given in Table 4. Asian (north) and Amerindians haplogroups A, B and D are absent among Kadar, Paniya, Irula and Kota. However haplogroup A is present only among Kurumbas, with a very low (3.3%) frequency. Haplogroup M was found to be the most frequent – 76.25% of the individuals in the pooled sample belonged to this haplogroup. The frequency of this haplogroup was found to be the lowest among the Irulas (53.3%) and highest among the Kotas (96.7%). Haplogroup U was also found to occur in all the populations except the Kotas, and the frequency of this haplogroup in the pooled sample was about (8.75%).

The early history of Indian populations is like a jigsaw puzzle with many missing pieces; however, there is enough anthropological and archaeological evidence to show that from time immemorial people of different ethnic stocks, cultures and languages entered India and

Table 3. mtDNA haplotypes based on ten restriction site and one insertion/deletion polymorphism in five tribal populations of South India

Haplotype	Kadar (n = 40)	Paniyan (n = 30)	Irula (n = 30)	Kota (n = 30)	Kurumba (n = 30)	Total (n = 160)
00111101010	22	18	14	29	23	106
	55.0	60.0	46.66	96.66	76.66	66.25
00110001010		10	7	1	4	22
		33.33	23.33	3.33	13.33	13.75
00111101000	8		1			9
	20.0		3.33			5.62
00110011010		2	1		2	5
		6.66	3.33		6.66	3.12
00110011000	3		6			9
	7.5		20.0			5.62
00111100110	4		1			5
	10.0		3.33			3.12
00110000110	1					1
	2.5					0.625
00111101011	1					1
	2.5					0.625
00111101001	1					1
	2.5					0.625
10110001010					1	1
					3.33	0.625

Order of loci: *Hae*III nt 663, *Hpa*I nt 3592, *Alu*I nt 5176, *Alu*I nt 7025, *Dde*I nt 10394, *Ahd* nt 10397, *Hinf*I nt 12308, *Hinc*III nt 13259, *Alu*I nt 13262, *Hae*III nt 16517, 9-bp deletion.
1, Presence of restriction site; 0, absence of restriction site.

Table 4. Frequencies (%) of various haplogroups in five tribal populations

Population	Haplogroup frequency (%)			
	A	C	M	U
Kadar		4 (10.0)	36 (90.0)	3 (7.5)
Paniya			18 (60)	2 (6.67)
Irula		1 (3.3)	16 (53.3)	7 (23.3)
Kota			29 (96.7)	
Kurumba	1 (3.3)		23 (76.7)	2 (6.7)
Pooled	1 (0.625)	5 (3.125)	122 (76.25)	14 (8.75)

contributed to the present-day gene pool of the subcontinent[15]. Tribal populations, because of their long isolation, are well differentiated from the non-tribal communities. It is clear that the south Indian tribals form a large and heterogeneous population, made up mostly of relic populations, but perhaps also of later arrivals that were never totally absorbed into the Indian culture[2].

Ballinger *et al.*[16] have proposed that the COII/tRNA^{Lys} 9-bp deletion originated in central China and spread to the southeast Asian populations and to coastal and island populations of the Pacific. In our study, only the Kadar population harboured the 9-bp deletion with a frequency of 0.05%. Earlier studies[6,17] have also indicated that this 9-bp deletion is extremely rare in India. The Dravidian tribes were found to possess the haplogroup M at a higher frequency (63.75%) compared with the Austro-Asiatics and the Tibeto-Burman tribes[18]. The comparison of our haplotype data with other tribal populations of India reveals interesting features. There is an extensive sharing of haplotypes across the ethnic groups; one haplotype (00111101010) was modal across all the population groups indicating the most ancient haplotype and therefore supporting the hypothesis proposed[18] that there was a small female founding population in India.

The presence of *Dde*I (10394) *Alu*I (10397) sites defined a specific haplogroup M. This halogroup was originally identified in southeast Asian populations[16,19], but later shown to be an ancient marker in India, predating the migration of Indo-European speakers into India[20]. Consistent with Roychoudhury *et al.*[6,18], all tribal populations of south India were found to possess this haplogroup in high frequencies (53.3–96.7%). As the tribal populations are considered by most anthropologists to be indigenous groups, it indicates that the haplogroup M is an ancient haplogroup in India. Recently, Quintana-Murci *et al.*[21] reported the presence of haplogroup M in Africa (Ethiopia) with a fairly high frequency (18%) and proposed that this haplogroup originated in Africa. On the contrary, Roychoudhury *et al.*[8,16] showed that haplogroup M is ubiquitous throughout India and its high frequency possibly indicates that this haplogroup has arisen in India and was carried to Ethiopia. The high frequency of haplogroup M found in the south Indian tribal populations in the present study also supports this hypothesis.

Haplogroup U has been reported as the second most common haplogroup in Europe[22]. Kivisild *et al.*[23] have reported its presence in west Eurasian and Indian populations. Hence it may serve as a good marker for identifying Caucasoid admixture in Indian populations. However, it is also found at low frequencies in western and eastern African populations[24], and therefore may have also been introduced into India and Africa. The presence of haplogroup U in south Indian tribal populations is rather interesting. It is consistent with the theory that Dravidian-speaking populations were more widespread in India and that the Aryan-speakers pushed them to their present habitat in southern India. However, this haplogroup may have been present among Dravidian-speakers even when they arrived in India with agriculture from the Fertile Crescent region. Further data on autosomal and Y chromosomal polymorphisms will provide information about early inhabitants of the Indian subcontinent.

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