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FOR THE RECORD

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Mitochondrial DNA Hypervariable Region I and II Sequence Polymorphism in the Dravidian Linguistic Group of India

POPULATION: Blood samples were collected from 131 unrelated individuals of four predominant and endogamous populations of Karnataka: Iyengar Brahmin, Lingayats, Gowda, and Bhovi. The population size for all of the four communities varied in between eight to eighty hundred thousand approximately (3).

KEYWORDS: forensic science, mitochondrial DNA, hypervariable region I and II, Gowda, Iyengar Brahmin, Lingayat, Bhovi, India.

Mitochondrial DNA is an important tool for studying maternal lineage that has been widely used in the study of human identification and population structure as well. In this study, HV I and HV II sequence polymorphism is studied in four important populations belonging to the Dravidian linguistic family of Karnataka, India. The information on some polymorphic autosomal STR loci is already available for these populations (1).

Genomic DNA was extracted from the whole blood samples following phenol-chloroform extraction method (4). A total of 10–15 ng of the isolated DNA was used as template for amplification of the Hypervariable (HV I and II) segments of the control region using two sets of primers as listed in Table 1. PCR was carried out in a 25 μL reaction volume consisting of 0.5 μM primer, 250 μM each dNTP, 2 μg/μL BSA, 0.5mM $MgCl_2$ and 5U Taq Polymerase (GIBCO BRL). Amplification was carried out in a PE 2400 thermocycler, with initial denaturation at 95°C for 1 min, followed by a cycling condition of 95°C for 10 sec, 60°C for 30 sec, and 72°C for 30 sec for 30 cycles.

ExoSap-IT™ (USB Corp.) was used to clean the amplified product following the manufacturer's instructions. Sequencing was performed at 96°C for 15 sec, 50°C for 1 sec, 60°C for 1min, followed by a final hold for 10 min at 15°C for 25 cycles using the BigDye™ Terminator Cycle Sequencing ready reaction (Applied Biosystems, Foster City, CA). The amplified product was ethanol precipitated before being run on the ABI 377 automated sequencer (Applied Biosystems, Foster City, CA).

The sequences were edited between positions 16050 to 16400 for HV I region and 90 to 415 for HV II region, which were then aligned and compared with the Anderson reference sequence (5) using BioEdit software (6). Gene and nucleotide diversity (7,8) were calculated using the Arlequin package (9).

Results obtained from the sequencing of the HV I and HV II segments of mt. DNA for each of the four populations are presented as haplotype data in Table 2. The values for gene and nucleotide diversity are given in Table 3. The Gene diversity for all studied populations was 1.00 while the nucleotide diversity varied from 0.037 for Lingayat to 0.066 for Gowda population. A total of 127 haplotypes were observed out of 131 samples. The Bhovis share two haplotypes 7 and 10 with the Gowda, and also haplotype 3 and 17, with Brahmin and Lingayat, respectively. The data generated in this study will add on to the mitochondrial control region sequence polymorphism marker databases available for other world populations.

Acknowledgment

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TABLE 1—*Primers used for PCR amplification and sequencing of hypervariable regions I and II of mitochondrial DNA.*

Primer	Nucleotide Sequence
L 15997	5′-CACCATTAGCACCCAAAGCT-3′
H 16391	5′-GAGGATGGTGGTCAAGGGAC-3′
L 048	5′-CTCACGGGAGCTCTCCATGC-3′
H 408	5′-CTGTTAAAGTGCATACCGCCA-3′

TABLE 2A—*Mitochondrial DNA HV-I and HV-II control region sequence polymorphism in 4 populations of Karnataka (*N*=131).*

SAMPLE	HAPLOTYPE Number	16069	16086	16093	16095	16102	16111	16126	16129	16145	16152	16153	16154	16157	16158	16168	16169	16170	16172	16176	16179	16184	16185	16186	16188	16189	16192	16193	16198	16199	16201	16206	16207	16209	16213	16214	16217	16218	16221	16222	16223	16227	16230	16231	16234	16239	16240	16242
Anderson		C	T	T	C	T	C	T	G	G	T	G	T	T	A	C	C	A	T	C	C	C	C	C	C	C	T	C	C	T	T	C	A	T	G	C	T	C	C	C	C	A	A	T	C	C	A	C
B1	1								A																																T							
B2	2																			T																					T							
B3	3																																								T							
B4	4								A																																T							
B5	5																																								T							
B6	6																																								T							
B7	7																																															
B8	8																								T																T							
B9	9								A																																T							
B10	10																																								T					T		
B11	11																																								T							
B12	12								A																											A											
B13	13								A																																							
B14	14																																															
B15	15																	G					T																								T		
B16	16		C								C																																					
B17	17																																								T							
B18	18																								T																T			C				
B19	19																																	C												T		
B20	20																																								T							
B21	21																																															
B22	22																													C											T							
B23	23						T																																	T							
B24	24								A																																T							
B25	25																						T																		T							
B26	26																																								T							
B27	27																																	C							T							
B28	28						T																																	T							
B29	29																																															
B30	30																																								T							
Bh1	31																																															
Bh2	32														G																										T							
Bh3	33			C																																				T							
Bh4	34																																								T							
Bh5	35																												C								C										
Bh6	36																												C								C										
Bh7	37								A																																T				T			
Bh8	38																																															
Bh9	39			C																																				T							
Bh10	3																																								T							
Bh11	40																																													T		
Bh12	41																																								T							
Bh13	42																																					T										T
Bh14	43																																														
Bh15	44																																								T							
Bh16	45																					T											G														
Bh17	46																			T																				T							

B- Bhovi, Bh- Brahmin, L- Lingayat, G- Gowda. 'D' – deletion , an insertion is designated by the appropriate position followed by a dot.

TABLE 2B—*Continued.*

SAMPLE	HAPLOTYPE Number	16245	16247	16250	16254	16256	16259	16260	16261	16262	16264	16265	16266	16270	16271	16274	16275	16278	16287	16289	16291	16292	16294	16296	16297	16298	16301	16304	16309	16310	16311	16312	16316	16318	16319	16320	16325	16327	16330	16343	16352	16353	16355	16356	16359	16360	16362	16366
Anderson		C	A	C	A	C	C	C	C	C	C	A	C	C	T	G	A	C	C	A	C	C	C	C	C	T	T	C	T	A	G	T	A	A	A	G	C	T	C	T	A	T	C	C	T	T	C	T	C
B1	1																																																
B2	2																																																
B3	3																	T																															
B4	4										T	C																																					
B5	5																											C																				
B6	6																				T																												
B7	7									T																													C									
B8	8																																															
B9	9																				T																												
B10	10																																															
B11	11																																															
B12	12																																															
B13	13																					T																											
B14	14												T							G								C																				
B15	15																				T																												
B16	16			T																																			C									
B17	17																																															
B18	18																																											T			C	
B19	19																																									C	T					
B20	20										T																																					
B21	21		G		G																																											
B22	22																																															
B23	23																G														C																
B24	24																																															
B25	25							T																		C																					
B26	26																																															
B27	27																																		A													
B28	28																																															
B29	29												T															C									C						T	C			
B30	30																																										T					
Bh1	31																									C													T								
Bh2	32																																															
Bh3	33																																															
Bh4	34																													A																	
Bh5	35																																				C											
Bh6	36																																				C											
Bh7	37																																															
Bh8	38						T		T																																						
Bh9	39																																															
Bh10	3																	T																														
Bh11	40																				T																									C		
Bh12	41																																	T													
Bh13	42																											C																			
Bh14	43							T																											A												
Bh15	44																																															
Bh16	45																																	T									C				
Bh17	46															A																			A									C				

B- Bhovi, Bh- Brahmin, L- Lingayat, G- Gowda. 'D' – deletion, an insertion is designated by the appropriate position followed by a dot.

TABLE 2C—*Continued.*

SAMPLE	HAPLOTYPE Number	16356	16359	16360	16362	16366	16368	16380	16381	111	140	146	150	151	152	153	174	182	194	195	196	199	200	204	214	224	227	234	239	242	246	250	263	264	271	279	293	295	309.1	309.2	315.1	335	343	372	373
Anderson		T	T	C	T	C	T	C	T	A	C	T	C	C	T	A	A	C	C	T	T	T	A	T	A	T	A	A	T	C	T	T	A	C	C	T	T	C				A	C	T	A
B1	1											C		T		G													C				G						C			C			
B2	2														C																		G						C			C			
B3	3																																G									C			
B4	4																																G						C			C			
B5	5											C								C												G									C			
B6	6														C																	G									C			
B7	7																															G						C			C			
B8	8											C																				G						C			C			
B9	9														C																	G									C			
B10	10													T	C																	G						C			C			
B11	11																															G									C			
B12	12																															G						C			C			
B13	13																															G						C	C	C			G
B14	14																						G									G						C			C			
B15	15																															G						C			C			
B16	16																															G						C			C			
B17	17														C																	G									C			
B18	18				C										C			T		C												G						C			C			
B19	19														C					C												G									C			
B20	20																															G									C			
B21	21											C			C																	G									C			
B22	22																					C										G						C			C			
B23	23																		T					C								G						C			C			
B24	24														C																	G									C			
B25	25																					C										G									C			
B26	26																		T												C	G									C			
B27	27												T		C																	G									C			
B28	28											C			C		T															G									C			
B29	29	C																														G						C			C			
B30	30																															G						C			C			
Bh1	31																															G									C			
Bh2	32																															G									C			
Bh3	33																															G						C			C			
Bh4	34														C																	G						C			C			
Bh5	35														C						C											G						C			C			
Bh6	36																															G						C	C	C			
Bh7	37														C																	G						C			C			
Bh8	38																					C										G									C			
Bh9	39																					C										G									C			
Bh10	3																															G									C			
Bh11	40	C																	T												C	G									C			
Bh12	41																															G						C			C			
Bh13	42										T																					G						C			C			
Bh14	43											C							T													G						C			C			
Bh15	44														C																	G						C	C	C			
Bh16	45											C																G				G									C			
Bh17	46														C																	G						C			C			

B- Bhovi, Bh- Brahmin, L- Lingayat, G- Gowda. 'D' – deletion, , an insertion is designated by the appropriate position followed by a dot.

TABLE 2D—*Continued.*

SAMPLE	HAPLOTYPE Number	16069	16086	16093	16095	16102	16111	16126	16129	16145	16152	16153	16154	16157	16158	16168	16169	16170	16172	16176	16179	16184	16185	16186	16188	16189	16192	16193	16198	16199	16201	16206	16207	16209	16213	16214	16217	16218	16221	16222	16223	16227	16230	16231	16234	16239	16240	16242
Anderson		C	T	T	C	T	C	T	G	G	T	G	T	T	A	C	C	A	T	C	C	C	C	C	C	C	T	C	C	T	T	C	A	A	T	G	C	T	C	C	C	C	A	A	T	C	C	A	C
Bh18	47																												C												T								
Bh19	48																												C												T								
Bh20	49																																																
Bh21	50																																																
Bh22	51												C																			C											G					
Bh23	52																																																
Bh24	53																																								T								
Bh25	54																																								T								
Bh26	55																																															
Bh27	56																					T																			T								
Bh28	57																																								T								
Bh29	58																																								T								
Bh29	59																																								T								
Bh31	60																																															
• Bh32	61																																								T								
Bh33	62									A				C																										T								
Bh34	63																																								T								
Bh35	64															T									T																T			C	T				
Bh36	65																																								T								
Bh37	66					C																																		T								
L1	67																																								T								
L2	68																										T														T								
L3	69																																																
L4	70																																								T								
L5	71																																								T								
L6	72																																																
L7	73																																								T								
L8	74																																																
L9	75						T																																	T								
L10	76																																																
L11	77																T																								T								
L12	78																																																
L13	79																																								T								
L14	80																										T														T								
L15	81																														T										T								
L16	17																																								T								
L17	82																											T													T								
L18	83																																																
L19	84																																							T								
L20	85																					T																			T								
L21	86																																																
L22	87																																								T								
L23	88																																																

B- Bhovi, Bh- Brahmin, L- Lingayat, G- Gowda. 'D' – deletion, , an insertion is designated by the appropriate position followed by a dot.

TABLE 2E—*Continued.*

SAMPLE	HAPLOTYPE Number	16245	16247	16250	16254	16256	16259	16260	16261	16262	16264	16265	16266	16270	16271	16274	16275	16278	16287	16289	16291	16292	16294	16296	16297	16298	16301	16304	16309	16310	16311	16312	16316	16318	16319	16320	16325	16327	16330	16343	16352	16353	16355
Anderson		C	A	C	A	C	C	C	C	C	C	A	C	C	T	G	A	C	C	A	C	C	C	C	T	T	C	T	A	G	T	A	A	A	G	C	T	C	T	A	T	C	C
Bh18	47																																										
Bh19	48																																										
Bh20	49																					T			C																		
Bh21	50																						T																				
Bh22	51												T					T																										
Bh23	52																						T	T																				
Bh24	53													T																														
Bh25	54																																										
Bh26	55												T															C	G								C						
Bh27	56					T																								C												
Bh28	57																																										
Bh29	58																	T																										
Bh29	59																									T						G											
Bh31	60																																										
Bh32	61																																										
Bh33	62																																										
Bh34	63																																				C						
Bh35	64																														C												
Bh36	65													T																	C				A					C			
Bh37	66																																										T
L1	67																																										
L2	68																																										
L3	69																																										
L4	70																																										
L5	71																																										
L6	72																											C															
L7	73																											C															
L8	74																						T														C						
L9	75																														C												
L10	76																														C												
L11	77									T																																	
L12	78																											C			C												
L13	79																						T																				
L14	80																	T																										
L15	81											G																																
L16	17																																											
L17	82																																											
L18	83																														C												
L19	84																																											
L20	85																																											
L21	86																																											
L22	87																																											
L23	88	T											T					T																										

B- Bhovi, Bh- Brahmin, L- Lingayat, G- Gowda. 'D' – deletion, , an insertion is designated by the appropriate position followed by a dot.

TABLE 2F—*Continued.*

SAMPLE	HAPLOTYPE Number	16356	16359	16360	16362	16366	16368	16380	16381	111	140	146	150	161	152	153	174	182	194	195	196	199	200	204	214	224	227	234	239	242	246	250	263	264	271	279	293	295	309.1	309.2	315.1	335	343	372	373
Anderson		T	T	C	T	C	T	C	T	A	C	T	C	C	T	A	A	C	C	T	T	T	A	T	A	T	A	A	T	C	T	T	A	C	C	T	T	C				A	C	T	A
Bh18	47														C																		G						C		C				
Bh19	48																																G						C		C				
Bh20	49														C																		G								C				
Bh21	50																																G						C		C				
Bh22	51													T	C																		G						C		C				
Bh23	52																			C													G								C				
Bh24	53				C				C			C			C																		G								C				
Bh25	54		C	T			C																										G		T			T			C				
Bh26	55																					C											G								C				
Bh27	56				C										C																		G								C				
Bh28	57					D		D												C													G								C			C	
Bh29	58											C												C									G						C		C				
Bh29	59														C					C													G						C	C	C				
Bh31	60																																G								C				
Bh32	61				C										C																		G						C		C				
Bh33	62																																G						C		C				
Bh34	63											C	T																				G						C		C				
Bh35	64				C										C																		G						C	C	C				
Bh36	65														C																		G						C		C				
Bh37	66											C			C				T														G						C		C				
L1	67														C																		G						C		C				
L2	68														C																		G								C				
L3	69				C																								C				G						C		C				
L4	70																					C											G								C				
L5	71				C							C			C																		G						C		C				
L6	72																																G						C		C				
L7	73																					C											G								C				
L8	74																			C													G						C		C				
L9	75																																G						C		C				
L10	76																																G						C		C				
L11	77																							C									G						C		C	G			
L12	78																																G						C		C				
L13	79												T																				G						C		C				
L14	80														C												G						G				C				C				
L15	81																									C							G						C		C				
L16	17														C																		G								C				
L17	82																			C													G						C		C				
L18	83																										G						G								C				
L19	84																																G								C				
L20	85																																G	T					C		C				
L21	86				C																												G						C		C				
L22	87				C																												G								C				
L23	88																														C		G						C		C				

B- Bhovi, Bh- Brahmin, L- Lingayat, G- Gowda. 'D' –deletion, , an insertion is designated by the appropriate position followed by a dot.

TABLE 2G—*Continued.*

SAMPLE	HAPLOTYPE Number	16069	16086	16093	16095	16102	16111	16126	16129	16145	16152	16163	16154	16157	16158	16168	16169	16170	16172	16176	16179	16184	16185	16186	16188	16189	16192	16193	16198	16199	16201	16206	16207	16209	16213	16214	16217	16218	16221	16222	16223	16227	16230	16231	16234	16239	16240	16242
Anderson		C	T	T	C	T	C	T	G	G	T	G	T	T	A	C	C	A	T	C	C	C	C	C	C	T	C	C	T	C	A	A	T	G	C	T	C	C	C	C	A	A	T	C	C	A	C
G1	89									A																							C						T										
G2	90																																							T				C				
G3	91																																							T		G						
G4	92	T		C				C		A									C																				T								
G5	93																																															
G6	94																																							T								
G7	95																																							T								
G8	96																																							T								
G9	10																																															
G10	97							C													T																					G			T			
G11	98																																															
G12	99																																															
G13	100																		C																													
G14	101																																							T			C					
G15	102																																															
G16	7																																							T								
G17	103																																							T								
G18	104																																															
G19	105																																							T								
G20	106						T																																	T								
G21	107																																							T								
G22	108																																							T								
G23	109																																	C											T		
G24	110																																															
G25	111			C																																				T								
G26	112																																							T								
G27	113																						T			C		D												T								
G28	114																																							T								
G29	115									A																														T								
G30	116																																															
G31	117							C																T									C														
G32	118																																	C											T		
G33	119																																			T				T								
G34	120							C													T																							T			
G35	121		C																																				T						T		
G36	122																		C								T												T								
G37	123																																							T								
G38	124	T																										T												T								
G39	125											A																												T								
G40	126																																	C											T		
G41	127				T		T												C																					T							

B- Bhovi, Bh- Brahmin, L- Lingayat, G- Gowda. 'D' – deletion, an insertion is designated by the appropriate position followed by a dot.

TABLE 2H—*Continued.*

SAMPLE	HAPLOTYPE Number	16245	16247	16250	16254	16256	16259	16260	16261	16262	16264	16265	16266	16270	16271	16274	16275	16278	16287	16289	16291	16292	16294	16296	16297	16298	16301	16304	16309	16310	16311	16312	16316	16318	16319	16320	16325	16327	16330	16343	16352	16353	16355
Anderson		C	A	C	A	C	C	C	C	C	C	A	C	C	T	G	A	C	C	A	C	C	C	C	T	T	C	T	A	G	T	A	A	A	G	C	T	C	T	A	T	C	C
G1	89							T	T																						C				A						G			
G2	90																														C												
G3	91													T															G														
G4	92									T																																	
G5	93												T															C			C												T
G6	94																																										
G7	95													T																													
G8	96													T																													
G9	10																														C												
G10	97																																										
G11	98					T										A																											
G12	99																																	T									
G13	100																	T																									
G14	101																														C												
G15	102												T															C									C						
G16	7								T																						C												
G17	103																																										
G18	104												T												C						C												T
G19	105																														C												
G20	106																														C												
G21	107															A																			A	T							
G22	108																															G											
G23	109																						T																			C	T	
G24	110												T															C			C												
G25	111																				D					C												C	T				
G26	112																											C															
G27	113							T																		C																	
G28	114																				T																							
G29	115														C																													
G30	116		G		G																																						
G31	117																																										
G32	118																																									C	T	
G33	119																		T								T	C																
G34	120		G																																								
G35	121								T																																		
G36	122																	T													C						C						
G37	123																																										
G38	124																														C												
G39	125																																										
G40	126																																									C	T	
G41	127										T			T																	C				A								

B- Bhovi, Bh- Brahmin, L- Lingayat, G- Gowda. 'D' – deletion, , an insertion is designated by the appropriate position followed by a dot.

TABLE 2I—*Continued.*

SAMPLE	HAPLOTYPE Number	16356	16359	16360	16362	16366	16368	16380	16381	111	140	146	150	151	152	153	174	182	194	195	196	199	200	204	214	224	227	234	239	242	246	250	263	264	271	279	293	295	309.1	309.2	315.1	335	343	372	373
Anderson		T	T	C	T	C	T	C	T	A	C	T	C	C	T	A	A	C	C	T	T	T	A	T	A	T	A	A	T	C	T	T	A	C	C	T	T	C				A	C	T	A
G1	89																			C													G						C		C				
G2	90	C			C																												G								C				
G3	91																																G						C		C				
G4	92																													T			G					T			C				
G5	93	C													C																		G								C				
G6	94															G																	G			C					C				
G7	95																							C									G						C	C	C				
G8	96														C																		G						C	C	C				
G9	10																																G						C		C				
G10	97																								G								G						C		C				
G11	98																																G								C				
G12	99																														C		G								C				
G13	100																																G								C				
G14	101	C			C																												G						C		C				
G15	102	C																															G								C				
G16	7																					C											G								C				
G17	103																																G						C		C				
G18	104	C											T									C											G								C				
G19	105																																G						C		C				
G20	106																			C													G						C		C				
G21	107				C																												G						C		C				
G22	108														C																		G						C	C	C				
G23	109											C																G					G								C				
G24	110	C																	T														G						C		C				
G25	111				C																												G						C		C				
G26	112																					C											G								C				
G27	113																																G								C				
G28	114																																G								C				
G29	115																																G								C				
G30	116												T																				G								C				
G31	117																				C												G								C				
G32	118														C																		G								C				
G33	119											C																					G						C		C		T		
G34	120											C																					G								C		C		
G35	121																																G								C				
G36	122														C																		G						C		C				
G37	123											C																G					G								C				
G38	124													T															C				G								C				
G39	125																			C													G								C				
G40	126											C			C													G					G						C	C	C				
G41	127									C		C										C									C	G								C				

B- Bhovi, Bh- Brahmin, L-Lingayat, G- Gowda. 'D' – deletion, an insertion is designated by the appropriate position followed by a dot.

TABLE 3—*Gene and nucleotide diversity of mitochondrial DNA hypervariable regions I and II in the four studied populations of Karnataka.*

Population	Bhovi	Brahmin	Lingayat	Gowda
	HV I + HV II	HV I + HV II	HV I + HV II	HV I + HV II
Gene diversity	1.000 ± 0.008	1.000 ± 0.006	1.000 ± 0.012	1.000 ± 0.005
Nucleotide diversity	0.054 ± 0.027	0.056 ± 0.029	0.037 ± 0.021	0.066 ± 0.034

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