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GENETIC CHARACTERIZATION OF MITOCHONDRIAL DNA CONTROL REGION OF MUGHAL ETHNIC GROUP IN PAKISTAN

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ABSTRACT

Studying populations at the genetic level is crucial for identifying population-specific trends. Numerous fields, including forensic science and health, use population-specific genetic patterns for investigation and analysis. A given ethnic group's maternal lineages, as well as its evolutionary and emigrational histories, can be gleaned from the examination of mtDNA control regions. The given ethnic group's maternal lineages, as well as its evolutionary and emigrational histories, can be obtained from the examination of mtDNA control regions. No such work has been reported on this ethnic group and for studying the genetic variability this study was designed. Therefore, in this study, the mitochondrial DNA haplotypes of the Mughal population from Pakistan's CR (control region) from 16024 base pairs to 576 base pairs are described.

To examine the genetic variance caused by maternally inherited DNA in this community, 100 unrelated samples of Mughals from upper Pakistan had their mtDNA control region sequences compared to the rCRS (revised Cambridge reference sequence). Out of a total of 91 haplotypes, the results showed that 88 were found to be unique. This population shared only 3 haplotypes. Nearly 62% of the haplotypes belonged to South Asian haplogroups, with evidence of Northren Asian and Northren European haplogroups (about 7%) also present, indicating admixed maternal genetics in this community.

The Mughal population has a high level of genetic diversity (0.999), the lowest rate of random matches (0.0100), and a high level of discriminatory capacity (0.9900). This study states the presence of different people from different regions of the world which refers to scientifically describe the origin and the settlement of these people from all over the world.

Key Words: Mitochondrial DNA (mtDNA), Control Region Sequencing, Mughal Population, Genetic Diversity, Maternal Lineages, Haplotypes, Population Genetics, Forensic Genetics, South Asian Haplogroups.

INTRODUCTION

DNA is the life's architectural blueprint. The genetic information necessary for eukaryotic and prokaryotic species, including bacteria and viruses, for their development, growth, reproduction, and operations, is carried by the DNA molecule (Lehninger et al., 2000). DNA analysis is a fundamental instrument in biochemical and genetic research that offers vital information about biology, useful details about ancestry, and evidence for legal cases (Jafari & Ansari 2019). Although DNA is found in the chromosomes of the nucleus in a compact form, mitochondrial DNA, also known as mtDNA, is found in the mitochondria. With 16,569 base pairs encoding 37 genes, mitochondrial DNA was the first organelle of the human genome to be sequenced (Vieira et al., 2016). A single cell contains several thousands of copies of the mitochondrial genome. The situation of the presence of identical copies of mtDNA inside a cell is known as homoplasmy while the presence of two or more different copies of the mtDNA inside a cell is known as heteroplasmy. Mitochondrial DNA is recognized as the dynamic genetic organelles which are capable to divide, self-replicate, move, and fuse (Vieira et al., 2016). In genomic studies, uniparental DNA markers are used for the mtDNA and Y-chromosome study, while autosomal markers are predominant (Cano et al., 2014). These autosomal markers have a huge parameter of variations inside the autosomal genome therefore are considered a strong tool for human identification (Ma et al., 2014). The use of mtDNA in the biological as well as evolutionary studies has attained huge importance (Pilipenko et al., 2018). The mtDNA analysis helps in forensic science for almost completely degraded samples like bones, cigarette butts, nails, teeth, stains, and the hair shafts that have a minute amount of nuclear DNA in them for their genetic analysis (Hoseinzadeh et al., 2016). The circular shape and the location of human mtDNA have proven its significance in the scientific investigation due to self-replication and energy production mechanisms (Cano et al., 2014) Displacement loop (D-loop) is the control region of the mtDNA covering approximately 7% (1122 bp) of the total genome of mitochondria (Hoseinzadeh et al., 2016). This loop has three highly variable regions (HVS) that are HVSI (342bp), HVSII (268bp), and HVSIII (137bp). From these, HVSI and HVSII are regions with a higher level of polymorphism (Cano et al., 2014). Anderson formed the Cambridge Reference Sequence (CRS) by sequencing the whole mtDNA genome of human (Anderson et al., 1981) which is now being used for standard comparisons. Any distinction when observed between the Anderson and the sequences of the individual sample is referred to as polymorphism (Cano et al., 2014). The haplogroups of the mitochondrial DNA are the genome markers that are inherited maternally. The analysis of mutations in the mtDNA has shown the presence of alike mutations in individuals who share a similar maternal line (Ma et al., 2014). Heteroplasmy is considered the form of polymorphism where it contains two different forms of mitochondrial DNA either in a cell or the individual (Barbieri et al., 2014). There exist seven macro-haplogroups that are L0, L1, L2, L3, L4, L5, and L6. These are specific for different area wise populations like Africans, the L haplogroup was reported in western Africa in 2014 (Barbieri et al., 2014). Other haplogroups like M, N, and R groups are subgroups of the macrohaplotypes (Goto et al., 2011). The studies conducted in Pakistan include mtDNA analysis for determining Pathan lineage (Rakha et al., 2011), Makrani (Siddiqi et al., 2015), Kalash (Ayub et al., 2015), and Saraiki (Hayat et al., 2015). Mughal population is predominantly present all over Pakistan. Famous historian Christophe Jaffrelot believes that the origin of the Mughal community is still unknown but some people say that the Mughal population has a connection with Turkish people. Mughals or Moguls form a distinct population in Pakistan (Van Schendel, 2020). In the west, the word Mughal was used for the emperor of the state (Fontana, 2011). This word was derived from the Persian and Arabic corruption of Mongolian that showed the Mongol origin of the Timurid dynasty (Van Schendel, 2020). Due to low or no similarities, the ancestors of Mughal ruler Babur were different from Mongol instead they were oriented towards Persian culture. Turks claim Mughal to be their descendants (Barfield, 2023)

In this study we aim to present the atlas of maternally inherited DNA of Mughals living in Pakistan. It will highlight mtDNA based genetic characterization of the population, the diversity and evolutionary aspects. The outcome of study is beneficial medical applications and for human identification. The data is also a contribution towards national mtDNA data of Pakistani population which will be helpful in criminal investigations.

METHODOLOGY

Blood samples from 100 maternally unrelated individuals were taken from different areas of Pakistan from Mughal ethnic group. Mughal people were identified based on their different ethnicity, their settlement, and other details in the different areas of Pakistan. All the details were recorded in the consent form that was filled and signed by the donor. EDTA vacutainers were used for preserving the blood samples. All the ethical aspects were kept in a note while conducting the sampling.

DNA extraction

DNA Mini Kit (Qiagen, Hilden, Germany) was used for the DNA Extraction from the preserved blood samples previously stored in the EDTA tubes. DNA extraction Protocol was adopted that was given by the manufacturer's instructions.

PCR Amplification and Sequencing

Quantification of the genomic DNA was done with the help of Nano Drop™ 1000 Spectrophotometer (Thermo Scientific, USA). For amplification of the entire mt DNA's D-loop, forward and reverse primers were used (Rakha *et al.*, 2011) given in Table 1. The amount of genomic DNA used was 1-2 ng. In each PCR reaction mix, 0.4 µM was the concentration of the primers used. Ampli Taq Gold® 360 PCR Master Mix (Applied Biosystems, Foster City, CA, USA) of volume 50 µL was used for performing PCR amplification. The overall process of PCR amplification consists of; the first step that is pre-denaturation conducted for 11 minutes at 95°C, the second step that is 35 cycles of denaturation for the 30s at 95°C, the third step of annealing for 30s at 56°C, the fourth step of extension for 90s at 72°C and fifth and final step of final extension for 7 minutes at 72°C. Sequencing of whole mitochondrial DNA D-loop or control region having the sequence range from nt 16024-16569 and 1-576 was done with the help of Big Dye® Terminator v3.1 Cycle sequencing kit (Applied Biosystems) was used according to the standard protocol and instructions, given by the manufacturer by using ABI 3100 Genetic Analyzer (Applied Biosystems). Along with these, commercially available facilities were also used for sequencing purposes. Bidirectional sequencing of the mtDNA control region was done with the same forward and reverse primers that were used for the PCR amplification are given in Table 2.

Table 1 Primers sequences used for PCR amplification and sequencing of mtDNA control region

Sr. No.	Primer	Sequence (5'_3')	TM (ºC)
1	Forward Primer (F15975)	CTCCACCATTAGCACCCAAA	56
2	Reverse Primer (R635)	GATGTGAGCCCGTCTAAACA	55.6

Abbreviation: Melting Temperature= TM

Table 2: The composition of reaction mixture for Amplification PCR.

Sr #	Components	Quantity
1	4mM $MgCl_2$ (Thermo scientific #EP0402)	4 µl
2	Taq Buffer (Thermo scientific #EP0402)	2.5 µl
3	7.5mM dNTPs (Thermo scientific #EP0402)	2.5 µl
4	Taq Polymerase (Thermo scientific #EP0402)	0.4 µl
5	Forward Primer	1.5 µl
6	Reverse Primer	1.5 µl
7	Template DNA	1.5 µl
8	Nuclease free water (IDT Technologies, INC)	11.1 µl
Total Reaction Volume		**25µl**

Analysis of the data

All the samples were sequenced bidirectionally and aligned using sequence analysis software Geneius (Version 7.1.5, Biomatters Ltd, New Zealand). A comparison of the sequences and results was done in both the directions with rCRD (Anderson *et al.*, 1981: Andrew et al., 1999). In order to determine the haplotype of each sequence, the sequences were subjected to be analysed by MITOMASTER, a tool present on the MITOMAP platform. The application of MITOMASTER takes each mtDNA control region sequence as input file and compares it with rCRS for the assignment of respective haplotype and haplogroups. The tool utilizes the HaploGrep2 with Phylotree 17 for haplogroup determination. The statistical parameters like genetic diversity (GD), random match probability (RMP) and power of discrimination (PD) were calculated statistically (Fisher & Garner, 2020).

RESULTS AND DISCUSSION

The study highlights very important genetic an evolutionary dynamics of the Mughal population. Out of the 100 samples under the study, it turned up with total 91 haplotypes. The 88 haplotypes out of 91 observed haplotypes were found to be as unique. The only 3 haplotypes were found to be shared with other haplotypes within the population under study. The haplotypes corresponded to respective haplogroups. The haplogroups give the idea of genetic relatedness of population to specific geographic locations. In the Mughal population, F1c is the most prevalent haplogroup was found in the 17 samples (17%) marking the connection with East Asian and South Asian origins. It was followed by the haplogroup M4a found in the 14 samples (14%). This haplogroup (M4a) is found within South Asian countries but also it was reported to be found in the Eastern Saudi Arabia. The haplogroups M3 and M6a were found to be 9.5% and 6.3% respectively, defining these samples be related with South Asian origins. The majority of the samples from this population were observed to be genetically close with South Asian populations. The haplogroup U4a has been observed with frequency of 7% within the population pointing out its connection with Northren Asian and Northren European haplogroups. The frequency of other occupant haplogroups have been displayed in the graph. The population comes with the higher genetic diversity (GD) 0.9999 signifying hugely diverse population as far as mtDNA control region is concerned. The higher genetic diversity also gives rise to higher Power of discrimination (PD) 0.9900. It also implies the very less random match probability (RMB) as 0.0100 making the persons from this population be easily distinguished from other population without consuming excessive sources.

The sites having polymorphism are given as haplotypes in Table 3. Data of mtDNA results for its diversity as calculated statistically for the respective population are given in Table 5. Population study of Mughals have shown great diversity in comparison with the other reported population of Pakistan except Kalash (Nesheva, 2014). Genetic diversity is due the reflection of unique haplotypes distribution. The numbers of unique haplotypes identified in the present studied population were 63%, which were found somehow consistent with Burusho 78%, Hazara 76%, Makrani 76%, Baluchi 69% and Brahui 68% among the other reported population of Pakistan, while moderately lower from Saraiki 92%, Sindhi 90% and Pathan 81%. Members of Gujars population revealed high frequency (42%) of South Asian lineage. The proportion of South Asian lineages in the other reported Pakistani populations were 48% in Sindhi, 39.1% in Pathan, 36% Pashtun, 29.4% in Saraiki and 24% in Makrani (Metspalu et al., 2004). Low frequency of South Asian lineages among the major ethnic groups of Afghanistan have also been reported with the prevalence of 15% in Hazara, 13.3% in Baluch and 7.1% in Pashtun, while absent in Tajik (Whale, 2012). The presence of south Asian mtDNA haplogroups in the present study population revealed that the population residing in this region are the true inhabitants and are remolded in the past by local demographic events. The West Eurasian haplogroup was the second most prevalent haplogroup accounting for (37%) in the individuals of the present study population. Its frequency among the Pathans of Pakistan was reported 55% and 26% in Makranis. Furthermore, the frequency of West Eurasian haplogroup in Indian Punjabis population was reported from (40-50%), in Kashmiris and Gujrathis 30%, while the least were observed in Indian Uttar Pradesh and West Bengal. Greater proportion of West Eurasian lineages were also reported among the major ethnic groups of Afghanistan with the frequencies of 40% in Hazara, 89% in Tajik,

74% in Baluch and 64% in Pashtun (Ayub et al., 2009). The presence of these lineages revealed that, the gene flow in the past to this region may occur from the west through Iran or from the North through Central Asia, through the invasion by different invaders *i.e.* Alexander, Arabians, Muslims and the British . The mega haplogroup R, M and N identified in the Gujars population are said to be South Asian in origin and has been originated approximately 60000-75000 years ago in South Asia (Akbar et al., 2014), suggesting their maternal gene pool as South Asian in origin

Genetic characterization of different populations of Pakistan is done for determining the frequency of various haplogroup among those populations. **Pathans** After studying the Pathan population's mtDNA control region the macrohaplotype magnitude was 8.7% M3, 10.4% HV, 11.3% U7, 30.09% M, 7.8% N, and 61.3 R. The results showed the ancestral relation of Pathans with South Asia, East Asia and West Eurasia (Rakha *et al.*, 2011). According to the Makrani people's mtDNA analysis, they have African haplotypes such L1a, L2a, L3b, and L3d. (Siddiqi *et al.*, 2015). **Saraiki** In Saraiki people, haplotypes of South Asia are most important (Hayat *et al.*, 2015). Because haplotype U4 is present in Kalashi people 34% more frequently than in other populations, Kalash Studies have shown that Western Eurasian haplogroups are found to be predominant in this population. (Ayub *et al.*, 2015). Studies on Gujars in Khyber Pakhtunkhwa (KPKSwat)'s district revealed that R, M, and N mega haplotypes predominate with 48%, 45%, and 7% of the total population, respectively**.** (Ullah *et al.*, 2017).

Table 3 The estimated haplotypes and haplogroups in Mughal population of Pakistan along with sampling areas

Sequence	Predicted Haplogroup	Haplotypes Frequency	Variants
mtDNA_MG_0001	H1q(H1q3)	2	A73G, G103A, G225A, A263G, T310C, T310TTC, C324G, A361d(=A357d†), T393d(=T391d†), T401d(=T398d†), G16036GG, A16037G, T16352C, G16526A
mtDNA_MG_0002	M6a(M6a1b)	5	A73G, T146C, A263G, C269d(=C268d†), C325d(=C324d†), C338d, A361C, CA362-, A374d(=A373d†), A385d(=A384d†), T393d(=T391d†), T401d(=T398d†), G16036GG, C16188T, C16223T, T16231C, T16362C, T16519C
mtDNA_MG_0003	M4a(M4a)	14 (Mg03)	A73G, A263G, C315CC, C325d(=C324d†), A352d(=A350d†), G16145A, C16176T, C16223T, C16261T, C16266T, C16291T, T16311C, T16325C, T16519C
mtDNA_MG_0004	M4a(M4a)	14	A73G, A263G, C315CC, A352C, CA356-, G16036GG, G16145A, C16176T, C16223T, C16261T, C16266T, C16291T, T16311C, T16325C, T16519C
mtDNA_MG_0005	M3(M3)	10	A73G, A263G, C315CC, A352C, CA356-, T393d(=T391d†), T401d(=T398d†), G16036GG, T16126C, A16158G, C16169T, C16223T, T16519C
mtDNA_MG_0006	M3(M3)	10	A73G, A263G, C315CC, A352C, CA356-, T393d(=T391d†),

			T401d(=T398d†), G16036GG, T16126C, A16158G, C16169T, C16223T, T16519C
mtDNA_MG_0007	M3(M3)	10	A73G, A263G, A361C, CA362-, A376d, T16126C, A16158G, C16169T, C16223T, T16519C
mtDNA_MG_0008	M4a(M4a)	14	A73G, T204G, T206G, C231A, A232C, A241C, T252G, C256G, A257G, A263G, C268T, A270T, A274C, C277A, A281T, C296A, A297C, C299A, C309T, C317G, G16145A, C16176T, C16223T, C16261T, C16266T, C16291T, T16311C, T16325C, T16519C
mtDNA_MG_0009	H1q(H1q3)	2	A73G, G103A, G225A, A263G, T310C, T310TTC, C324G, A361d(=A357d†), T393d(=T391d†), T401d(=T398d†), G16036GG, A16037G, T16352C, G16526A
mtDNA_MG_0010	R30b(R30b)	2	A73G, A263G, C315CC, C324G, A361d(=A357d†), A373G, C378d(=C377d†), C387d(=C386d†), T401d(=T398d†), G16036GG, G16129A, C16290T, A16318G, C16320T, T16362C, T16519C
mtDNA_MG_0011	M2a(M2a1a)	1	A73G, T195C, T204C, A263G, C309CC, C325d(=C324d†), A361d(=A357d†), T393d(=T391d†), T401d(=T398d†), G16036GG, C16223T, C16270T, G16319A, T16352C, T16519C
mtDNA_MG_0012	F1c(F1c1a)	17	A73G, T152C, A234G, A249d(=A248d†), A263G, C315CC, A365AA, G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0013	F1c(F1c1a)	17	A73G, T152C, A234G, A249d(=A248d†), A263G, G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0014	F1c(F1c1a)	17	A73G, T152C, A234G, A249d(=A248d†), A263G, G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0015	F1c(F1c1a)	17	A73G, T152C, A234G, A249d(=A248d†), A263G, C315CC, G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0016	M3a(M3a1+204)	9	A73G, T204C, T217C, A263G, G16036GG, T16126C, C16223T, C16266T, T16311C, T16519C

mtDNA_MG_0017	M30(M30)	4	A73G, T195A, A263G, C315CC, C324G, G16036GG, C16188CC, C16223T, A16293G, C16295T, T16519C
mtDNA_MG_0018	M4a(M4a)	14	A73G, A263G, C315CC, C324G, C330G, G16036GG, G16145A, C16176T, C16223T, C16261T, C16266T, C16291T, T16311C, T16325C, T16519C
mtDNA_MG_0019	M4a(M4a)	14	A73G, T152C, A263G, C315CC, C324G, G16036GG, G16145A, C16176T, C16223T, C16261T, C16266T, C16291T, T16311C, T16325C, T16519C
mtDNA_MG_0020	H27(H27)	2	A263G, A286C, T310C, T310TTC, G16036GG, A16316G, T16519C
mtDNA_MG_0021	H27(H27)	2	A263G, A286C, T310C, T310TTC, G16036GG, A16316G, T16519C
mtDNA_MG_0022	M3(M3)	10	A73G, A263G, C315CC, G16036GG, T16126C, A16158G, C16169T, C16223T, T16519C
mtDNA_MG_0023	M3(M3)	10	A73G, A263G, G16036GG, T16126C, A16158G, C16169T, C16223T, T16519C
mtDNA_MG_0024	M3(M3)	10	G71GG, A73G, A263G, C309d(=C303d†), G16036GG, T16126C, A16158G, C16169T, C16223T, T16519C
mtDNA_MG_0025	M6a(M6a1b)	5	A73G, T125G, T131G, T146C, A176d(=A175d†), C186G, A189d(=A188d†), G16036GG, C16188T, C16223T, T16231C, T16362C, T16519C
mtDNA_MG_0026	M4a(M4a)	14	A73G, A263G, C315CC, A361d(=A357d†), T401d(=T398d†), T414d, A426d(=A425d†), CA438-, A446d, G16036GG, G16145A, C16176T, C16223T, C16261T, C16266T, C16291T, T16311C, T16325C, T16519C
mtDNA_MG_0027	HV2(HV2)	7	A73G, T152C, A263G, T310TTC, T310C, C325d(=C324d†), C338d, A361d(=A357d†), C371d(=C369d†), C375d, A388d, T393d(=T391d†), T401d(=T398d†), G16036GG, T16217C, T16311C, T16519C
mtDNA_MG_0028	M3(M3)	10	A73G, T208G, A263G, C269d(=C268d†), C296d(=C295d†), C303A, C315CC, C325d(=C324d†), C338d, G16036GG, C16040G,

			T16126C, A16158G, C16169T, C16223T, T16519C
mtDNA_MG_0029	F1c(F1c1a)	17	A73G, T152C, A234G, A249d(=A248d†), A263G, C315CC, C324G, A361C, CA362-, A374d(=A373d†), C387d(=C386d†), T393d(=T391d†), T401d(=T398d†), T414d, C418d, A426d(=A425d†), CA438-, C445d, G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0030	F1c(F1c1a)	17	A73G, T152C, A234G, A249d(=A248d†), A263G, C315CC, A352C, CA356-, C371d(=C369d†), G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0031	H2a(H2a2a1)	1	G16023GG, G16036GG
mtDNA_MG_0032	M3a(M3a1+204)	9	A73G, T204C, A263G, C315CC, C324G, A374C, A376d, C387d(=C386d†), T401d(=T398d†), G16036GG, T16126C, C16223T, T16311C, T16519C
mtDNA_MG_0033	R30b(R30b)	2	A73G, A263G, C315CC, C324G, A361d(=A357d†), A373G, C378d(=C377d†), C387d(=C386d†), T401d(=T398d†), G16036GG, G16129A, C16290T, A16318G, C16320T, T16362C, T16519C
mtDNA_MG_0034	HV2(HV2)	7	A73G, T152C, A263G, T310TTC, T310C, C325d(=C324d†), C338d, A361d(=A357d†), C371d(=C369d†), C375d, A388d, T393d(=T391d†), T401d(=T398d†), G16036GG, T16217C, T16311C, T16519C
mtDNA_MG_0035	F1c(F1c1a)	17	A73G, T152C, A234G, A249d(=A248d†), A263G, C315CC, A352C, CA356-, A365d(=A363d†), C371d(=C369d†), C387d(=C386d†), AT397-, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0036	M5d(M5d)	6	A73G, T146C, A263G, T310TTC, T310C, C324G, A376d, T393d(=T391d†), T401d(=T398d†), T414d, A419d, A426d(=A425d†), CA438-, C445d, G16036GG, T16093C, G16129A, C16223T, T16311C, T16519C
mtDNA_MG_0037	M2a(M2a1a3)	2	A73G, T195C, C198T, T204C, A263G, A352C, CA356-, A365d(=A363d†), G16036GG,

			T16157C, C16223T, C16270T, G16319A, T16352C, T16519C
mtDNA_MG_0038	M3a(M3a1+204)	9	A73G, T204C, A263G, C315CC, C324G, A352d(=A350d†), T16126C, C16223T, T16311C, T16519C
mtDNA_MG_0039	H32(H32)	2	A73G, T152C, T208G, C231A, A263G, C269d(=C268d†), A281T, A291d(=A286d†), T319d(=T318d†), C325d(=C324d†), C330G, A339d, A352d(=A350d†), G16036GG, C16294T, A16318T, T16519C
mtDNA_MG_0040	M4a(M4a)	14	A73G, A263G, A352d(=A350d†), G16145A, C16176T, C16223T, C16261T, C16266T, C16291T, T16298C, T16311C, T16325C, T16519C
mtDNA_MG_0041	M4a(M4a)	14	A73G, A263G, C269T, A270d, A281T, C324G, A326d, C330G, C338d, A352C, CA356-, A368d(=A367d†), A374C, A376d, A385d(=A384d†), A397T, G16036GG, G16145A, C16176T, C16223T, C16261T, C16266T, C16291T, T16298C, T16311C, T16325C, T16519C
mtDNA_MG_0042	M30(M30+16234)	1	A73G, C194T, T195A, A263G, A376d, G16036GG, C16147G, C16223T, C16234T, T16519C
mtDNA_MG_0043	U7(U7)	3	A73G, T152C, A263G, C315CC, C16294T, A16318T, T16519C
mtDNA_MG_0044	F1c(F1c1a)	17	A73G, T152C, A234G, A249d(=A248d†), A263G, A352C, CA356-, A365d(=A363d†), A376d, G16036GG, C16111T, G16129A, T16304C, T16519C (No similarity with previous yellow)
mtDNA_MG_0045	M4a(M4a)	14	A73G, A263G, C315CC, A352d(=A350d†), G16036GG, G16145A, C16176T, C16223T, C16261T, C16266T, C16291T, T16298C, T16311C, T16325C, T16519C
mtDNA_MG_0046	M4a(M4a)	14	A73G, T206G, T208G, A263G, C269d(=C268d†), C315CC, C317G, C327d, C330G, C338d, C343T, A352C, CA356-, AC368-, A374d(=A373d†), C381G, A385d(=A384d†), T393d(=T391d†), T414d, G16036GG, G16145A, C16176T, C16223T, C16261T,

			C16266T, C16291T, T16298C, T16311C, T16325C, T16519C
mtDNA_MG_0047	M30(M30)	4	A73G, T195A, T206G, A263G, C309d(=C303d†), C325d(=C324d†), A352C, CA356-, A16051G, C16223T, T16519C
mtDNA_MG_0048	M3a(M3a1+204)	9	A73G, T204C, A263G, T310TTC, T310C, C324G, C330G, C338d, A352C, CA356-, C371d(=C369d†), C375d, C387d(=C386d†), T393d(=T391d†), T401d(=T398d†), G16036GG, T16126C, C16223T, T16311C, T16519C
mtDNA_MG_0049	HV2(HV2)	7	A73G, T152C, A263G, T310TTC, T310C, C324G, A365d(=A363d†), A376d, T393d(=T391d†), T401d(=T398d†), T414d, C418d, T430d, CA438-, A446d, G16036GG, T16217C, T16311C, T16519C
mtDNA_MG_0050	U4a(U4a2a3)	7	A73G, T152C, A263G, T310TTC, T310C, A361d(=A357d†), A376d, G16036GG, C16294T, A16318T, T16519C
mtDNA_MG_0051	M5d(M5d)	6	A73G, T146C, A263G, T310TTC, T310C, C324G, A365d(=A363d†), A376d, T393d(=T391d†), T401d(=T398d†), A16013C, G16036GG, T16093C, G16129A, C16223T, T16311C, T16519C
mtDNA_MG_0052	M4a(M4a)	14	A73G, A263G, A352d(=A350d†), G16145A, C16176T, C16223T, C16261T, C16266T, C16291T, T16298C, T16311C, T16325C, T16519C
mtDNA_MG_0053	M4a(M4a)	14	A73G, A263G, C315CC, A361d(=A357d†), C371d(=C369d†), A376d, C387d(=C386d†), T393d(=T391d†), T401d(=T398d†), G16036GG, G16145A, C16176T, C16223T, C16261T, C16266T, C16291T, T16311C, T16325C, T16519C
mtDNA_MG_0054	M6a(M6a1b)	5	A73G, T146C, A263G, T310TTC, T310C, A352d(=A350d†), G16036GG, C16188T, C16223T, T16231C, T16362C, T16519C
mtDNA_MG_0055	N9b(N9b1c)	1	T55G, T63C, T72G, A73G, T74G, G94A, T119G, T125A, T131G, T133C, G185GG, T246C, A249AA, A263G, T267TT, A291AA, A302AA, C315CC, C320T, C324G, C348G,

			A361d(=A357d†), G389A, A390AA, T414G, C418G, C427A, T430C, A440C, A446C, G16023GG, G16036GG, T16189C, C16223T, C16245G, T16304C, G16319A, G16384A, T16413G, G16434A, T16437G, T16484G, T16505G, T16519C, G16558A
mtDNA_MG_0056	M3(M3)	10	A73G, A263G, T310TTC, T310C, C324G, A352C, CA356-, A365d(=A363d†), C371d(=C369d†), C387d(=C386d†), AT397-, G16036GG, T16126C, C16223T, T16519C
mtDNA_MG_0057	F1c(F1c1a)	17	A73G, T152C, T208G, A234G, A249d(=A248d†), A263G, C269d(=C268d†), A281T, C296d(=C295d†), C309d(=C303d†), C324G, C330G, C338d, G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0058	M3a(M3a1+204)	9	A73G, T204C, A263G, C269T, A270d, A281T, C296d(=C295d†), A302AAC, G316C, C324G, A326d, C330G, C338d, A352d(=A350d†), G16036GG, T16126C, C16223T, T16311C, T16519C
mtDNA_MG_0059	M3a(M3a1+204)	9	A73G, T204C, A263G, T310TTC, T310C, A361d(=A357d†), C371d(=C369d†), A376d, T393d(=T391d†), T401d(=T398d†), T16126C, C16223T, T16311C, T16519C
mtDNA_MG_0060	F1c(F1c1a)	17	A73G, T152C, A234G, A249d(=A248d†), A263G, C315CC, T393d(=T391d†), T401d(=T398d†), G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0061	M6a(M6a1b)	5	A73G, T146C, A263G, T310TTC, T310C, A361d(=A357d†), A376d, T401d(=T398d†), G16036GG, C16188T, C16223T, T16231C, T16362C, T16519C
mtDNA_MG_0062	M3a(M3a1+204)	9	A73G, T204C, A263G, T310TTC, T310C, A361d(=A357d†), C371d(=C369d†), A376d, T393d(=T391d†), T401d(=T398d†), T16126C, C16223T, T16311C, T16519C
mtDNA_MG_0063	M4a(M4a)	14	A73G, A263G, C325d(=C324d†), A352C, CA356-, A365d(=A363d†),

			A376d, G16036GG, G16145A, C16176T, C16223T, C16261T, C16266T, C16291T, T16298C, T16311C, T16325C, T16519C
mtDNA_MG_0064	M3a(M3a1+204)	9	A73G, T204C, T208G, C261G, A263G, C268T, A281T, C296A, A297C, C299A, C324G, C330G, C338A, A361d(=A357d†), A368d(=A367d†), A374d(=A373d†), A385d(=A384d†), T401d(=T398d†), G16036GG, T16126C, C16223T, T16311C, T16519C
mtDNA_MG_0065	M5d(M5d)	6	A73G, T146C, A263G, A352C, CA356-, A376d, T16093C, G16129A, C16223T, T16311C, T16519C
mtDNA_MG_0066	F1c(F1c1a)	17	A73G, T152C, A234G, A249d(=A248d†), A263G, A270d, C315CC, C324G, C330G, C338A, A361d(=A357d†), A374C, A376d, A385d(=A384d†), AT397-, G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0067	U4a(U4a2a3)	7	A73G, T152C, A263G, T310TTC, T310C, C324G, A376d, T393d(=T391d†), T401d(=T398d†), T414d, C427d, AC432-, C445A, C447d, T455d(=T452d†), C463d(=C461d†), T471d, T482d, G16036GG, C16294T, A16318T, T16519C
mtDNA_MG_0068	M5d(M5d)	6	A73G, T146C, A263G, T310C, T310TTC, C324G, C330G, C338d, A361d(=A357d†), C371d(=C369d†), C375d, T401d(=T398d†), T414d, A419d, A426d(=A425d†), CA438-, C447d, G16036GG, T16093C, G16129A, C16223T, T16311C, T16519C
mtDNA_MG_0069	M5d(M5d)	6	A73G, T146C, A263G, C325d(=C324d†), C338d, A361d(=A357d†), A368d(=A367d†), A374d(=A373d†), A385d(=A384d†), T393d(=T391d†), T401d(=T398d†), T16093C, G16129A, C16223T, T16311C, T16519C
mtDNA_MG_0070	M2a(M2a1a3)	2	A73G, T195C, C198T, T204C, A263G, C315CC, C324G, A352C, CA356-, A368d(=A367d†), A374C, A376d, A385d(=A384d†), T401d(=T398d†), T414d, A426d(=A425d†), G16036GG,

			T16157C, C16223T, C16270T, G16319A, T16352C, T16519C
mtDNA_MG_0071	F1c(F1c1a)	17	A73G, T152C, T204G, T208G, T220TT, A234G, A249d(=A248d†), C261A, A263G, C269T, A270d, A281T, C296A, A297C, C324G, C327d, C330G, C338A, A339C, A352C, CA356-, C371T, TA372-, A376C, A385d(=A384d†), AT397-, G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0072	F1c(F1c1a)	17	A73G, T152C, A234G, A249d(=A248d†), A263G, C325d(=C324d†), A361d(=A357d†), A368d(=A367d†), C375d, C387d(=C386d†), T393d(=T391d†), T401d(=T398d†), T414d, G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0073	U7(U7)	3	A73G, T152C, A263G, C315CC, C325d(=C324d†), A361d(=A357d†), C371d(=C369d†), A376d, T393d(=T391d†), T401d(=T398d†), G16036GG, C16294T, A16318T, T16519C
mtDNA_MG_0074	U4a(U4a2a3)	7	A73G, T152C, A263G, T310TTC, T310C, C324G, C330G, C338A, G16036GG, C16294T, A16318T, T16519C
mtDNA_MG_0075	U4a(U4a2a)	1	A73G, A263G, T310C, T310TTC, A352d(=A350d†), C362d, C378d(=C377d†), T401A, T403d, C411G, T414d, C418G, A428G, T430d, G16036GG, T16519C
mtDNA_MG_0076	M4a(M4a)	14	A73G, A263G, C315CC, A361d(=A357d†), T393d(=T391d†), T401d(=T398d†), G16036GG, G16145A, C16176T, C16223T, C16261T, C16266T, C16291T, T16298C, T16311C, T16325C, T16519C
mtDNA_MG_0077	F1c(F1c1a)	1	A73G, T152C, A176d(=A175d†), A189d(=A188d†), T204G, T206G, T208G, A234G, A249d(=A248d†), C256G, A257G, A263G, A270d, A274C, C277A, A281T, C296A, A297C, G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0078	M5d(M5d)	6	A73G, T146C, A263G, T310TTC, T310C, C324G, C348G, C371d(=C369d†), A376d,

			T393d(=T391d†), T401d(=T398d†), G16036GG, T16093C, G16129A, C16223T, T16311C, T16519C
mtDNA_MG_0079	F1c(F1c1a)	17	T55G, A73G, C144G, T152C, A176d(=A175d†), A189d(=A188d†), T204G, T206G, T208G, A210G, T212A, A234G, A249d(=A248d†), T252G, C256G, A257G, A263G, T267TT, A272d, A281T, C296d(=C295d†), C303A, G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0080	D4j(D4j14)	1	A73G, T146C, A263G, T310TTC, T310C, C324G, A352C, CA356-, A376d, T401d(=T398d†), T414d, A426d(=A425d†), CA438-, C445d, G16036GG, C16188T, C16223T, T16231C, T16362C, T16519C
mtDNA_MG_0081	M3a(M3a1+204)	9	A73G, T204C, T217C, A263G, C315CC, C324G, A352C, CA356-, A376d, T393d(=T391d†), T401d(=T398d†), T414d, A419d, C438d(=C433d†), C445d, T16126C, C16223T, C16266T, T16311C, T16519C
mtDNA_MG_0082	M3(M3)	10	A73G, A263G, C315CC, C324G, A352d(=A350d†), A376d, T393d(=T391d†), T401d(=T398d†), T414d, A419d, A426d(=A425d†), CA438-, C445d, G16036GG, T16126C, A16158G, C16169T, C16223T, T16519C
mtDNA_MG_0083	U4a(U4a2a3)	7	A73G, T152C, A263G, A281T, T310TTC, T310C, C325d(=C324d†), C330A, C338d, C362d, A374d(=A373d†), G16036GG, C16294T, A16318T, T16519C
mtDNA_MG_0084	U4a(U4a2a3)	7	A73G, T152C, A263G, T310C, T310TTC, A376d, T393d(=T391d†), T401d(=T398d†), T414d, G16036GG, C16294T, A16318T, T16519C
mtDNA_MG_0085	F1c(F1c1a)	17	A73G, T152C, A234G, A249d(=A248d†), A263G, C309T, T310C, A361d(=A357d†), A376d, T393d(=T391d†), T401d(=T398d†), C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0086	HV2(HV2)	7	A73G, T152C, A263G, T310TTC, T310C, C324G, A361d(=A357d†), C375d, C387d(=C386d†),

			T393d(=T391d†), T401d(=T398d†), G16036GG, T16217C, T16311C, T16519C
mtDNA_MG_0087	H1c(H1c3)	1	A73G, T125A, T131G, T152C, T204G, T206G, T208G, T213A, T217d(=T216d†), T223G, A232d, T239A, T252G, A257G, A263G, C268T, C273A, C277A, C285A, C296d(=C295d†), C309CC, G316C, C324G, A326d, C330A, C332A, C338A, C340A, C343T, C362A, G366A, T16519C
mtDNA_MG_0088	HV2(HV2)	7	A73G, T152C, A263G, T310C, T310TTC, C324G, A368d(=A367d†), A376d, T401d(=T398d†), G16036GG, T16217C, T16311C, T16519C
mtDNA_MG_0089	F1c(F1c1a)	17	T55G, A73G, T152C, A176d(=A175d†), A189d(=A188d†), T204G, T206G, T208G, T209TG, C231A, A234G, A249d(=A248d†), T252G, C256G, A257G, A263G, C264G, C264CG, C271d, C277A, A281T, C296d(=C295d†), C303A, G16036GG, C16111T, G16129A, T16304C, T16519C
mtDNA_MG_0090	U4a(U4a2a3)	7	A73G, T152C, A263G, T310C, T310TTC, C325d(=C324d†), C371d(=C369d†), C387d(=C386d†), T401d(=T398d†), G16036GG, C16294T, A16318T, T16519C
mtDNA_MG_0091	HV2(HV2)	7	A73G, T152C, A263G, T310TTC, T310C, C325d(=C324d†), C338d, A361d(=A357d†), C371d(=C369d†), C375d, A388d, T393d(=T391d†), T401d(=T398d†), G16036GG, T16217C, T16311C, T16519C
mtDNA_MG_0092	M30(M30)	4	A73G, T195A, A263G, C315CC, T393d(=T391d†), T401d(=T398d†), T414d, A419d, A426d(=A425d†), C438d(=C433d†), C445A, C447d, G16036GG, A16038G, A16051G, C16223T, T16325C, T16519C
mtDNA_MG_0093	HV2(HV2)	7	A73G, T152C, A263G, T310TTC, T310C, A361d(=A357d†), T401d(=T398d†), T414d, A432C, CA438-, A446d, G16036GG, T16217C, T16311C, T16519C
mtDNA_MG_0094	M3(M3)	10	A73G, A263G, C315CC, A361d(=A357d†), T393d(=T391d†), T401d(=T398d†), T414d, C420T,

			TA424-, C438d(=C433d†), A446d, G16036GG, T16126C, A16158G, C16169T, C16223T, T16519C
mtDNA_MG_0095	U4a(U4a2a3)	7	A73G, T152C, A263G, T310TTC, T310C, A352d(=A350d†), A365d(=A363d†), A376d, T393d(=T391d†), T401d(=T398d†), G16036GG, C16294T, A16318T, T16519C
mtDNA_MG_0096	P2(P2)	1	A73G, A263G, C269T, A270d, C309CC, T318C, C324G, C330G, C338d, A352C, CA356-, G366A, C371d(=C369d†), C375d, G16036GG, T16519C
mtDNA_MG_0097	U7(U7)	3	A73G, T152C, T206G, A263G, C268T, C315CC, C324G, C330G, C338d, A352C, CA356-, C371T, TA372-, T393d(=T391d†), T401d(=T398d†), G16036GG, C16294T, A16318T, T16519C
mtDNA_MG_0098	H32(H32)	2	A73G, T125A, T152C, T204G, T206G, T208G, T223G, A263G, C269T, A270d, C299d(=C298d†), C16294T, A16318T, T16519C
mtDNA_MG_0099	M6a(M6a1b)	5	A73G, T146C, A263G, T310TTC, T310C, C324G, A361d(=A357d†), C375d, T393d(=T391d†), T401d(=T398d†), C16188T, C16223T, T16231C, T16362C, T16519C
mtDNA_MG_0100	M30(M30)	4	A73G, C150T, T195A, A263G, T310C, T310TTC, A361d(=A357d†), A376d, T393d(=T391d†), T401d(=T398d†), T414d, CA438-, C445d, C16179d, C16223T, T16519C

Total Number of samples = 100

Table 4. Haplogroups, their sample size and occurrence in Geographical areas of world

Sr No	Haplogroup	Samples	Corresponding geographic Region
1	F1c(F1c1a)	17	East Asian, South Asian
2	M4a(M4a)	14	South Asia also in Eastern Saudi Arabia
3	M3(M3)	10	South Asia, NW India
4	M3a(M3a1+204)	9	South Asia, NW India
5	U4a(U4a2a3)	7	Northern Asia, Northern Europe
6	HV2(HV2)	7	Central Asia
7	M5d	6	South Asian
8	M6a(M6a1b)	5	South Asian
9	M30(M30)	4	South Asia
10	U7(U7)	3	West Eurasian
11	H32(H32)	2	West Asia
12	M2a(M2a1a3)	2	South Asia

13	H1q(H1q3)	2	Western European
14	H27(H27)	2	West Asia
15	R30b(R30b)	2	South Asia
16	P2(P2)	1	South East Asia
17	H2a(H2a2a1)	1	Western Asia
18	M2a(M2a1a)	1	South Asia
19	M30(M30+16234)	1	South Asia
20	N9b(N9b1c)	1	Southern Asia
21	D4j(D4j14)	1	American
22	H1c(H1c3)	1	Western European
23	U4a(U4a2a)	1	West Asia

Table 5. Genetic Properties of Haplogroups

Total Number of samples	100
Total number of Haplotypes	91
Unique haplotypes	88
Shared haplotypes	5
Genetic Diversity	0.999
Random Match Probability	0.0100
Power of discrimination	0.9900

Table 6: Percentage occurrence of the Mutations found

No.	Mutations	Percentage occurrence
1	Transversion	30.22
2	Transition	34.7
3	Insertion	8.4
4	Deletion	26.7

Table 7: Comparative Analysis of mtDNA sequence and genetic findings of Other Ethnic Group Studies with Mughal Population (current study)

Populations	Wakhi (25)	Makrani [1]	Saraiki [2]	Pathan [4]	Four KPK Tribes [19]	Sindhi [20]	Punjabi [15]	Kashmiri [21]	Hazara [23]	Kho (Chitral) [22]	Gujar (HVR1 & 2) [24]	Mughal (This study)
Sample size	40	100	85	230	100	88	100	317	319	16	73	100
Random match probability	0.026	0.0408	0.0542	0.0065		0.018	0.0085	0.0054	0.0085		0.0903	0.0100
Power of discrimination	0.974	0.9592	0.9458	0.9978		0.981	0.8819	0.7918	0.9915	0.202	0.9097	0.9900
Average Genetic diversity	0.998	0.9688	0.957	0.993	0.945	0.992	0.9633	0.9977	0.9945	0.215	0.9223	0.999

CONCLUSION

After comparison with the rCRS, observed mutations in population samples under study were, 78 transition (34.7%) 68 transversions (30.22%), 19 insertions (8.4%) and 60 deletions (26.7%). Total number of 91 different haplotypes were identified in 100 samples with 225 polymorphic sites. Out of 91, 88 of them are unique and 3 haplotypes were shared by one or more than one individuals. The most frequently observed haplotypes in this population were haplotypes F1c. and M4a each of these constitutes 17 % and 14% population as shown in Table 4. In this study, the South Asian haplogroups have clear dominance including M4a (14.%), M3 (10%), M3a (9%), M5d (6%), M6a (5%), M30 (4%), M2a (2%), R30b (2%), M2a (1%), M30(M30+16234) (1%). A comparison of genetic diversity of this Mughal population's mtDNA control region is done with other studied distinct ethnic groups of Pakistan. It is found that among all the studies ethnic group Pathans population was the most diversified and the Kalash population was least diversified having haplotype diversity 0.993 and 0.851 respectively as given in Table 6 (Rakha *et al.,* 2011). Here, it is found that F1c Haplogroups have clear dominance over other haplogroups. It means the distinct geographical location of the Mughal population may be because of heterogonous mitochondrial DNA haplogroups make-up (Sounier *et al.,* 2009). This study of the analysis of mitochondrial DNA will be useful in studying Biochemical genetics, population studies, and forensic sciences.

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