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Discovery of Three Newly Described Single Nucleotide Polymorphisms in Mitochondrial DNA Hypervariable Region I (HVI) and Estimation of Variants and Haplotypes Encompassing Nucleotide Positions 16024-16365

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Abstract

The aims of this research are to study the mitochondria noncoding region by using the Sanger sequencing technique and establish the degree of variation characteristic of a fragment. FTA® Technology (FTA™ paper DNA extraction) utilized to extract DNA. A portion of a noncoding region encompassing positions 16024-16365 amplified in accordance with the Anderson reference sequence. PCR products purified by EZ-10 spin column then sequenced and detected by using the ABI 3730xL DNA Analyzer. The most frequent variant differed in the single position A16227G, according to the CRS sequence. A new polymorphic position 16046, 16105 and 16141 are described may in future be suitable sources for identification purpose. The data obtained can be used to identify variable nucleotide positions characterized by frequent occurrence most promising for identification variants.

Keywords: Encompassing nucleotide positions 16024-16365; HVI; Iraq; Mitochondrial DNA; Polymorphism

Introduction

The mitochondrial DNA (mtDNA) is a small circular genome located within the mitochondria in the cytoplasm of the cell. The mitochondrial genome can be divided into two sections: a large coding region, which is responsible for the production of various biological molecules involved in the process of energy production in the cell, and a smaller 1.2 kilobase pair fragment, called the control region. It is found to be highly polymorphic and harbors three hypervariable regions HVI, HVII and HVIII [1]. Mitochondrial DNA comprising of about 37 genes coding for 22 tRNAs, two rRNAs and 13 mRNAs are a small circle of DNA [2]. Mitochondrial DNA does not recombine and thus there is no change between parent and child, unlike nuclear DNA [3,4]. There is more sequence divergence in mitochondrial than in nuclear DNA [5,6]. This may be caused by a faster mutation rate in mtDNA that may result from a lack of repair mechanisms. Sequencing of highly polymorphic segments of the control region of mitochondrial DNA (mtDNA) is today a routine method of analysis of biological traces which are not suitable for STR analysis due to insufficient concentration of nuclear DNA or heavy degradation processes [7,8]. A promising approach in this context seems to be analysis of selected single nucleotide polymorphisms (SNPs) that are useful for identification purposes. The aim of this study was to sequence the portion of the noncoding region of mtDNA in order to ascertain the degree of variation present in this fragment and to find those particular polymorphic positions that fulfill the conditions necessary for their future application in the identification process.

Materials and Methods

Population data

Unrelated 324 healthy blood samples from the middle and south of Iraq provinces. The age of the donors was between 20 to 30 years old, due to the mtDNA will get more mutation after 30 years old in human (Figure 1).

Extraction of DNA

Blood samples were taken by FTA (FTATM paper DNA extraction) cards and sent it to the genetics laboratory.

Amplification of mitochondrial DNA

A portion of noncoding region encompassing positions from 16024 to 16365 amplified in accordance with the Anderson reference sequence [9] GenBank: J01415. This portion of DNA was amplified in two primers: the first one is HVI-F (16024-16045) 5'-TTCTTTCATGGGGAAGCAGATT-3' and the second HVI-R has a position (16365-16345) 5'-AGTCAAATCCCTTCTCGTCCC-3'. Add in 20 μ L of Master Mix into PCR tube. Change the pipette tip and add 20 μ L of Primer Mix into PCR tube. Add 10 μ L of extracting DNA into the PCR tube after changing the pipette tip again. Allow all the liquid settles at the bottom of the tube, and not elsewhere. Check the volume in the PCR tube using the PCR tube with 50 μ L in it. 95°C hold for 10 minutes, 30 cycles of: 94°C for 30 seconds, 52.5°C for 30 seconds, 65°C for 1 minute. 72°C hold for 10 minutes. 4°C hold, ∞ infinity is the cycling protocol for amplification of mtDNA PCR.

Evaluation of mitochondrial DNA

To confirm whether the mtDNA PCR product is amplified, we will

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Received November 05, 2013; Accepted December 16, 2013; Published December 19, 2013

Citation: Imad H, Abeer F, Cheah Y, Mohammed J, Aamera O (2013) Discovery of Three Newly Described Single Nucleotide Polymorphisms in Mitochondrial DNA Hypervariable Region I (HVI) and Estimation of Variants and Haplotypes Encompassing Nucleotide Positions 16024-16365. J Forensic Res 5: 209. doi:10.4172/2157-7145.1000209

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scrutinize the products of the amplification. Gel electrophoresis is used to do this. Here the electricity is used to force the movement of DNA fragments through a special gel. Since the DNA is negatively charged, it will move to the positive electrode in the electric field. The electric force causes the shorter portions of the DNA to move faster than the longer ones.

Purification of mitochondrial DNA

Using a special binding buffer, EZ-10 spin column purification kits using a silica gel membrane selectively absorb up to 10 μg of DNA fragments. Since Nucleotides, oligos (<40-Meir), enzymes, mineral oil and other impurities do not EZ-10 Spin Column bind to the membrane, they are just removed. Here the DNA fragments can be separated in small amounts and can be used in further applications without any further treatment.

Cycle sequencing and sequence analysis

The DNA Sequencing of the PCR products was done using the BigDye TM Terminator. Utilizing POP-7 polymer (Applied Biosystems) polymer lot number 1206453. The separation of the cycle sequencing products was carried out. Detection was by using the ABI 3730×L DNA Analyzer, cap array size 96, cap array length 50. The

J Forensic Res ISSN: 2157-7145 JFR, an open access journal reference sequence described by Anderson $\left[9\right]$ was compared to the data observed.

Cycle sequencing interpretation guidelines

Within the noncoding region Mitochondrial DNA, sequencing results are studied from a consensus sequence derived from multiple sequence results. Data were analyzed by Sequencher[™] (SEQUENCHER[™] 4.7 User Manual for Windows[®] 1991-2007) [10] and aligned with the Anderson sequence using the sequence Navigator software. They are accepted by stating the nucleotide position followed by the code for the polymorphic base (for example, 263G).

Statistical analysis

Genetic diversity for the analyzed DNA fragment was calculated according to the formula: $D=1-\sum p^2$.

Where p: frequencies of the observed haplotypes [11].

Results

The basic aim of this work was to assess the degree of variation characterizing a selected segment of the noncoding region of mtDNA. The study enabled identification of 103 different haplotypes and 28

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Table 1: Continued.

	16030	16032	16038	16041	16042	16051	16052	16064	16084	16105	16117	16119	16120	16124	16129	16141	16154	16156	16197	16221	16227	16253	16266	16303	16312	16334	16346	16349)
Anderson	С	т	Α	Α	G	Α	с	т	G	т	т	Α	Α	т	G	Α	т	G	С	С	Α	Α	С	G	Α	т	G	Α	No. of Individual
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H72										С													Т						1
H73								С														G							1
H74						G													Т										2
H75		С													Α														2
H76																								Α					1
H77									Α																				4
H78																				Т			Т						3
H79					Α										С								G						1
H80																	С												2
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H82																	С												3
H83						G																							1
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H98																G			Т										1
H99				G																									3
H100														С								G							1
H101												G										G						•	1
H102										С											G								2
H103											С								Т										1
Total																													324

H*: Haplotype; G: Guanine; T: Thiamine; C: Cytosine; A: Adenine.

polymorphic nucleotide Positions Table 1. The most frequent variant (H1) was consistent with the Anderson sequence. Substitutions determined during the study are transitions and transversion. This fact is consistent with abundant literature data revealing significant domination of transitions over transversions [12,13]. Eleven polymorphic positions, 16030, 16032, 16025, 16105, 16120, 16129, 16197, 16266, 16303, 16346 and 16349 have transverse substitution Table 2. All the other substitutions determined during the analysis are transitions. The most frequent variant differed in the single position A16227G, according to the CRS sequence.

Comparative analysis of our results with previously published Iraqi data revealed significant differences in varying patterns [14,15]. This observation supports the thesis that different SNP-type polymorphisms can be strongly associated with a given population. Table 3 presents a summary of the Iraqi data in comparison with other global populations [16-18]. Significant assistance for the research was provided by Mitomap computer database, which contains information concerning human mtDNA [19]. This database includes data about currently known variable positions, their possible association with genetic diseases, and references to the literature. There is also a simple program called Mito Analyzer attached to the database which enables convenient access to information concerning polymorphic positions.

Discussion

The presence of more than one mitochondrial DNA (mtDNA) variant within a cell, tissue, or individual is emerging as an important component of eukaryotic genetic diversity. Yet the variations may vary from person to person. Therefore, to understand the polymorphisms at

different sites, it is of critical importance to investigate the sequencing of mtDNA coding region's transmission. The first entire human mtDNA sequence was explained by Anderson et al. [9]. Cambridge Reference Sequence CRS is the name given to the published sequence used as a reference standard. For the coding function of the analyzed DNA fragment. Sequencing of the mitochondrial DNA coding region in the 300 unrelated donors showing a new polymorphic position 16046, 16105 and 16141 are described may in future be suitable sources for identification purpose. Sequence the portion of the noncoding region of mtDNA is in order to verify the degree of variation present in the fragment. It is also to identify those particular polymorphic positions that meets the conditions necessary for their future use in the identification process.

Earlier writings for a detailed description of molecular biology, genetics, sequence determination procedures, interpretation practices, and utility of mtDNA sequence analysis in forensic casework and human identification [20,21]. In cases where there is an abundance in the sample, for example mass graves in mass disasters, there are newly discovered forensically validated methods such as ESI-MS [22] Certainly, all such applications should have a strong grasp of the mtDNA variation that is present in the populations concerned. As an example, describing and frequency estimates of common mtDNA types and any population sub-structuring must be at hand [23]. Consequently, this may also increase the pool of samples with degraded and insufficient nuclear DNA for mitochondrial DNA analysis.

Conclusion

It will become easier to handle minute amounts of DNA or DNA

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Positions	Mutation	Type of mutation	Presence in Mitomap	Frequency	Frequency%		
16030	Transversion	C-G	Presence	0.003	0.3%		
16032	6032 Transversion		Presence	0.020	20/		
16032	Transition	T-C	Presence	0.030	370		
16038	Transition	A-G	Presence	0.024	2.4%		
16041	Transition	A-G	Presence	0.047	4.7%		
16042	Transition	G-A	Presence	0.058	5.8%		
16051	Transition	A-G	Presence	0.036	3.6%		
16052	Transversion	C-G	Presence	0.042	4 20/		
16052	Transition	C-T	Presence	0.043	4.3%		
16064	Transition	T-C	New*	0.030	3%		
16084	Transition	G-A	Presence	0.061	6.1%		
16105	Transversion	T-A	New	0.045	4 50/		
16105	Transition	T-C	Presence	0.045	4.5%		
16117	Transition	T-C	Presence	0.024	2.4%		
16119	Transition	A-G	Presence	0.037	3.7%		
16120	Transversion	A-T	Presence	0.034	3.4%		
16124	Transition	T-C	Presence	0.068	6.8%		
16129	Transition	G-A	Presence	0.040	4.00/		
16129	Transversion	G-C	Presence	0.043	4.3%		
16141	Transition	A-G	New	0.033	3.3%		
16154	Transition	T-C	Presence	0.042	4.2%		
16156	Transition	G-A	Presence	0.047	4 70/		
16156	Transition	G-T	Presence	0.047	4.7%		
16197	Transversion	C-G	Presence	0.045	4 50/		
16197	Transition	C-T	Presence	0.045	4.5%		
16221	Transition	C-T	Presence	0.052	5.2%		
16227	Transition	A-G	Presence	0.047	4.7%		
16253	Transition	A-G	Presence	0.058	5.8%		
16266	Transversion	C-G	Presence	0.050	E 00/		
16266	Transition	C-T	Presence	0.058	5.8%		
16303	Transition	G-A	Presence	0.000	0.00/		
16303	Transversion	G-C	Presence	0.036	3.6%		
16312	Transition	A-G	Presence	0.023	2.3%		
16334	Transition	T-C	Presence	0.046	4.6%		
16346	Transition	G-A	Presence	0.045	4.50/		
16346	Transversion	G-C	Presence	0.045	4.5%		
16349	Transition	A-G	Presence	0.040	40/		
16349	Transversion	A-T	Presence	0.040	4%		
			Genetic diversity*	D=1- ∑p²=0.963=96.3%			

New*: new polymorphic positions; Genetic diversity* Genetic diversity for the analysed DNA fragment was calculated according to the formula: D=1- $\sum p^2$

Table 2: Types of mutations and Frequency in a variable positions.

that is badly degraded with the coming of more techniques. Individual nucleotides may differ, and on top of that their number may differ. A new polymorphic positions are described may in future be suitable sources for identification purpose.

Acknowledgements

I sincerely wish to thank Prof. Issam for providing me the opportunity to work on this project. I am indebted to you for choosing the project, your enthusiasm for helping me and your patience and guidance as I progress to put this project together. I am thankful to you for helping me through the various analysis stages, and for providing helpful criticism and feedback throughout the writing process. I also would like to thank Dr. Khalifa from the Institution of medico-legal for all time put in to discuss the project and helping me to put the project together. I would also like to thank Zainab Al-Habubi from the Department Biology for her guidance and help in the laboratory work.

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Population	Iraq ¹	India ²	Malaysia ³	Africa ⁴	German⁵	US Caucasian ⁶
Sample size	320	98	195	111	200	604
No. of variant sites	28	83	149	97	153	233
A→G	64	233	473	323	330	1112
G→A	34	66	81	78	55	219
T→C	40	145	461	382	308	1007
C→T	28	117	321	486	199	688
% Transition	93.26	94.85	92.16	95.77	95.61	97.61
A→T	2	1	2	0	4	2
A→C	0	23	81	15	5	47
G→T	0	0	0	18	0	1
G→C	6	0	3	0	1	6
C→A	0	0	30	17	11	12
C→G	2	4	1	6	19	6
T→A	2	7	5	0	1	0
T→G	0	0	3	0	0	0
% Transversion	6.74	5.15	7.84	4.23	4.39	2.39
Insertion	0	168	322	140	291	983
Deletion	0	0	28	6	6	14

Note: % of transitions and transversions were calculated as number of observations divided by total substitution times 100.

¹This study. ²Reference: [16],

³Reference: [17],

⁴Reference: [17],

⁵Reference: [18]

⁶Reference: [17]

 Table 3: Comparisons of the characteristics across HV1 of the D-loop region in different human population groups.

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