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Original Research

Mitochondrial DNA markers in Arabic Iraqi population

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ABSTRACT

Background: The D-loop region mitochondrial DNA (mtDNA) typing is an excellent tool for forensic applications because has universal primers exist that can be applied to almost any unknown sample and generate a result from D-loop region variations are much concentrated in these regions. The aim of the present study was to determine the variations of D-loop region using Sanger DNA sequencing techniques in Arabic Iraqi population. **Materials and Methods:** mtDNA isolation for used as a template to overlapping extended primers to generates four partially overlapping PCR amplicons on the mtDNA D-loop hypervariable regions, which is ready to direct DNA sequencing. **Results:** This study record 147 polymorphic positions found within the D-loop of the unrelated 100 Arab Iraqi mtDNA samples. The frequency of transitional polymorphic nucleotides were observed highest at positions 263, 73 and 16519 with 0.82, 0.66 and 0.51, respectively, as well as, frequency of insertion C is 0.71 and 0.5 at positions 315 and 309, respectively. The two samples were bearing insertion of double cytosine at positions 315 and 309, respectively. One point mutation heteroplasmy was detected located at position 16233. A statistical estimate this population showed the random match probability and the genetic diversity of 0.0294% and 99.8%, respectively. **Conclusion:** The variations of mtDNA D-loop region in this study that included point mutation, insertion, and heteroplasmy that consider as added the data in forensic genetics scope in Arabic Iraqi population.

KEY WORDS: Forensic sciences, forensic genetics, arabic population, DNA sequencing, Iraq, mitochondrial DNA marker

INTRODUCTION

Iraq is an Arabian country located in the Middle East. The Iraqi population consists of 75-80% Arabs and 20-25% others, which their official language is Arabic. Each population has a unique genetic structure which is determined by the genome analysis of its individuals; however, the genetic structure of a population has extracted the markers from frequency DNA variations. The genetic variation of D-loop sequence in populations has an important role in the forensic application of maternal mitochondrial DNA (mtDNA) identification and in studies of population history, origin, migration, or evolution.

The forensic investigation of mtDNA is better with the sequence DNA variation of two hypervariable regions within the control region tend to mutate 5-10 times faster than nuclear DNA [1]. This region has been estimated the polymorphic positions approximately 1-3% among unrelated persons [2]. The high mutation rate is making this region as a genetic marker in human identity testing [3]. The analysis of mtDNA widely used in forensic investigations which are mostly focus on control region since variations are very much concentrated in this region. There are several laboratories including the FBI Laboratory (Washington DC, USA), the Armed Forces DNA Identification Laboratory (Rockville, USA), and Forensic Science Service (Birmingham, UK) are using mtDNA examination in cases where nuclear DNA is degraded and not sufficient [4].

MATERIALS AND METHODS

The present study was randomly selected 100 blood samples of unrelated Iraqi volunteers of three sequential generations. mtDNA was isolated by using mtDNA extraction Kit (Biovision, USA). PCR amplification of the mtDNA D-loop region generates four partially overlapping PCR amplicons (~350 bp for each one) spanning the four hypervariable fragments ranging from 15997 to 16236, 16159 to 16527, 29 to 285, and 172 to 419 nucleotide position in the mtDNA D-loop; the four amplified products were subjected to cycle sequencing by using ABI 3730 xL DNA analyzer (Applied Biosystem, USA). The DNA Sequence Data were analyzed using BioEdit Software and then aligned with the revised Cambridge Reference Sequence (GenBank sequence NC_012920) by added the CLUSTAL W to the same software. A statistical estimate was included the diversity and random match probability of this population. The random match probability was defined according to Stoneking study [5] and the genetic diversity was calculated according to Tajima study [6].

RESULTS

Diversity parameters of the D-loop among the unrelated 100 Arab Iraqi population displays the variations compared with the Cambridge reference sequence and record 147 polymorphic positions found within the D-loop mtDNA samples. The majority of the polymorphism nucleotide positions in the D-loop region 16% of which are located within four overlapping D-loop regions. Variations of nucleotide sequencing are caused by nucleotide transition, substitutions, and insertions. In this study, nucleotide transitions make up the major value of 81.7% and low frequency of transversion of 3.7%, whereas single or double cytosine (2C) insertion show significant frequency 14.6% of the total number of mutations. 147 polymorphic sites of these were observed eight positions 152, 309, 315, 16183, 16193, 16265 16286, and 16304 show two polymorphic sequences in each position. The highest frequency of polymorphic nucleotides are observed at positions 263, 73 (transition from A to G), and 16519 (transition from T to C) with 0.82, 0.66 and 0.51, respectively, as well as, frequency of insertion C is 0.71 and 0.5 at positions 315 and 309, respectively. Two samples bear insertion of 2C at positions 315 and 309, respectively.

One point mutation heteroplasmy was detected in Arab Iraqi sample by direct sequencing of the control region fragments. It was located at position 16233 that showed both nucleotide Y = C/T at this position. The longest C-stretch region observed within the four samples contained 12 serial cytosine residues, occurred by nucleotide transversion A to C at position 16183, the transition at position 16189 and insertion C at position 16193. In this study, the same region contains 10 serial cytosine residues in six samples. The Sequencing data alignment of one sample show C-stretch region spans from 303 to 315 which occur by transition located at position 310 T to C.

The important parameters for statistical evaluation of mtDNA typing are based on the random match probability and genetic diversity in forensic casework. The probability of two randomly selected individuals from a population having identical mtDNA haplotype is 0.0294 and the genetic diversity is 99.8%, by using mtDNA manger software.

DISCUSSION

The variations of the D-loop among Arab Iraqi population through compared with the Cambridge reference sequence detected the 147 polymorphic positions which are classified into transitions 81.7% and transversions 3.7% within the D-loop mtDNA samples. There are previous studies included that higher occurrence of nucleotide transitions than substitutions in transitions to transversions ratio were 87:7 [7] and 24:1 [8]. The sequence variations within mtDNA non-coding regions have forensic value, where mutation rate of mtDNA control region is about 5-10 times higher than nuclear DNA [1] so that this region among unrelated individuals has been estimated to vary 1-3% [2]. However, the main reason for the occurrence of these variations is the low efficiency of repair and the production of replication error in the non-coding region [9] so that these variations have suitable as forensic genetic marker. Human mtDNA D-loop was described as complex evolution pattern with higher frequency of nucleotide transitions than trans versions; so that higher rate of pyrimidine transitions in the light strand than purine transitions and substitution rates which vary among nucleotide positions [10,11]. The distribution of DNA point mutation is show nucleotide transitional change is highest rate that significant difference is due to the chemical structure of the nucleotides and the chemical properties of complementary base pairing. High frequency of transition may be because of different mutational mechanisms in the genomic regions [12]. This study shows that hypermutability of transition cytosine to thyamine and vice versa [Table 1]. Methylation of the cytosine base generated the spontaneous deamination of methyl-C rise to a thymine. This transitional frequency occurs about 10 times faster than other point mutations in mammals [13]. This study estimates 120 insertion of one cytosine at three positions. The large data set of mtDNA sequence variation in human populations has been accumulated and is a valuable marker for human population and evolutionary studies [14]. The nucleotide position 16233 that showed point mutation heteroplasmy. Heteroplasic T and C at this specific position is not able to be detected by direct sequencing, in spite of the quality score of this position which approved confirmatory results of this heteroplasmic point mutation the light and heavy strand examination was carried out. In general, the heteroplasmy is considered a disadvantage in forensic casework, it can complicate and invalidate data interpretation, but in this study sample at position 16233 record haplotype A4, whereas in the study of Al-Zahery, the results showed position 16233C in Arab Iraqi case, which recorded mtDNA haplotype T2e.

Table 1: The nucleotide transition, transversion and insertions observed in the hypervariable regions of the mitochondrial DNA in Arab Iraqi population

Mutation type	Number of positions	Total number of mutations
Transitions		
Pyrimidine-pyrimidine		
T→C	41	252
C→T	42	181
Purine-purine		
G→A	30	218
A→G	15	64
Total	128	715
Transversions		
Purine-pyrimidine		
C→G	3	13
A→C	8	12
Pyrimidine-purine		
T→A	4	9
A→T	4	7
C→A	2	5
A→G	2	3
G→T	1	1
G→C	1	1
Total	31	49
Insertions		
Cytosine insertion		
+1C	3	120
+2C	2	2
Total	5	122

This special feature help improved data interpretation and probability of match [15].

Homopolymeric cytosine region containing serial repeated cytosine residues was observed in positions 16184-16193 when a nucleotide transition of the sample from T to C occurred at position 16189 to compare with the Cambridge reference sequence. The second similar case occurs when transition of T to C at position 310, so the C-stretch has extended from 303 to 315 [16]. The important topics in forensic genetics investigation are C-stretches regions which include the sequence spans nucleotides position 16184-16193 by change T at position 16189 and the C-stretch region spans positions 303-315 by change T at position 310, according to Cambridge reference sequence. This transition is from T to C in the samples which produce homopolymeric C-stretch due to polymerases slippage that rise errors in complementary strand synthesis to the mtDNA template [17]. The length variant of homopolymeric cytosine is considered a power of discrimination in forensic mtDNA investigation.

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