



Short communication

mtDNA sequence diversity of Hazara ethnic group from Pakistan



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ABSTRACT

The present study was undertaken to investigate mitochondrial DNA (mtDNA) control region sequences of Hazaras from Pakistan, so as to generate mtDNA reference database for forensic casework in Pakistan and to analyze phylogenetic relationship of this particular ethnic group with geographically proximal populations. Complete mtDNA control region (nt 16024–576) sequences were generated through Sanger Sequencing for 319 Hazara individuals from Quetta, Baluchistan. The population sample set showed a total of 189 distinct haplotypes, belonging mainly to West Eurasian (51.72%), East & Southeast Asian (29.78%) and South Asian (18.50%) haplogroups. Compared with other populations from Pakistan, the Hazara population had a relatively high haplotype diversity (0.9945) and a lower random match probability (0.0085). The dataset has been incorporated into EMPOP database under accession number EMP00680. The data herein comprises the largest, and likely most thoroughly examined, control region mtDNA dataset from Hazaras of Pakistan.

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1. Introduction

Hazaras are among few ethnic groups whose origin is obscure. Ancestral home of Hazaras is known as Hazarajat (also referred as Hazaristan), which lies in the central highlands of Afghanistan, among the Koh-i-Baba mountains and the western extremities of the Hindu Kush [1]. Hazaras speak “Hazaragi” (or Aazragi) language [2] and are overwhelmingly Shia Muslims constituting the third largest ethnic group in Afghanistan [3].

According to historical records, several Turk and Mongol tribes constituted the Hazaras ethnically, hence, these are considered of Turko-Mongol origin. The Hazaras identity is believed to be originated after 1221 CE siege of Bamiyan by the Mongol Empire, led by Genghis Khan in pursuit of Jalal ad-Din Mingburnu, the last ruler of the Khwarezmian Empire, and his forces in Afghanistan. It is commonly believed that the soldiers of army left behind by Genghis Khan to guard important places at Hindu Kush, married to the local Tajik women and gave rise to the ancestors of the Hazaras [2]. Few Mongol words in vocabulary of the language of Hazaras

supports their Mongol ancestry. Moreover, there are also lines of evidence that some of the remote tribes of Hazaras spoke Mongol language till last century. Their central Asian facial features including sparse beards, high cheekbones and epicanthic eye folds further supports their Mongol origin [2].

Hazaras have faced history of horrendous persecution in Afghanistan. It dates back to 16th century, with Babur from Kabulistan. It is reported that during reign of Emir Abdur Rahman (1880–1901), thousands of Hazaras were killed, expelled and enslaved [4]. Syed Askar Mousavi, a contemporary Hazara writer, claims that half the population of Hazaras was displaced, shifted to neighboring Baluchistan province of Pakistan [5] and Khorasan Province of Iran [4].

Population data on Hazaras from both countries, Afghanistan and Pakistan, have only been restricted to HVS-I [6,7]. Forensic casework mtDNA analysis requires relevant and reliable databases for estimating the probability of a given haplotype in a population [8]. This population study constitutes high quality entire mitochondrial DNA (mtDNA) control region sequence data of 319 randomly selected individuals from population of Hazara, resident of Baluchistan province of Pakistan.

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2. Materials and methods

Blood samples were collected from 319 Hazara individuals (males $n = 185$); (females $n = 134$) residing in Hazara town, an area on the western outskirts of Quetta, city of Baluchistan Province of Pakistan (Fig. 1). All participants were unrelated individuals within at least three generations.

Written informed consent was obtained from all volunteer donors. Samples were fully anonymized. Ethical approval was obtained from Institutional Review Board of University of Health Sciences, Lahore, Pakistan.

2.1. DNA extraction, amplification and sequencing

Genomic DNA was extracted with Axygen[®] AxyPrep[™] Blood Genomic DNA Miniprep Kit according to the manufacturer's protocol (Axygen Biosciences; CA, USA). Same set of primers and PCR conditions were used to amplify the entire control region (nt 16024–576) as in our previous study [9]. Purification of PCR products was done with Exonuclease I (TaKaRa) and Shrimp Alkaline Phosphatase (TaKaRa) in 10× Exonuclease I Buffer (TaKaRa) following reaction compositions recommended by the manufacturer. The reaction mixture was incubated at 37 °C for 45 min, followed by enzyme deactivation at 95 °C for 15 min. Sequencing primers from [9] were used to perform capillary electrophoresis using the BigDye[™] Terminator v3.0 Ready Reaction Cycle Sequencing Kit on Applied Biosystems 3730×1 DNA Analyzer (Thermo Fisher Scientific) according to manufacturer's instructions.

2.2. Haplogroup assignment

Forward and reverse sequences were aligned and compared using by SeqManNGen[®] version 12.0 (DNASTAR, Madison, WI). Sequence variations in each sequence were scored relative to the revised Cambridge Reference Sequence (rCRS) [10]. Quality of sequences was examined manually, and two analysts independently annotated deviations from the reference sequence. The recommended nomenclature for mtDNA typing was used for

alignment of variants [11]. The haplogroup assignments were carried out using Mitotool (www.mitotool.org) [12,13], Haplogrep (www.haplogrep.uibk.ac.at) [14], and EMMA (www.empop.online) [15] based on PhyloTree build 17 [16]. Haplogroup assignments were re-evaluated by manual inference and conservative most recent common ancestor (MRCA) status was assigned to each sequence to improve the prediction. All 319 sequences generated here were incorporated to EMPOP database under accession number EMP00680 [17]. The sequences are also available on GenBank via accession numbers KX448813–KX449131.

2.3. Data analysis

Indels at nucleotide positions 16193, 309, 315 and 573 were not taken into account for analysis unless otherwise mentioned. The number of haplotypes were calculated by direct counting method. Discrimination capacity was estimated by dividing the number of different haplotypes by total number of individuals [18]. Haplotype diversity was estimated by DnaSP 5.10.1 [19] and random match probability was calculated as sum of squares of observed haplotype frequencies [18].

Based on the distribution patterns [20] and the latest version of the PhyloTree [16], haplogroups were assigned into three ancestry groups: West Eurasian (H, HV, I, J, K, N, R, R0, T, U1a, U5a, U7, U8a, W, X), South Asian (M*, U2, U4), and East Asian (A, B, C, D, F, G) [7,16,21,22]. Molecular diversity indices, pairwise differences between and within populations, haplotype frequencies, analysis of molecular variance (AMOVA) and pairwise F_{ST} values were calculated using DnaSP 5.10.1 [19] and Arlequin 3.5 (Computational and Molecular Population Genetics Lab, Institute of Ecology, and Evolution, University of Berne, Bern, Switzerland) [23]. F_{ST} calculations were made under the Kimura 2-parameter model [24]. In addition, the data was compared with previously published data of other ethnic groups from Pakistan (Kashmiri [9], Pathan [25], Makrani [26]) and worldwide populations (Uzbek, Kazakh, Turkmen, Kyrgyz, Afghan, Russian, Tajik [27], Chinese [28], Thai [29], Vietnamese [30], Laos [31], Kuwaiti [32], Iraqi [33], Egyptian [34], Dubai [35], Ashkenazi Jews [36], Roma [37], Sze 'kely [37], Greeks [38] Moroccan [39] and Iranian [40]) for which entire



Fig. 1. The red dotted area refers to geographic location of sampling from Quetta of Pakistan. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

control region (nt 16024–576) data could be obtained from the literature. Additional comparative analysis was performed with populations for those available sequences consisted of at least nt 16024–16569 range i.e, Afghanistan (Hazara, Pashtun, Tajik, Turkmen, and Uzbek [7]), China (Kazakh [41] and Mongol [42]), Iran (Iranian [40]), Uzbekistan (Uzbek, Kazakh, Turkmen, Kyrgyz, Afghan, Russian, Tajik [27]), Pakistan (Kashmiri [9], Pathan [25], Makrani [26], Baluch (EU565791–EU565815) and Brahui (EU565766–EU565790)). In order to have uniformity and non-arbitrary comparative analysis for the published data, consistent treatment of selecting base on the basis of phylogenetic weightage was applied for the sequence heteroplasmic positions. Pairwise F_{ST} matrix was imported into R statistical package (<http://www.R-project.org/>) for plotting heatmap (ComplexHeatmap) and principal components analysis (FactoMineR). This study followed the requirements of this journal [43,44] and the ISFG recommendations for forensic population data publication [43].

3. Results and discussion

To establish reference database for mtDNA applications in forensic, here we present a high quality mtDNA control region sequences dataset of 319 Hazara individuals sampled from Baluchistan Province of Pakistan. mtDNA haplotypes of 319 individuals are given in Table S1. Data showed higher haplotype diversity (0.9945) for Hazaras as compared to Makrani (0.9905) [26] but lower than Kashmiri (0.9977) and Pathan populations [25]. Further, data showed lower random match probability (0.0085) among Hazaras as compared to Makrani population, while a higher value than Kashmiri and Pathan populations (Table 1). Point heteroplasmies were identified in two individuals at two different positions (16230R and 16256Y).

3.1. Haplogroup information

A total of 189 distinct haplotypes were observed in all 319 individuals (Table S2). The most common haplotype among all was shared by 9 individuals (2.82%). Out of 189 haplotypes, 124 were unique while 65 were shared by 2–9 individuals. All these haplotypes were assigned to 156 different haplogroups/sub-haplogroups based on PhyloTree Build 17 [16]. The haplogroups were mainly assigned into three continental groups, namely West Eurasian (51.72%), East & Southeast Asian (29.78%) and South Asian (18.50%) [7,16,21,22]. The most frequent haplogroups were H (14.11%), K (14.73%), D (9.72%) and HV (9.40%). Hazaras had higher percentage of East and South East Asian haplogroups among all other Pakistani populations (Fig. S1). Phylogeographical cross-comparison of mtDNA and Y-chromosomal data from Hazara had already showed that male descendants of Mongols were accompanied by women of East Asian ancestry while settling through Central Asia [6]. Our results indicate that the Hazara are characterized by relatively high frequencies of West Eurasian

mtDNAs which are virtually fewer or otherwise absent in bordering West Asian populations.

3.2. Pairwise F_{ST} comparisons and population structure

Comparison between different Pakistani populations (Kashmiri [9], Pathan [25] and Makrani [26]) on the basis of pairwise F_{ST} values using complete mtDNA control region sequences showed the highest distance between Hazara and Makrani followed by Hazara and Pathan populations (Table 2). Analysis of Molecular Variance (AMOVA) showed that 97.95% of the variance was within populations and 2.05% of the variance represented among Pakistani populations (Table S3).

Hazara control region (nt 16024–576) sequence data was compared with regional populations (Uzbek, Kazakh, Turkmen, Kyrgyz, Afghan, Russian, Tajik [27], Chinese [28], Thai [29], Vietnamese [30], Laos [31], Kuwaiti [32], Iraqi [33], Egyptian [34], Dubai [35], Ashkenazi Jews [36], Roma [37] Sze 'kely [37], Greeks [38] Moroccan [39] and Iranian [40]). AMOVA was used to test for significant variation in the mtDNA distribution among regional and global populations. 95.83% of the variance observed within these populations was attributable to differences within populations, and 4.17% of the variance represent differences among populations (Table S4). Heatmap matrix (Fig. S2) and corresponding pairwise F_{ST} values (Table S5) showed that Hazaras, along with other populations of northern areas of Pakistan (i.e. Kashmiri and Pathan), were clustered more closely to Central Asian Turkmen, Uzbek, Kazakh and Tajik populations. Similar results were obtained through Principal Component Analysis (PCA) (Fig. S3). These results supported the fact about Hazaras having their roots in Afghanistan that shares a long common history with Central Asian [45]. More than 2000 km of joint borders unite Afghanistan with Turkmenistan, Tajikistan and Uzbekistan and their multifaceted links might have influenced the mtDNA diversity of Hazaras [45]. It can also be noticed that Afghan and Russian from Uzbekistan [27] do not cluster with rest of the surrounding Central Asian populations including Hazara (Fig. S3). One potential reason could be relatively smaller sample size studied for these two populations.

In order to include available sequences (nt 16024–16569) from Afghanistan (Hazara, Pashtun, Tajik, Turkmen, and Uzbek [7]), an additional comparative analysis was performed by trimming the sequence data from China (Kazakh [41] and Mongol [42]), Iran (Iranian [40]), Uzbekistan (Uzbek, Kazakh, Turkmen, Kyrgyz,

Table 2

Pairwise F_{ST} distances of mtDNA control region sequences among different ethnic groups of Pakistan.

	Kashmiri [9]	Hazara	Makrani [26]	Pathan [25]
Kashmiri	*	0.012	0.031	0.014
Hazara	0.000	*	0.035	0.024
Makrani	0.000	0.000	*	0.032
Pathan	0.000	0.000	0.000	*

Pairwise F_{ST} values (above diagonal) and associated P -values (below diagonal).

Table 1

Forensic parameters and diversity indices of mtDNA control region sequences from Pakistani ethnic groups.

	Hazara	Kashmiri [9]	Pathan [25]	Makrani [26]
No. of Samples	319	317	230	99
No. of Haplotypes	189	251	192	71
No. of Unique haplotypes	124	201	128	54
No. of Polymorphic sites including indels	217	230	215	142
Discrimination Capacity	0.5925	0.7918	0.8348	0.7172
Random Match Probability	0.0085	0.0054	0.0066	0.0195
Haplotype Diversity (Hd)	0.9945	0.9977	0.9978	0.9905
Average Number of Pairwise differences	10.431	10.447	11.144	12.110

Afghan, Russian, Tajik [27]) and Pakistan (Pathan [25], Kashmiri [9], Makrani [26], Baluch (EU565791–EU565815) and Brahui (EU565766–EU565790)). PCA illustrated that Hazaras of Pakistan are more closely related to the Hazaras of Afghanistan while showed comparatively distal clustering with Mongols but in same quadrante (Fig. S4).

Comparative analysis of shared haplotypes of different populations was performed. Although number of shared haplotypes strongly depends on sample size, however, Hazaras shared more haplotypes with Tajiks of Afghanistan as compare to Tajiks from Uzbekistan (Table S6). These findings did not correspond to the historical claim of partial Tajik origin of Hazaras [2].

4. Conclusion

This is the first report of a reasonably sized forensic database from Hazaras including entire mtDNA control region sequences. The Hazaras are genetically found to be heterogeneous and show a higher similarity to the Central Asian populations as compared to Western Asian populations.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fsigen.2017.07.004>.

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Supplementary material: mtDNA sequence diversity of Hazara ethnic group from Pakistan

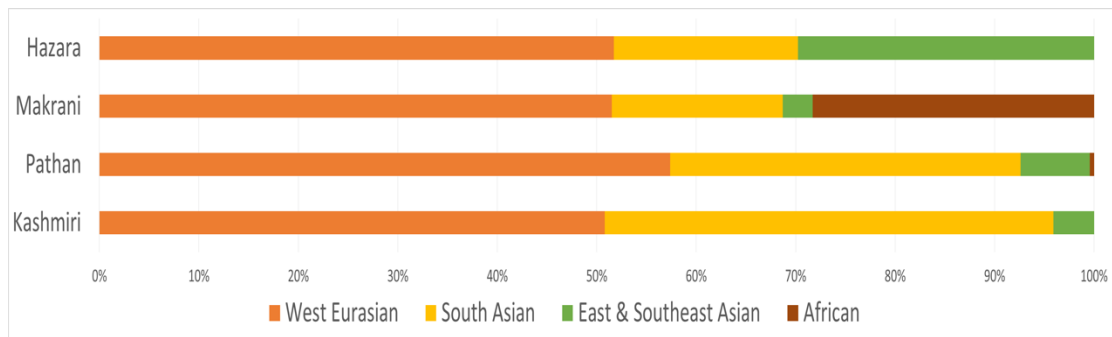


Figure S1. The matrilineal composition of four Pakistani populations based on complete control region sequences (Hazara (319), Kashmiri (317) [9], Pathan (230) [25] and Makrani (99) [26])

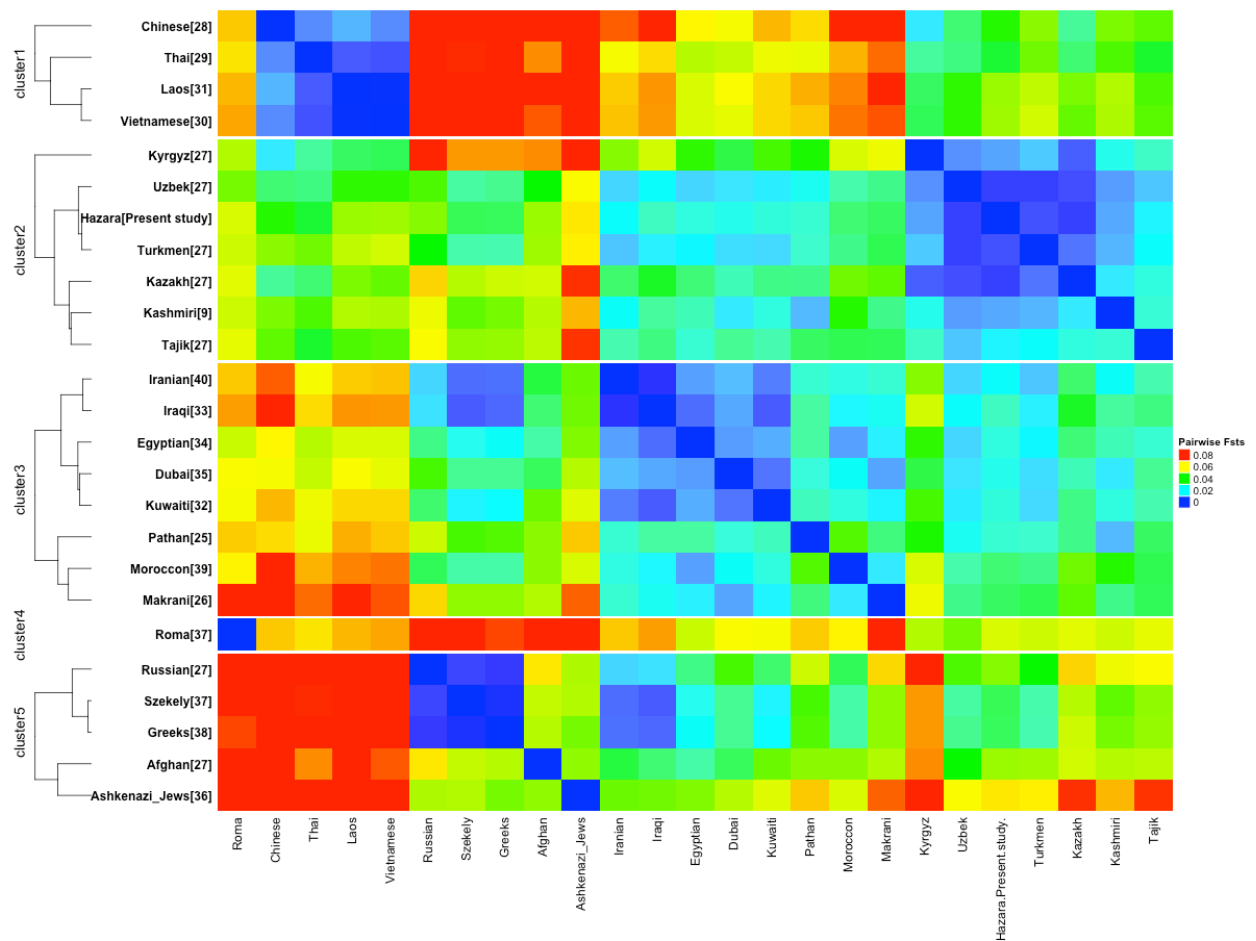


Figure S2. Heatmap matrix of pairwise F_{ST} values for 25 populations including Hazara

Data Sources (mtDNA Complete Control Region):

Populations	References	No. of samples	Populations	References	No. of samples
Hazara	Present study	319	Chinese	[28]	377
Kashmiri	[9]	317	Thai	[29]	190
Pathan	[25]	230	Vietnamese	[30]	187
Makrani	[26]	99	Laos	[31]	214
Uzbek	[27]	328	Kuwaiti	[32]	381
Turkmen	[27]	249	Iraqi	[33]	182
Tajik	[27]	244	Egyptian	[34]	277
Russian	[27]	151	Dubai	[35]	249
Kyrgyz	[27]	249	Ashkenazi Jews	[36]	173
Kazakh	[27]	256	Roma	[37]	205
Afghan	[27]	98	Greeks	[38]	319
Szekely	[37]	182	Iranian	[40]	352
Moroccan	[39]	509			

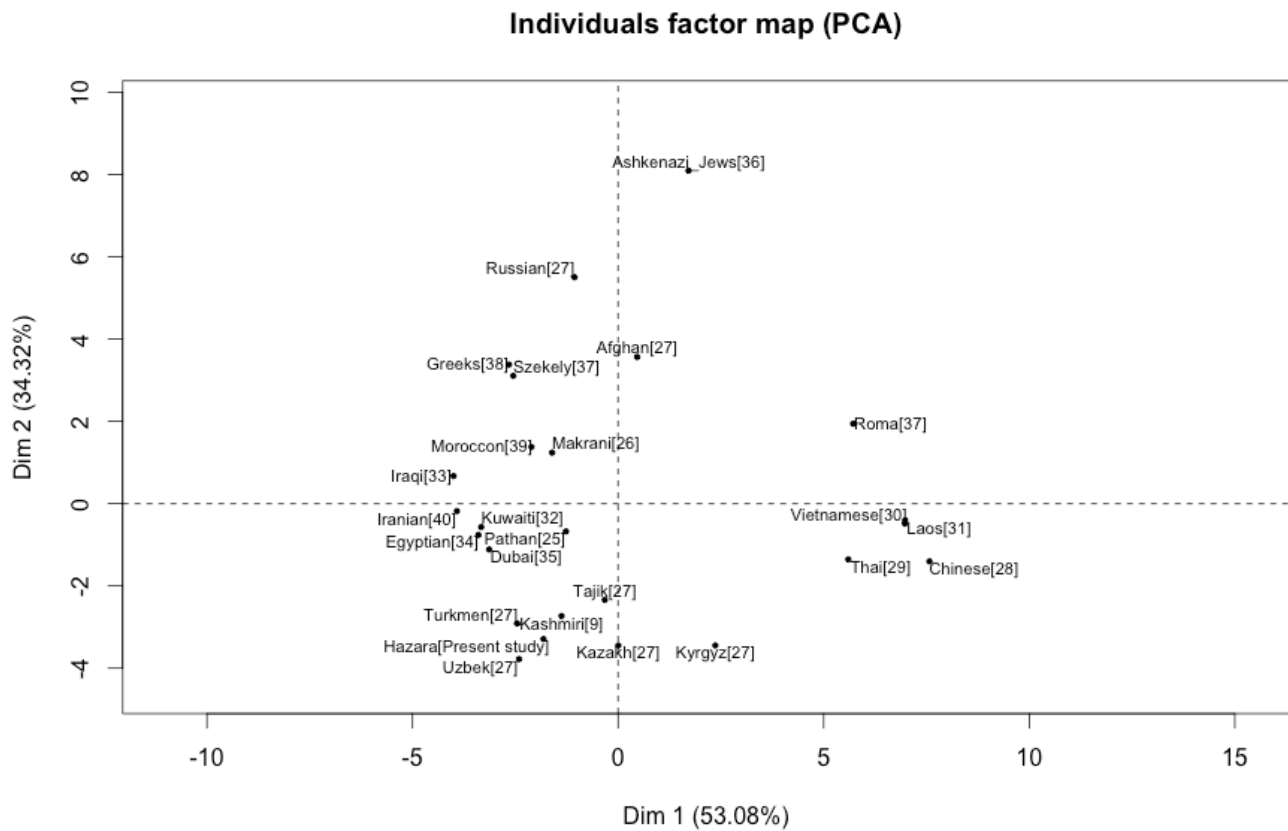


Figure S3: Principal component analysis (PCA) based on pairwise F_{ST} values defined by complete control region sequences

Data Sources:

Populations	References	No. of samples	Populations	References	No. of samples
Hazara	Present study	319	Chinese	[28]	377
Kashmiri	[9]	317	Thai	[29]	190
Pathan	[25]	230	Vietnamese	[30]	187
Makrani	[26]	99	Laos	[31]	214
Uzbek	[27]	328	Kuwaiti	[32]	381
Turkmen	[27]	249	Iraqi	[33]	182
Tajik	[27]	244	Egyptian	[34]	277
Russian	[27]	151	Dubai	[35]	249
Kyrgyz	[27]	249	Ashkenazi Jews	[36]	173
Kazakh	[27]	256	Roma	[37]	205
Afghan	[27]	98	Greeks	[38]	319
Szekely	[37]	182	Iranian	[40]	352
Moroccan	[39]	509			

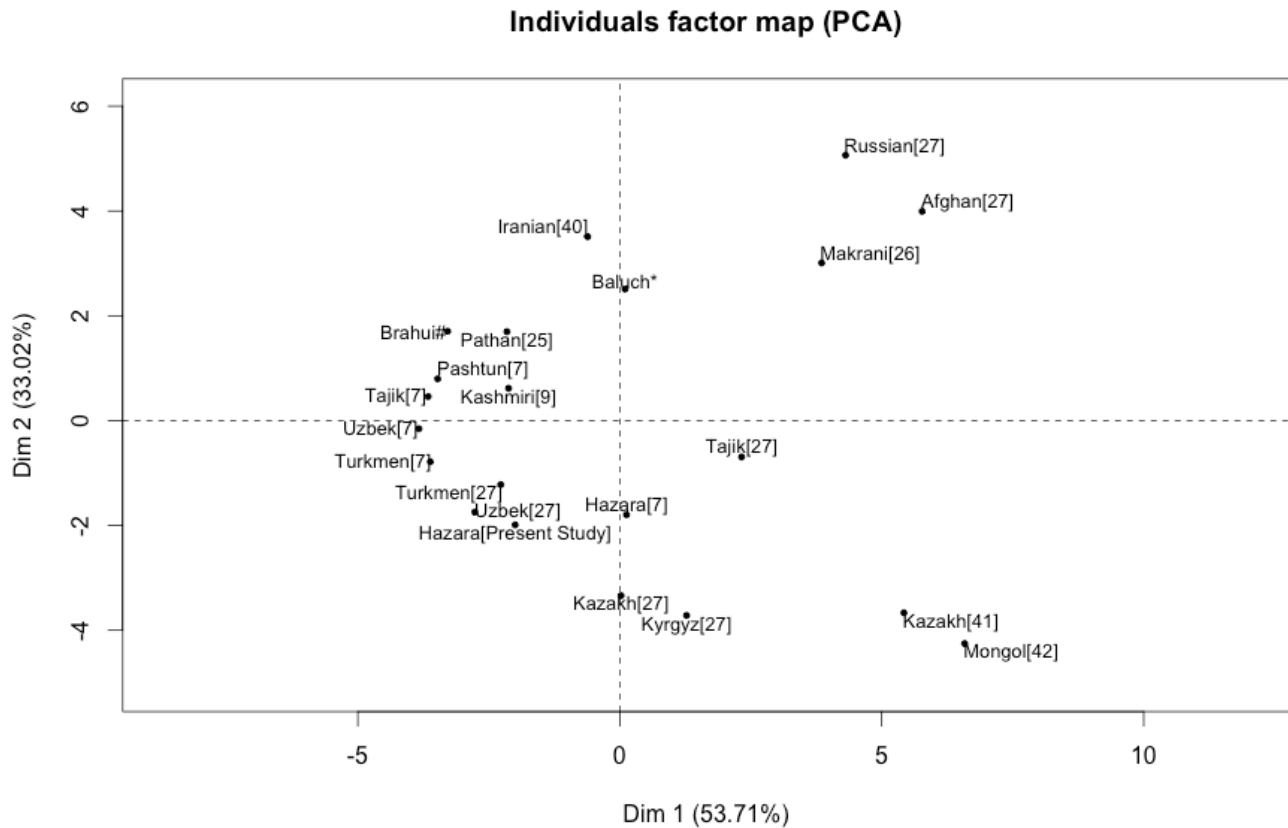


Figure S4: Principal component analysis (PCA) based on pairwise F_{ST} values defined by HVI sequences

Data Sources:

Populations	References	No. of samples	Populations	References	No. of samples
Hazara	Present study	319	Mongol	[44]	201
Kashmiri	[9]	317	Kazakh	[43]	160
Pathan	[25]	230	Tajik	[7]	146
Makrani	[26]	99	Hazara	[7]	78
Uzbek	[27]	328	Turkmen	[7]	75
Turkmen	[27]	249	Uzbek	[7]	127
Tajik	[27]	244	Baluch*	EU565791-EU565815	25
Russian	[27]	151	Brahui#	EU565766-EU565790	25
Kyrgyz	[27]	249	Iranian	[40]	352
Kazakh	[27]	256	Afghan	[7]	90
Afghan	[27]	98			

Table S2. mtDNA haplogroup frequencies of Hazara in Pakistan

	Number	Percent	Haplogroup	Number	Percent (%)
A	18	5.6	A+152+16362	10	3.1
			A+152+16362+16189	2	0.6
			A15	2	0.6
			A16	3	0.9
			A23	1	0.3
B	11	3.4	B4b1a+207	1	0.3
			B4b1a3a	3	0.9
			B4c1b+16335	3	0.9
			B4c1c1	1	0.3
			B5a	2	0.6
			B5b2c	1	0.3
C	20	6.3	C	3	0.9
			C4a1a+195	8	2.5
			C4a1a4a	2	0.6
			C4a2	2	0.6
			C4b8a	1	0.3
			C5c	4	1.3
D	31	9.7	D2b	1	0.3
			D4+195	2	0.6
			D4b1	2	0.6
			D4b1a1	1	0.3
			D4b2b	1	0.3
			D4c2a	3	0.9
			D4c2b	7	2.2
			D4e5a	1	0.3
			D4e5b	1	0.3
			D4g2	1	0.3
			D4h1	2	0.6
			D4i	2	0.6
			D4j+16286	1	0.3
			D4l2	1	0.3
			D4m2a	4	1.3
D4p	1	0.3			
F	7	2.2	F1a1	1	0.3
			F1a1a	1	0.3
			F1b1+@152	3	0.9
			F1b1b	1	0.3
			F1d	1	0.3

Supplementary material: mtDNA sequence diversity of Hazara ethnic group from Pakistan

G	6	1.9	G2a+152	5	1.6		
			G2a2a	1	0.3		
H	45	14.1	H1+16189	1	0.3		
			H10+16093	2	0.6		
			H11a1	1	0.3		
			H13a1b	1	0.3		
			H13a1c	1	0.3		
			H14a	4	1.3		
			H14b2a	1	0.3		
			H1b	2	0.6		
			H1e1a4	8	2.5		
			H1m1	1	0.3		
			H1o	1	0.3		
			H29	1	0.3		
			H2a1	4	1.3		
			H2a1f	1	0.3		
			H2a2a	13	4.1		
			H2a5b1	1	0.3		
			H3b6	1	0.3		
H5	1	0.3					
HV	30	9.4	HV14	2	0.6		
			HV19	2	0.6		
			HV1b+152	7	2.2		
			HV2	5	1.6		
			HV2a	5	1.6		
			HV4b	1	0.3		
			HV6	8	2.5		
J	14	4.4	J1b1b	1	0.3		
			J1b8	3	0.9		
			J1c	2	0.6		
			J1c2i	1	0.3		
			J1d	3	0.9		
			J1d5	2	0.6		
			J2b1a	1	0.3		
			JT	1	0.3		
			7	2.2	K1a4c1	3	0.9
					K1b1a1+199	1	0.3
		K2a5	3	0.9			
K	47	14.7	M10a1a1b	1	0.3		
			M12a1b	2	0.6		
			M18a	1	0.3		
			M2a1a	1	0.3		

Supplementary material: mtDNA sequence diversity of Hazara ethnic group from Pakistan

			M2a2	1	0.3
			M30	1	0.3
			M30b	1	0.3
			M30c1	1	0.3
			M30e	1	0.3
			M3a1+204	1	0.3
			M43a1	4	1.3
			M54	3	0.9
			M5a2a1a	3	0.9
			M65a+@16311	4	1.3
			M7b1a1	1	0.3
			M8a2+152	2	0.6
			M8a2a	2	0.6
			M9	4	1.3
			M9a1a1a	13	4.1
N	4	1.3	N1a1a1a1a	1	0.3
			N1b1	2	0.6
			N9a1	1	0.3
R0	6	1.9	R0a+60.1T	3	0.9
			R0a1a	3	0.9
R0	8	2.5	R2	3	0.9
			R30b2	2	0.6
			R7	1	0.3
			R8a1a1d	1	0.3
			R8a1a3	1	0.3
T	10	3.1	T	2	0.6
			T1a1'3	2	0.6
			T2a1b	1	0.3
			T2b34	1	0.3
			T2c1a	4	1.3
U	46	14.4	U1a	3	0.9
			U1a1a	3	0.9
			U1a1a1	3	0.9
			U2b	1	0.3
			U2b1	1	0.3
			U2b2	1	0.3
			U2c'd	3	0.9
			U2e1h	1	0.3
			U2e2	2	0.6
			U4'9	2	0.6
			U4b3	1	0.3
			U5a1	1	0.3

Supplementary material: mtDNA sequence diversity of Hazara ethnic group from Pakistan

			U5a1+@16192	3	0.9
			U5a1a1+152	1	0.3
			U5a2a	10	3.1
			U7	1	0.3
			U7a	5	1.6
			U8b1b	2	0.6
			U9a1	2	0.6
W	4	1.3	W+194	1	0.3
			W3a1+199	1	0.3
			W6	2	0.6
X	3	0.9	X2	3	0.9
Y	1	0.3	Y1a+16189	1	0.3
Z	1	0.3	Z+152	1	0.3

@ corresponds to mutations that are reversions to an ancestral state (back mutations) and are indicated as @ in HaploGrep output

Table S3: Analysis of molecular variance for 4 Pakistani populations based on mtDNA complete control region sequences

Source of variation	d.f	Sum of Squares	Variance components	Percentage of variation	Data
Among population	3	91.189	0.10937 Va	2.05	
Within population	961	5021.488	5.22527 Vb	97.95	
Total	964	5112.677	5.33464		

Sources:

Populations	References	No. of samples
Kashmiri	[9]	317
Pathan	[25]	230
Makrani	[26]	99
Hazara	Present Study	319

Table S4: Analysis of molecular variance for global populations based on mtDNA complete control region sequence

Source of variation	d.f	Sum of Squares	Variance components	Percentage of variation
Among population	26	1579.624	0.22973 Va	4.17
Within population	6531	34490.714	5.28108 Vb	95.83
Total	6557	36070.338	5.51081	

Data Sources:

Populations	References	No. of samples	Populations	References	No. of samples
Hazara	Present study	319	Chinese	[28]	377
Kashmiri	[9]	317	Thai	[29]	190
Pathan	[25]	230	Vietnamese	[30]	187
Makrani	[26]	99	Laos	[31]	214
Uzbek	[27]	328	Kuwaiti	[32]	381
Turkmen	[27]	249	Iraqi	[33]	182
Tajik	[27]	244	Egyptian	[34]	277
Russian	[27]	151	Dubai	[35]	249
Kyrgyz	[27]	249	Ashkenazi Jews	[36]	173
Kazakh	[27]	256	Roma	[37]	205
Afghan	[27]	98	Greeks	[38]	319
Szekely	[37]	182	Iranian	[40]	352
Moroccan	[39]	509			

Table S5. Pairwise differences among twenty regional populations based on mtDNA complete control region sequences as calculated using Arlequin v3.5

Population Pairwise F _{ST}	Afghan [7]	Baluch *	Brahui *	Hazara (Current Study)	Iranian [40]	Kazakh [27]	Kyrgyz [27]	Kashmiri [9]	Kazakh [41]	Makrani [26]	Mongol [44]	Pashtun [27]	Pathan [25]	Russian [27]	Tajik [27]	Turkmen [27]	Tajik [7]	Turkmen [7]	Uzbek [7]	Uzbek [27]	
Population Sample Size	90	25	25	319	352	256	249	317	160	99	201	98	230	151	244	249	146	75	127	328	
Afghan [7]	0																				
Baluch *	0.05343	0																			
Brahui #	0.03789	0.00431	0																		
Hazara (Current Study)	0.03777	0.02576	0.01563	0																	
Iranian [40]	0.03302	0.01788	-0.00245	0.02428	0																
Kazakh [27]	0.05245	0.03807	0.02369	0.00433	0.03878	0															
Kyrgyz [27]	0.06399	0.03985	0.02704	0.00916	0.04492	0.00378	0														
Kashmiri [9]	0.04284	0.01283	-0.00033	0.01311	0.01655	0.0226	0.02362	0													
Kazakh [41]	0.08377	0.04888	0.04668	0.01981	0.06454	0.01091	0.01255	0.03793	0												
Makrani [26]	0.05363	0.03813	0.01469	0.03616	0.02858	0.04398	0.0505	0.02951	0.06937	0											
Mongol [42]	0.07762	0.06808	0.05135	0.02103	0.07311	0.01025	0.00691	0.04315	0.02074	0.06639	0										
Pashtun [27]	0.0298	0.00598	0.0013	0.00847	0.008	0.02034	0.02654	0.00586	0.04003	0.027	0.04544	0									
Pathan [25]	0.03443	0.00758	-0.00584	0.01609	0.01267	0.02757	0.03211	0.00276	0.04218	0.02886	0.05377	0.00436	0								
Russian [27]	0.04289	0.0439	0.02918	0.03437	0.01028	0.05446	0.06912	0.03858	0.08801	0.05984	0.09745	0.01866	0.03027	0							
Tajik [27]	0.04991	0.05372	0.02468	0.0209	0.0319	0.02618	0.02626	0.03233	0.05168	0.04076	0.03372	0.02437	0.03622	0.0475	0						
Turkmen [27]	0.04205	0.02364	0.00978	0.00708	0.01696	0.00965	0.01269	0.01342	0.02881	0.03561	0.02966	0.00753	0.01617	0.03461	0.02	0					
Tajik [7]	0.02859	0.00908	0.00027	0.00588	0.00787	0.01708	0.02291	0.00572	0.03831	0.02784	0.04178	-0.00153	0.00515	0.01514	0.02147	0.00719	0				
Turkmen [7]	0.0279	0.00908	0.00933	0.00384	0.01591	0.01137	0.01558	0.00816	0.02467	0.0333	0.0328	-0.00167	0.0065	0.02756	0.0225	0.00261	-0.00022	0			
Uzbek [7]	0.03278	0.01209	-0.00301	0.00526	0.00846	0.01219	0.01651	0.0035	0.03207	0.02298	0.03505	0.00084	0.00386	0.02636	0.02324	0.00357	0.00025	0.00002	0		
Uzbek [27]	0.03629	0.02482	0.00559	0.00425	0.01863	0.00539	0.00814	0.01158	0.01922	0.03273	0.02139	0.00876	0.01221	0.03301	0.01717	0.00401	0.00638	0.00336	0.00293	0	
F _{ST} p-value	Afghan [7]	Baluch *	Brahui *	Hazara (Current Study)	Iranian [40]	Kazakh [27]	Kyrgyz [27]	Kashmiri [9]	Kazakh [41]	Makrani [26]	Mongol [42]	Pashtun [27]	Pathan [25]	Russian [27]	Tajik [27]	Turkmen [27]	Tajik [7]	Turkmen [7]	Uzbek [7]	Uzbek [27]	
Afghan [7]	*																				
Baluch *	0.00000+0.0000	*																			
Brahui #	0.00781+0.0024	0.34180+0.0169	*																		
Hazara (Current Study)	0.00000+0.0000	0.00098+0.0010	0.01855+0.0046	*																	
Iranian [40]	0.00000+0.0000	0.01172+0.0033	0.61230+0.0154	0.00000+0.0000	*																
Kazakh [27]	0.00000+0.0000	0.00098+0.0010	0.00684+0.0027	0.00098+0.0010	0.00000+0.0000	*															
Kyrgyz [27]	0.00000+0.0000	0.00000+0.0000	0.00586+0.0022	0.00000+0.0000	0.00000+0.0000	0.00977+0.0026	*														
Kashmiri [9]	0.00000+0.0000	0.03027+0.0053	0.46484+0.0140	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	*													
Kazakh [41]	0.00000+0.0000	0.00000+0.0000	0.00098+0.0010	0.00000+0.0000	0.00000+0.0000	0.00098+0.0010	0.00000+0.0000	0.00000+0.0000	*												
Makrani [26]	0.00000+0.0000	0.00391+0.0019	0.07227+0.0064	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	*											
Mongol [42]	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00293+0.0016	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	*										
Pashtun [27]	0.00000+0.0000	0.15527+0.0103	0.38230+0.0128	0.00098+0.0010	0.00488+0.0020	0.00000+0.0000	0.00000+0.0000	0.01172+0.0036	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	*									
Pathan [25]	0.00000+0.0000	0.11816+0.0091	0.81836+0.0103	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.01562+0.0037	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.03027+0.0051	*								
Russian [27]	0.00000+0.0000	0.00000+0.0000	0.00293+0.0016	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	*							
Tajik [27]	0.00000+0.0000	0.00000+0.0000	0.00586+0.0022	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	*						
Turkmen [27]	0.00000+0.0000	0.00977+0.0026	0.09766+0.0075	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00781+0.0028	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	*					
Tajik [7]	0.00000+0.0000	0.08398+0.0075	0.43652+0.0128	0.00098+0.0010	0.00098+0.0010	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.72363+0.0138	0.00098+0.0010	0.00000+0.0000	0.00000+0.0000	0.00098+0.0010	*				
Turkmen [7]	0.00000+0.0000	0.12402+0.0086	0.10742+0.0088	0.06841+0.0075	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00293+0.0016	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.68262+0.0166	0.01860+0.0037	0.00000+0.0000	0.00000+0.0000	0.11821+0.0107	0.48340+0.0161	*			
Uzbek [7]	0.00000+0.0000	0.03996+0.0058	0.64844+0.0139	0.00391+0.0019	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.02246+0.0040	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.33203+0.0149	0.02051+0.0038	0.00000+0.0000	0.00000+0.0000	0.05469+0.0053	0.38672+0.0148	0.43164+0.0156	*		
Uzbek [27]	0.00000+0.0000	0.00195+0.0014	0.16895+0.0100	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00000+0.0000	0.00293+0.0016	0.00098+0.0010	0.07617+0.0100	0.02734+0.0049	*	

* GenBank Accession 'EU565791-EU565815'
 # GenBank Accession 'EU565766-EU565790'

Table S6. Comparison (based on sequence n.p 16024-16569) of Haplotypes from Hazaras with Haplotypes from Central Asian Populations

Haplotype	Hazara (Present Study)	Baluch*	Hazara [7]	Kazakh[27]	Kyrgyz[27]	Kashmir[9]	Kazakh[41]	Mongo[42]	Pashtun[7]	Pathan[25]	Tajik[27]	Turkmen[27]	Tajik[7]	Turkmen[7]	Uzbek[7]	Uzbek[27]
Hap_27	14	0	3	6	9	17	11	1	4	11	2	23	10	6	4	11
Hap_134	10	0	0	2	2	0	0	0	0	0	0	0	3	0	0	0
Hap_109	9	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hap_122	9	0	0	2	0	0	1	0	0	0	0	0	0	0	0	1
Hap_120	8	0	0	7	1	0	2	3	0	0	4	3	0	0	1	2
Hap_140	7	0	0	1	0	1	0	0	0	0	0	0	3	0	0	2
Hap_202	6	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
Hap_36	5	0	1	7	16	0	9	9	1	5	0	6	0	1	1	8
Hap_31	4	0	0	1	3	0	0	1	1	3	0	0	1	0	0	2
Hap_75	4	0	2	8	6	0	2	0	0	0	0	2	1	3	2	5
Hap_94	4	0	1	1	0	0	0	0	0	0	5	2	0	0	0	2
Hap_157	4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Hap_182	4	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Hap_15	3	0	0	2	1	0	2	0	0	3	0	1	2	2	0	6
Hap_77	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hap_91	3	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0
Hap_105	3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Hap_123	3	0	0	0	3	0	0	0	0	0	0	2	0	0	0	1
Hap_124	3	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1
Hap_131	3	0	0	0	0	2	0	0	0	1	0	5	0	0	0	0
Hap_136	3	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0
Hap_153	3	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
Hap_16	2	0	3	1	1	0	0	0	2	1	0	0	0	0	0	2
Hap_46	2	3	0	0	0	0	0	0	2	2	0	2	0	2	1	1
Hap_82	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hap_111	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	3
Hap_112	2	0	1	1	1	0	1	2	0	0	0	1	0	0	0	0
Hap_118	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Hap_145	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Hap_147	2	0	0	0	0	1	0	0	0	2	0	0	0	0	1	2
Hap_148	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hap_154	2	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0
Hap_159	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hap_166	2	0	0	0	0	1	0	0	0	0	4	0	0	0	0	0
Hap_172	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hap_179	2	0	0	0	0	0	0	0	1	1	0	2	0	0	0	0
Hap_186	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hap_209	2	0	0	1	0	0	2	1	0	0	0	0	0	0	0	1
Hap_210	2	0	0	4	7	0	4	6	1	0	1	3	0	0	1	2
Hap_222	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Hap_5	1	2	0	0	1	7	0	0	2	3	0	0	1	0	1	1
Hap_14	1	0	0	0	1	0	1	1	0	0	15	0	0	0	1	2
Hap_18	1	0	0	1	0	0	0	3	0	1	0	1	5	0	1	0
Hap_19	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hap_28	1	0	0	1	1	2	2	0	0	0	0	0	0	0	0	0
Hap_40	1	0	0	2	5	3	2	1	0	8	0	3	2	1	3	6
Hap_44	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hap_72	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hap_73	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
Hap_74	1	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0
Hap_79	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hap_93	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1
Hap_96	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1
Hap_98	1	0	1	0	4	2	1	0	0	1	0	0	0	1	3	1
Hap_103	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Hap_108	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hap_121	1	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0
Hap_130	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Hap_132	1	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0
Hap_137	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Hap_138	1	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0
Hap_161	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hap_162	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
Hap_165	1	0	0	1	0	0	0	0	0	1	0	5	2	0	1	0
Hap_167	1	0	0	1	0	2	1	0	0	2	14	0	4	2	0	1
Hap_170	1	0	0	0	0	0	0	0	0	0	0	1	0	2	1	1
Hap_175	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Hap_187	1	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0
Hap_192	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Hap_193	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Hap_196	1	0	0	0	1	0	2	0	0	0	0	0	0	0	0	1
Hap_199	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
Hap_200	1	0	0	1	0	1	2	0	0	2	0	1	0	0	4	3
Hap_206	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
Hap_213	1	0	0	0	2	0	1	0	0	0	0	4	0	1	0	0
Hap_215	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Hap_219	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
Hap_223	1	0	0	0	1	1	0	0	0	0	0	0	3	0	0	0
Hap_227	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Hap_229	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Hap_233	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Hap_234	1	0	0	0	0	2	0	0	0	0	0	0	0	0	2	0
Hap_235	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Hap_239	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Hap_242	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1
Population Sample Size	319	25	78	256	249	317	151	196	90	230	244	249	146	75	127	328

* GenBank Accession 'EU565791-EU565815'