

Research Article

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Screening of Three Different Alleles of mtDNA (G709A, G3496T, A3537G) in Subpopulation of UKM Students

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ABSTRACT

Human DNA consists of nucleus DNA (nDNA) and mitochondrial DNA (mtDNA). Both are valuable in medicine and forensic genetics but in this project, single nucleotide polymorphisms (SNPs) in mtDNA are used to trace the mutation occurred. Mutations in the sequence of alleles can lead to haplogroup variation and also certain diseases. The purpose of this study is to screen of mutations on alleles G709A, G3496T, and A3537G in Malay population of The National University of Malaysia (UKM) students. These SNPs lie in the ND1 (nitrogen dehydrogenase subunit 1) coding region, and the reports state that these three alleles are prone to mutate. From MitoMap Web site, the mutations of these alleles are reported to have potential in causing several diseases with the collaboration of other SNPs mutation. Allele G709A is reported to have an association with hearing loss and Leber Hereditary Optic Neuropathy (LHON) while allele G3496T is associated to LHON only. Allele A3537G is related to diabetes. A total of 100 DNA samples were collected from Malay students of UKM and preserved on FTA card to be purified later. The concentration of the DNA on the purified FTA card was between 10µM to 20µM. An attempt was made by amplifying those three loci from the genomic DNA. The amplified product was detected and separated using 1% gel electrophoresis. Before sequencing, the PCR products were visualized under UV light using gel documentation system. All PCR products were sequenced to detect the mutation on every single position chosen. From the alignment of sequencing results, allele G709A and allele G3496T showed no mutation. Meanwhile four samples from alleles A3537G has the mutation. From the results obtained, it seems that mutations are rare in all selected alleles. It is recommended to increase the sample size and alleles selected in the future to increase the strength of the study. This study also should be applied to other populations in Malaysia such as Chinese and Indian.

Key words: mtDNA polymorphisms, mutation, Allele G709A, Allele 3496T and Allele A3537G

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INTRODUCTION

Mitochondria, a bi-membrane organelle, serve as an energy provider for eukaryotes. A variety of cellular chemical reaction is facilitated by mitochondria in its matrix. The distinct quality of mitochondria is it carries a separate genetic material known as mitochondrial DNA (Rensvold et al., 2013). The human mitochondrial genome was deciphered a published first in 1980's, after reviews and modification it was revised and become known as Revised Cambridge Reference Sequence (rCRS) (Bandelt et al., 2012). Human mtDNA is a circular shaped closed double-stranded helix containing 16569 bases which codes for polypeptides, transfer, and ribosomal RNA. The shorter sequence of bases contributes to its compact structure. MtDNA is more abundant in a cell since each mitochondrion has an average 4-5 copy and a cell has more than 25 mitochondria in average which yield more genetic material for analysis compared to nuclear DNA (Ghochani et al., 2010).

The admission of forensic DNA in an administration of justice is due to its uniqueness and its reliability in the differentiation of an individual. There is no two individuals have the same genetic material configuration except for

identical twins (Kurelac et al., 2012). DNA is also a viable source of evidence because it can be found in a variety of samples such as hair, body fluids and semen. Apart from nuclear DNA, mtDNA is also significant evidence in forensic science, available in larger quantity and in a better quality. In the event of no ante mortem sample of available for reference, samples can be obtained from maternal lineage. This is particularly useful in disaster victim identification (Prieto et al., 2011). Apart from samples obtained from a crime scene that are usually contaminated, degraded, small quantity and low quality, mtDNA analysis is more qualified to be analyzed compared to nuclear DNA (Pajnic et al., 2010).

MtDNA is ten times more prone to mutate compared to nuclear DNA especially in coding regions so it can provide a suitable material for population studies. This event may be due to its high base substitution rate and its slow and less effective repair mechanism. The continuation change in the sequence has given rise to singular groups of specific polymorphism between individuals of a region. Mitochondrial inheritance is maternal since, during fertilization only the head of the sperm enters the ovum, the paternal mitochondria located at the neck region is left outside of the ovum and will be degraded (Levine & Elazar, 2011). Based on this, a mutation inherited in mtDNA is from maternal lineage and from the report, point mutations are commonly seen coding region (Fu et al., 2013).

MtDNA nucleotide position 709 is located in 12S rRNA region. This region is highly susceptible to mutation as it may lead to deafness syndrome or loss of hearing (Rydzanicz et al., 2009). These two health problems are reported to cause by mutation of SNP 709 from G to A. From previous studies on families inheriting the hearing loss disease, mutation of a base from G to A occurred in almost all individual in the family including eight individuals who suffered the disease. The previous research concluded that mutation in multiple alleles may cause the disease (Stalder et al., 2012). The remaining two SNPs which are SNP 3496 and SNP 3537 are located in MT-ND1, the coding region of nitrogen dehydrogenase subunit 1 (ND1). From the previous study, the mutation in SNP 3496 is secondary observed in LHON patients that the base changed from guanine G to T (Guo et al., 2012). Mutation in SNP 3537 has been seen in individuals with diabetes that the base changed from A to guanine G (Zuo et al., 2010). Screening of this SNP position is quite important as diabetes can be classified as a common disease among Malaysian (Sufiza et al., 2013).

METHODOLOGY

A total of 100 buccal swab samples were collected from Malay students of UKM using FTA cards and labeled with number 1 to 100 accordingly. The stain on the FTA cards was then punctured using Harris Micropunch to form 2.0mm sample discs. Prior to amplification process, samples were purified using FTA purification reagent and then air dried at room temperature or heated at 56°C. A total of two sets of designed primers were used in this study that can cover all the SNPs selected. For SNP 709, the forward and reverse primers that have been used were 5'TGCAAGCATCCCCGTTTC' and 5'CCTCCCCAATAAAGCTAAAACCTC'3 while for SNP 3496 and 3537, the forward and reverse primers used were 5'TAATGCTTACCGAACGAA3' and 5'CGTTTACTCAATCCTCTGA3'. These primers were designed to produce 300bp of DNA fragments and recorded as fragment I for SNP 709 and fragment II for SNP 3496 and SNP 3537. The samples were amplified in a 50µl of PCR mix that consisted of deionized distilled water, PCR buffer, Taq polymerase, dNTPs, magnesium chloride, reverse and forward primers and purified DNA sample. The PCR cycles involved were pre denaturation for 4 minutes at 95°C, followed by 30 cycles of denaturation for 45 seconds at 95°C, annealing at 73°C for 30 seconds and elongation for 1 minute at 72°C then completed by a final extension for 5 minutes at 73°C. The PCR products then were separated through gel electrophoresis for 40 minutes using 1% agarose gel in 1X TBE buffer. The separated DNA fragments were observed under the UV illuminator and the image were documented using gel documentation system. Before quantification, the PCR products were purified using DNA purification kit supplied by Qiagen. The purified products later were quantitated using nanodrop spectrometry to validate the purity and concentration. Finally, the samples were sent for sequencing in Malaysia Genome Institute (MGI). The sequencing results in the form of electropherogram were analyzed using available online Bioedit Sequence Alignment Editor V7.0 software.

RESULTS

From the observation of DNA separated on gel electrophoresis under UV illuminator, all of DNA samples for SNP 709, SNP 3496 and SNP 3537 manage to produce bands on gel electrophoresis. Both PCR products for fragment 1 and II were observed at 300bp. Figure 1 below shows results of gel electrophoresis with positive and negative controls for fragment I with a total of 10 DNA samples labeled from 1 to 10. All DNA bands appear at 300bp while figure 2 shows results of gel electrophoresis together with positive and negative controls for fragment 2 with a total of 10 samples labeled from 11 to 20 and the bands also appear at 300bp. The successful amplified DNA samples were quantitated before sending for sequencing in MGI. The sequencing results were then aligned and compared with revised Cambridge Reference Sequence (rCRS) using available online Bio edit software.

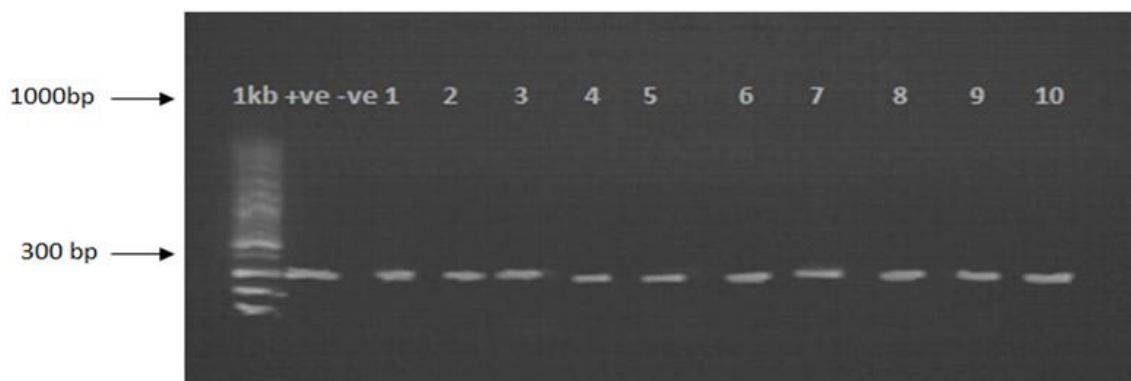


Figure 1

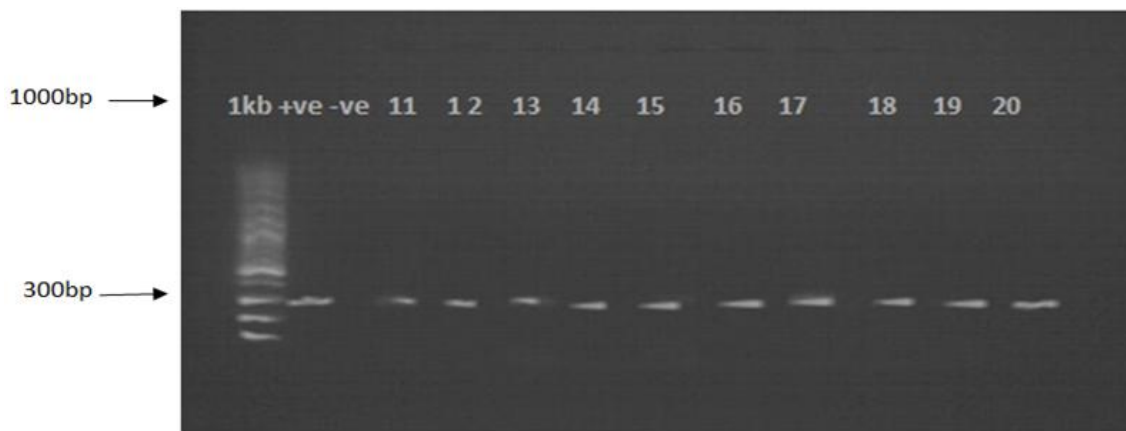


Figure 2

The rCRS from position 300bp to 900bp was used as reference sequence for fragment I and fragment II. All sequenced samples were aligned simultaneously together with the reference sequence to check if there were any changes to the selected nucleotide position. According to rCRS database, the original base for SNP 709 was guanine (G) and from the analysis there were no changes of the base at this particular nucleotide position. The reference sequence for fragment II was selected from nucleotide 3000 to 3800 base pairs. This reference sequence was used to be compared with SNP 3496 and SNP 3537. The original base for allele 3496 was guanine (G) and from the analysis, no changes of nucleotide were observed.

As for SNP 3537, the original base was adenine (A). Based on the comparison of the alignments, there were four samples numbered 1, 27, 59 and 71 were observed to have changes of a base from adenine (A) to guanine (G). This shows a 4% of the incidence of mutation for this allele A3537G among Malay students of UKM. Both forward and reverse of sequence comparison has been done to validate the nucleotide changes.

The comparison of electropherograms data showed that samples with necessary concentration and purity produced clear and sharp peaks with less noise and overlapping peaks. Meanwhile samples with low concentration and purity specification showed the opposite, short and low peaks with heavy noise and overlapping peaks.

DISCUSSION

From the electrophoresis and quantitation results, there were PCR products with low concentration. This could be a result of small quantity or loss and lack of DNA quality templates for amplification (Ruijter et al., 2009). Primer dimmers were also captured as DNA band and appeared around 100bp on gel electrophoresis. This may cause by non-optimum annealing temperature that lead them attached to each other, which can be eliminated by hot start PCR technique (Wang et al., 2012).

Table 4.1 Summary results of sequence alignment for all SNPs

Allele	Original base	Mutation/ Total	Samples with mutation occurred	Base changes
709	G	No	None	No
3496	G	No	None	No
3537	A	Yes/ 4	Samples 1,27,59 and 71	A>G

The results of sequence alignment are summarized in Table 4.1 above. There is no mutation observed in SNP 709 and SNP 3496. However mutation is observed in 4 samples of SNP 3537 which are sample 1, 27, 59 and 71.

From previous reports, SNP 709 is classified as a hot spot for polymorphism due to its incidence of mutation in various population groups, so it is one of the criteria for this allele to be selected for screening purpose. But from the screening, no mutation is recorded. Same occurs to SNP 3496. Out of 100 samples screened, no mutation observed. Based on the screening outcomes, it can be concluded that opportunity of Malay population to have deafness syndrome and LHON based on mutation of SNP 709 and SNP 3496 alone are low. Even though four samples have been found to mutate from A to G in SNP 3537 to produce 4% of mutation percentage, the number is still considered as low. As this SNP is related to diabetes, screening of this position brings results that Malay population is not exposed to diabetes based on allele mutation only. Maybe other factors contribute to high chances of Malay population to relate to diabetes.

CONCLUSION

Based upon the screenings conducted, mutation was observed in SNP 3537 only and none found in SNP 709 and SNP 3496. Screening of single SNP for disease relation maybe not enough as some genetic diseases may arise from a collaboration of several SNP mutations. Additional of several SNP positions in this study will be considered in the future.

Other than that, this study also can be expanded by including students of other races in UKM and also increasing the sample size. This will give us a clear perspective on distribution and inheritance of polymorphism between various populations in Malaysia. The study also can be included various locations for a consistent and precise data.

REFERENCES

- Bandelt H.J., Van Oven M and Salas A. 2012. Haplogrouping Mitochondrial DNA Sequences in Legal Medicine/ Forensic Genetics. *International Journal of Legal Medicine* 126(6): 901-916
- Fu Q., Mitnik A., Johnson P.L., Bos K., Lari M., Bollongino R., Sun C., Giemisch L., Schmitz R. and Burger J. 2013. A Revised Timescale for Human Evolution Based on Ancient Mitochondrial genomes. *Current Biology*
- Ghochani M., Nulton J., Salamon P., Frey T., Rabinovitch A and Baljon A. 2010. Tensile Forces and Shape Entropy Explain Observed Crista Structure in Mitochondria. *Biophysical Journal* 99(10): 3244-3254
- Guo H., Zhuang X.Y., Zhang A.M., Zhang W., Yuan Y., Guo L., Yu D.D., Liu J., Yang D.K. and Yao Y.G. 2012. Presence of Mutation M. 1448T> C in a Chinese Family with Maternally inherited Essential Hypertension but No Expression of LHON. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*
- Kurelac I., Lang M., Zuntini R., Calabrese C., Simone D., Vicario S., Santamaria M., Attimonelli M., Romeo G. and Gasparre G. 2012. Searching for a Needle in the Haystack: Comparing Six Methods to Evaluate Heteroplasmy in Difficult Sequence Context. *Biotechnology Advances* 30(1): 363-371
- Levine B. and Elazar Z. 2011. Inheriting Maternal mtDNA. *Science (New York, NY)* 334(6059): 1069
- Pajnic I.Z., Pogorelec B.G. and Balazic J. 2010. Molecular Genetic Identification of Skeletal Remains from the Second World War Kofin I Mass Grave in Slovenia. *International Journal of Legal Medicine* 124(4): 307-317
- Prieto L., Zimmermann B., Goios A., Paneto G., Alves C., Alonso A., Fridman C., Cardoso S. and Lima G. 2011. The Ghep-Empop Collaboration on mtDNA Population Data-A New Resource for Forensic Casework. *Forensic Science International: Genetics* 5(2): 146-151
- Rensvold J.W., Ong S.E., Jeevanathan A., Carr S.A., Mootha V.K. and Pagliarini D.J. 2013. Complementary RNA and Protein Profiling Identifies Iron as a Key Regulator of Mitochondrial Biogenesis. *Cell Reports* 3(1): 237-245
- Ruijter J., Ramakers C., Hoogars W., Karlen Y., Bakker O., Van Den Hoff M. and Moorman A. 2009. Amplification Efficiency: Linking Baseline and Bias in the Analysis of Quantitative PCR Data. *Nucleic Acids Research* 37(6): e45
- Rydzanicz M., Wrobel M., Dominica F., Wojciech G., Witold S. and Krzysztof S. 2009. Screening of the General Polish Population for Deafness-associated Mutations in Mitochondrial 12S rRNA and tRNA^{Ser}(UCN) Genes. *Genetic Testing and Molecular Biomarkers* 13(2): 167-172
- Stalder N., Yarol N., Tozzi P., Rotman S., Morris M., Fellmann F., Schwitter J. and Hullin R. 2012. Mitochondrial A3243G Mutation with Manifestation of Acute Dilated Cardiomyopathy. *Circulation Heart Failure* 5(1): e1-e3
- Suffiza N.A., Azuana R., Farida I. and Thomas P. 2013. Medication Adherence in Patients, with Type 2 Diabetes Mellitus Treated at Primary Health Clinics in Malaysia. *Patient Preference and Adherence* 2013 (7): 525-530
- Wang X., Spandidos A., Wang H. and Seed B. 2012. Primerbank: A PCR Primer Database for Quantitative Gene Expression Analysis, 2012 update. *Nucleic Acids Research* 40 (D1): D1144-D1149
- Zou Y., Jia X., Zhang A., Wang W.Z., Li S., Guo X., Kong Q.P., Zhang Q. and Yao Y.G. 2010. The Mt-Nd1 and Mt-Nd5 Genes are Mutational Hotspots for Chinese Families with Clinical Features of LHON but Lacking the Three Primary Mutations. *Biochemical and Biophysical Research Communications* 399(2): 179-185