Data Article

Data on haplotype diversity in the hypervariable region I, II and III of mtDNA amongst the Brahmin population of Haryana

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A R T I C L E   I N F O

Article history:
Received 23 November 2017
Received in revised form 6 January 2018
Accepted 10 January 2018
Available online 31 January 2018

Keywords:
Mitochondrial DNA
D-loop
Hypervariable regions
Forensic genetics

A B S T R A C T

Human mitochondrial DNA (mtDNA) is routinely analysed for pathogenic mutations, evolutionary studies, estimation of time of divergence within or between species, phylogenetic studies and identification of degraded remains. The data on various regions of human mtDNA has added enormously to the knowledge pool of population genetics as well as forensic genetics. The displacement-loop (D-loop) in the control region of mtDNA is rated as the most rapidly evolving part, due to the presence of variations in this region. The control region consists of three hypervariable regions. These hypervariable regions (HVI, HVII and HVIII) tend to mutate 5–10 times faster than nuclear DNA. The high mutation rate of these hypervariable regions is used in population genetic studies and human identity testing. In the present data, potentially informative hypervariable regions of mitochondrial DNA (mtDNA) i.e. HVI (np 16024–16365), HVII (np 73–340) and HVIII (np 438–576) were estimated to understand the genetic diversity amongst Brahmin population of Haryana. Blood samples had been collected from maternally unrelated individuals from the different districts of Haryana. An array of parameters comprising of polymorphic sites, transitions, transversions, deletions, gene diversity, nucleotide diversity, pairwise differences, Tajima’s D test, Fu’s Fs test,
mismatch observed variance and expected heterozygosity were estimated. The observed polymorphisms with their respective haplogroups in comparison to rCRS were assigned.

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Specifications Table

| Subject area            | Forensic Science          |
|-------------------------|---------------------------|
| More specific subject area | Forensic Genetics         |
| Type of data            | Tables and Figures        |
| How data was acquired   | DNA from blood samples were extracted by using PCI method [1]. PCR amplification was carried out using SureCycler 8800 (Agilent Technologies, USA). All the amplified samples were cleaned before sequencing by using Post PCR Clean-up Kits (Thermo Scientific, USA) and sequencing were carried out using Applied Biosystems DNA Sequencer (Life Technologies, CA, USA). Analysis part were carried out by using Arlequin software version 3.5 (Computational and Molecular Population Genetics Lab, Zoological Institute, Switzerland) [2], Mega 7 [3] and HaploGrep 2 software (Medical University of Innsbruck, Austria) [4] |
| Data format             | Analyzed                  |
| Experimental factors    | Genomic DNA was extracted and amplified from the blood samples |
| Experimental features   | PCR amplification of HVI, HVII and HVIII was carried out using SureCycler 8800 (Agilent Technologies, USA) and Sequencing were carried out using Applied Biosystems DNA Sequencer (by Life Technologies, CA, USA) |
| Data source location    | Haryana (A northern state of India) |
| Latitude: 29.0588°N     | Longitude: 76.0856°E      |
| Data accessibility      | The data is available with this article |

Value of the data

- The data report will provide baseline information to any future evolutionary and genetic studies based on control region of mtDNA of the Brahmin population of Haryana.
- The data produced may be helpful in finding new mutations or polymorphisms which will prove quite useful in personal identification.
- It is also of special relevance to the investigative agencies in particular and society in general.
- In cases of mass disasters, it is common for the government agencies to provide compensations to the deceased family members. So the present data can also aid in the identification process of such cases.
- The present data will enhance the DNA database of Brahmin population, which can be used for calculating probabilities of match based on mtDNA.

1. Data

Table 1 describes the primer pairs used for amplifications of extracted samples.
Table 2 describes the PCR reaction mixture used for amplification of HVI, HVII and HVIII region.
Table 3 summarizes the PCR cycling conditions adopted during experiment. Table 4 summarizes the molecular diversity as seen in the HVI region. Table 5 summarizes the molecular diversity as seen in the HVII and HVIII region. Table 6 summarizes the molecular diversity as seen in the HVI + HVII + HVIII region. Table 7 summarizes the frequency distribution of mtDNA haplotypes in Brahmin population. Table 8 summarizes the sequence polymorphism and their respective haplogroups in the Brahmin population. Table 9 GenBank accession numbers for Brahmin Population (Supplementary Table).

2. Experimental design, materials and methods

2.1. Sample collection and genomic DNA extraction

The present study was completed in different phases. The first phase comprised of blood samples collection followed by the second phase, which involved the molecular biology procedures for DNA extraction, PCR amplification, PCR clean-up and sequencing. The last phase consisted of statistical analysis and interpretation of the data generated.

Blood samples had been collected from maternally unrelated individuals from nearly all districts of Haryana belonging to the ethnic group of Brahmins, after following proper ethical guidelines. Total

| Mt DNA region | Nucleotide Position | Primers | Primer Sequence (5′-3′) | Tm Value | Size (bp) |
|---------------|---------------------|---------|-------------------------|----------|-----------|
| HVI           | 16024–16365         | L 15900 (F) | TACACCAGTCTTGTAAACC     | 49.1 °C  | 828       |
|               |                     | H 00159 (R) | AAATAATAGGATGAGGCAAGAATC | 52.5 °C  |           |
| HVII & III    | 73–576              | L 00015 (F) | CACCCTATTACCACCTACG     | 52.7 °C  | 585       |
|               |                     | H 00599 (R) | TTGAGGGATAGCTACATAA     | 50.3 °C  |           |

Table 1 Details of the primers used for amplification of the Hypervariable Regions.

| S.No. | Chemical                   | Quantity |
|-------|----------------------------|----------|
| 1     | 10 × PCR buffer            | 2.5 µl   |
| 2     | 2 mM each dNTPs            | 2.5 µl   |
| 3     | 10 mM forward primer       | 1.5 µl   |
| 4     | 10 mM reverse primer       | 1.5 µl   |
| 5     | 5 U/µl Taq DNA polymerase  | 0.5 µl   |
| 6     | D/DH2O                     | 15.5 µl  |
| 7     | 50 ng of template DNA      | 1 µl     |

Table 2 PCR reaction setup.

| Cycle step                | Temperature | Time duration |
|---------------------------|-------------|---------------|
| Hot start                 | 95 °C       | 7 min         |
| Denaturation              | 95 °C       | 15 s          |
| Annealing                 | 62 °C       | 30 s          |
| Elongation                | 72 °C       | 1 min         |
| End cycle Elongation      | 72 °C       | 10 s          |
| Hold                      | 4 °C        | ∞             |
genomic DNA was extracted from the samples using the Phenol-Chloroform method [1]. The extracted DNA was checked for its quality on 0.8% agarose gel and quantity was checked on the Nanodrop (Thermo scientific, USA).

Table 4
Molecular diversity as seen in the HVI region.

| Population | Brahmin |
|------------|---------|
| Sample size | 66 |
| No. of polymorphic sites | 69 |
| No. of observed transitions | 62 |
| No. of observed transversions | 10 |
| No. of observed substitutions | 72 |
| No. of observed indels | 1 |
| Nucleotide composition (%) C | 33.29 |
| T | 22.42 |
| A | 33.00 |
| G | 11.29 |
| Mean number of pairwise differences | 5.78 ± 2.8 |
| Heterozygosity/sample | 0.016 ± 0.05 |
| No of Haplotypes | 51 |
| Gene Diversity | 0.986 ± 0.006 |
| Nucleotide Diversity | 0.0167 ± 0.009 |
| Ss2 of haplotype frequencies (RMP) | 0.0285 |
| Alleles Frequency (Mean ± S.D) | 1.212 ± 0.437 |
| Sum of square deviation | 0.0011 |
| Harpending’s raggedness index | 0.0081 |
| Mismatch distribution observed mean | 5.78 |
| Mismatch observed variance | 7.1 |
| Tajima’s D test | −2.02 |
| Fu’s FS test | −25.31 |

Table 5
Molecular diversity as seen in the HVII & HVIII region.

| Population | Brahmin |
|------------|---------|
| Sample size | 66 |
| No. of polymorphic sites | 58 |
| No. of observed transitions | 43 |
| No. of observed transversions | 13 |
| No. of observed substitutions | 56 |
| No. of observed indels | 5 |
| Nucleotide composition (%) C | 34.54 |
| T | 22.79 |
| A | 30.46 |
| G | 12.22 |
| Mean number of pairwise differences | 5.3 ± 2.59 |
| Heterozygosity/sample | 0.091 ± 0.12 |
| No of Haplotypes | 48 |
| Gene Diversity | 0.986 ± 0.005 |
| Nucleotide Diversity | 0.0104 ± 0.005 |
| Ss2 of haplotype frequencies (RMP) | 0.285 |
| Alleles Frequency (Mean ± S.D) | 1.12 ± 0.34 |
| Sum of square deviation | 0.001 |
| Harpending’s raggedness index | 0.0087 |
| Mismatch distribution observed mean | 5.3 |
| Mismatch observed variance | 6.31 |
| Tajima’s D test | −2.03 |
| Fu’s FS test | −25.45 |
2.2. PCR amplification

The three hypervariable regions, i.e. the HVI lying between np16024 and 16365, HVII lying between np 73–340 and HVIII lying between np 438–576 were amplified using both forward and reverse primers. The primer pairs used by the Brandstatter et al. [5] were used for amplifications. They were synthesized at IDT (Integrated DNA Technologies (IDT), USA) (Table 1). Two sets of PCR reactions were used for each sample, i.e. one for HVI region alone and the other amplified both the HVII and HVIII regions together. All controls, i.e., control and – ve extraction controls and amplification controls along with a reagent blank control were used during the experiments. Controls were used to ensure that no contamination was present at any stage during all the experiments. The PCR reaction was carried out in a final volume of 25 µl given in Table 2. PCR was performed on (SureCycler 8800, Agilent Technologies, USA). The PCR cycling conditions used are given in Table 3. After PCR amplification, the amplified product was visualized on 1.6% agarose (Sisco Research Laboratory, India) gel. GeneRuler 100 bp ladder (Thermo scientific, USA) was used for reading the size of the amplified

| Table 6 | Molecular diversity as seen in the HVI + HVII + HVIII region. |
|---------|-------------------------------------------------------------|
| Population | Brahmni |
| Sample size | 66 |
| No. of polymorphic sites | 127 |
| No. of observed transitions | 105 |
| No. of observed transversions | 25 |
| No. of observed substitutions | 130 |
| No. of observed indels | 4 |
| Nucleotide composition (%) C | 34.04 |
| T | 22.64 |
| A | 31.48 |
| G | 11.84 |
| Mean number of pairwise differences | 11.09 ± 5.1 |
| Heterozygosity | 0.087 ± 0.1 |
| No. of Haplotypes | 64 |
| Gene Diversity | 0.999 ± 0.002 |
| Nucleotide Diversity | 0.0129 ± 0.006 |
| Ss2 of haplotype frequencies (RMP) | 0.016 |
| Alleles Frequency (Mean ± S.D) | 1.16 ± 0.26 |
| Sum of square deviation | 0.00034 |
| Harpending’s raggedness index | 0.003 |
| Mismatch distribution observed mean | 11.09 |
| Mismatch observed variance | 13.73 |
| Tajima’s D test | −2.09 |
| Fu’s FS test | −24.47 |

| Table 7 | Frequency distribution of mtDNA haplotypes in Brahmins population. |
|---------|-------------------------------------------------------------|
| Number of times a haplotype repeated | Numbers of Haplotypes |
|          | HVI | HVII + HVIII | HVI + HVII + HVIII |
| 1        | 45  | 37  | 64  |
| 2        | 1   | 7   | 2   |
| 3        | 2   | 2   | −   |
| 4        | 2   | 1   | −   |
| 5        | 1   | 1   | −   |
| Total    | 51  | 48  | 64  |
| Random match probability | 0.028 | 0.028 | 0.016 |
Table 8
Sequence polymorphism and their respective haplogroups in the Brahmin population.

| Sample ID | HVI region               | HVII region               | HVIII region               | Haplogroups |
|-----------|--------------------------|---------------------------|----------------------------|-------------|
| BR1       | 16154C 16206C 16230G 16311C | 73G 263G 309.1C 315.1C   | 524.1AC                    | U2a1a       |
| BR2       | 16129A 16223T 16343G      | 73G 189G 194T 195C 199C 204C 207A 263G 315.1C | 489C         | W3a1 + 199  |
| BR3       | 16189C 16223T            | 73G 212K 236C 263G 309.1C 315.1C | 489C         | M           |
| BR4       | 16126C 16223T 16311C     | 73G 204C 263G 315.1C     | 482C 489C                 | M3a1 + 204  |
| BR5       | 16167T 16172C 16318T     | 73G 151T 152C 263G 270G 275K 276M 315.1C | 523d 524d    | U7a1a       |
| BR6       | 16129A 16223T 16362C     | 73G 263G 315.1C         | 489C         | D4a         |
| BR7       | 16169.1C 16189C 16193.1C 16223T 16274A | 73G 152C 182T 195C 263G 309.1C 315.1C | 447G 489C 523d 524d | M2b         |
| BR8       | 16095T 16223T 16249C 16395C | 73G 114T 146C 263G 309.1C 315.1C | 489C         | M3a4        |
| BR9       | 16126C 16163G 16186T 16189C 16294T | 73G 152C 195C 263G 309.1C 315.1C | 489C         | T1a13       |
| BR10      | 16046.1A 16183C 16189C 16193.1C 16223T 16362C | 73G 195C 263G 299d 309.1C 315.1C 373G | 462T 489C    | J1b1a       |
| BR11      | 16069T 16126C 16145A 16172C 16222T 16261T | 73G 247T 263G 295T 315.1C | 523d 524d    | U7a         |
| BR12      | 16309G 16318T            | 73G 151T 152C 263G 315.1C | 489C         | M4A         |
| BR13      | 16145A 16176T 16209C 16223T 16261T 16311C | 73G 152C 263G 315.1C     | 489C         | T2d1b       |
| BR14      | 16126C 16294T            | 73G 146C 152C 263G 315.1C | 489C         | R7b1a       |
| BR15      | 16260T 16261T 16286T 16311C 16319A 16362C | 73G 146C 152C 263G 315.1C | 489C         | M37e        |
| BR16      | 16111T 16172C 16184T 16189C 16223T 16274A 16295T | 73G 263G 309.1C 315.1C | 489C         | N9b         |
| BR17      | 16189C 16223T            | 73G 195C 263G 315.1C     | 489C         | M66b        |
| BR18      | 16104T 16184T 16223T 16311C | 73G 150T 195C 198T 199C 207A 263G 309.1C 315.1C | 456T         | HV12a       |
| BR19      | 16172Y 16220C 16264T 16292T | 263G 309.1C 315.1C       | 489C         | U2b2        |
| BR20      | 16209C 16239T 16352C 16353T | 73G 146C 152C 234G 263G 309.1C 315.1C | 489C         | M4*G7 + 16311 |
| BR21      | 16223T 16311C            | 73G 114T 263G 309.1C 315.1C 357C 361.1A | 489C         | M37 + 152 + 151 |
| BR22      | 16309G 16318C            | 73G 151T 152C 263G 315.1C | 489C         | M33d        |
| BR23      | 16309G 16318T            | 73G 151T 152C 263G 315.1C | 489C         | M49d        |
| BR24      | 16178C 16223T 16288C 16293T | 73G 152C 204C 207A 263G 315.1C | 489C         | M5b2        |
| BR25      | 16243C                   | 73G 263G 309.1C 315.1C | 489C         | M6a1b       |
| BR26      | 16129A 16223T 16291T     | 73G 152C 263G 315.1C 334C | 489C         | M30 + 16234 |
| BR27      | 16188T 16223T 16231C 16362C | 73G 146C 152C 263G 309.1C 315.1C | 489C         | U7a         |
| BR28      | 16175G 16223T 16234T     | 73G 195A 263G 315.1C    | 489C         | M3          |
| BR29      | 16309G 16318T            | 73G 151T 152C 222A 263G 309.1C 315.1C | 476A 523d 524d | U7a         |
| BR30      | 16126C 16223T 16311C     | 73G 98A 146C 152C 207S 263G 315.1C | 523d 524d    | L3h1        |
| BR31      | 16111T 16184T 16193.1C 16223T 16258C 16274A 16319A | 73G 191A 194T 263G 315.1C | 447G 489C 523d 524d | M2b         |
| BR32      | 16209C 16318T            | 73G 152C 153G 195C 263G 315.1C | 482C 489C    | U2a         |
| BR33      | 16126C 16163G 16186T 16189C 16294T | 73G 152C 263G 309.1C 315.1C | 523d 524d    | K2a5        |
| BR40 | 16223T 16234T | 73G 195A 263G 315.1C | 489C 523d 524d | M30 + 16234 |
| BR41 | 16129A | 263G 309.1C 315.1C | H1e + 16129 |
| BR42 | 16111T 16169.1C 16189C 16223T 16274A 16319A 16320T | 73G 152C 182T 263G 315.1C | 447G 471C 523d 524d 530T | M2b1b |
| BR43 | 16111T 16184T 16223T 16266T 16296T | 73G 78T 120G 152G 167G 180G 263G 315.1C | 489C 523d 524d | M7b2 |
| BR44 | 16223T 16256T 16311C 16362C | 73G 263G 309.1C 315.1C | 489C | M43a1 |
| BR45 | 16183C 16193C 16223T | 73G 153G 195C 225A 226C 263G 315.1C | X2b + 226 |
| BR46 | 16179d 16223T | 73G 146C 242T 263G 295T 315.1C | 462T 489C 523d 524d | M7c |
| BR47 | 16223T 16327A | 73G 125G 146C 152C 195C 236G 315.1C | 489C 523d 524d | M24 |
| BR48 | 16126C 16163G 16186T 16189C 16294T | 73G 146C 152C 263G 315.1C | 523d 524d | T1a + 152 |
| BR49 | 16189C 16223T | 73G 146C 263G 315.1C | N9b |
| BR50 | 16145A 16176E 16223T 16261T 16311C | 73G 263G 309.1C 315.1C | 482C 489C | M4a |
| BR51 | 16126C 16170G 16223T 16311C | 73G 146C 152C 263G 309.1C 315.1C | 461T 489C 573.1CC | M6 |
| BR52 | 16051G 16254G | 73G 85C 180C 216G 263G 309.1C 315.1C | 462T 489C | U2 |
| BR53 | 16069T 16126C 16145A 16222T 16261T 16288C 16362C | 73G 195C 263G 309.1C 315.1C | 482C | M3 |
| BR54 | 16092C 16126C 16223T 16311C | 73G 263G 315.1C | 489C | M11a |
| BR55 | 16188G 16223T 16270T 16274A 16290T 16291T 16319A 16352C | 73G 146C 263G 315.1C | 489C | M7c |
| BR56 | 16126C 16248T 16292T 16294T 16296T 16325C 16327T | 73G 204C 263G 315.1C | 447G 489C | T2c1 |
| BR57 | 16126C 16234T | 73G 263G 309.1C 315.1C | 489C | M |
| BR58 | 16051G 16129C 16182C 16183C 16189C 16362C | 73G 195A 263G 309.1C 315.1C | 489C 523d 524d | U2e |
| BR59 | 16126C 16234T | 73G 152C 263G 315.1C | 489C | M1 + 152 |
| BR60 | 16126C 16223T 16311C | 73G 152C 217C 263G 309.1C 315.1C | 508G | N1a |
| BR61 | 16126C 16223T 16311C | 73G 146C 242T 263G 295T 315.1C | 462T 489C 523d 524d | J1b1a + 146 |
| BR62 | 16126C 16223T 16311C | 73G 152C 263G 309.1C 315.1C | 489C 523d 524d | M30 |
| BR63 | 16126C 16223T 16311C | 73G 152C 263G 309.1C 315.1C | 489C | L3h1 |
product. After electrophoresis, the gel was visualized under Gel Documentation System (Alpha Innotech).

2.3. Post PCR cleanup

All the samples were cleaned before sequencing by using Post PCR Clean-up Kits (Thermo Scientific, USA) to remove the PCR inhibitors, primer-dimer formation and impurities present in the template.

2.4. Sequencing

The sequencing was carried out in the Xcelaris Genomic Labs by using the ABI BigDye Terminator Cycle Sequencing Kit on ABI 3700 Genetic analyzer (Applied Biosystems). All the samples were sequenced with the same primers used in PCR amplification for HVI, HVII & HVIII regions. An additional primer (16410R- GAGGATGGTGGTGGTCAA) has also been used for hyper variable region I in samples where slippage due to ‘C’ stretch was observed.

2.5. Statistical analysis

The interpretation of the HVI, HVII and HVIII chromatogram was done as per the guidelines to improve the quality of the data [6–9]. The sequences were matched and aligned with the revised Cambridge reference sequences (rCRS) [10] by using Mega 7 [3]. The coding for heteroplasmic sites was done according to the IUPAC codes in the interpretation guideline to interpret the mtDNA data analysis [8]. Diversity indices and differentiation tests were computed. The gene diversity was calculated according to Tajima [11]. Population pairwise differences were calculated by using genetic distances [12]. Nucleotide diversity, haplotype diversity, mean pairwise difference, number of haplotypes, mismatch distributions, Harpending’s raggedness index, Tajima’s D test and Fu’s Fs statistics were calculated by using Arlequin software version 3.5.1.2 [2] as shown in Tables 4 and 5. A random match probability (RMP) was calculated according to Stoneking et al. [13] (Table 6). Haplogroups classification and phylogenetic tree was performed by using HaploGrep 2 software [4] as shown in Table 8 and Fig. 1.

Acknowledgements

This work was supported by the University Grant Commission (UGC), New Delhi, Govt. of India under Major Research Project (MRP) scheme [Grant no. F.No. 42-45/2013 (SR)].
Transparency document. Supplementary material

Transparency document associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.01.011.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.01.011.

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