Mitochondrially-Encoded Adenosine Triphosphate Synthase 6 Gene 
Haplotype Variation among World Population during 2003-2013

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Abstract

Background: Adaptation and natural selection serve as an important part of evolution. Adaptation in molecular level can lead to genetic drift which causes mutation of genetic material; one of which is polymorphism of mitochondrial DNA (mtDNA). The aim of this study is to verify the polymorphism of mitochondrially-encoded Adenosine Triphosphate synthase 6 gene (MT-ATP6) as one of mtDNA building blocks among tropic, sub-tropic, and polar areas.

Methods: This descriptive quantitative research used 3,210 mtDNA sequences, taken from GenBank, as secondary data from 27 different populations. The data were grouped into 3 population groups based on the climates of their location. After grouping, the sequences were then aligned and trimmed using Unipro EUGENE, and analysed by Arlequin and MitoTool.

Results: Results demonstrated 21 haplotypes distributed among 3 populations with variations between each climate population. In the tropic and sub-tropic populations, the dominant haplotype is h1 while h6 is dominant in the polar population.

Conclusions: There is a variation of haplotype polymorphism between tropic, sub-tropic, and polar climate population.

Keywords: climate, DNA, genetic testing, mitochondrial, MT-ATP6

Introduction

During Evolution, adaptation is essential for every living being for surviving the natural selection. This evolution also happened to humans during the migration from Africa according to Out of Africa theory.¹ Different altitude and latitude result in different climates; therefore adaptation to new climate is important to survive the natural selection.² When humans are exposed to the cold environment; their bodies automatically activate the thermogenesis system, which includes shivering and non-shivering thermogenesis (NST).² NST is done by increasing the metabolic activity of human body to produce heat and energy in adipose tissue, specifically inside the mitochondria.³⁴ Production of Adenosine Triphosphate (ATP) as a form of energy which happens inside the mitochondria requires several enzymes, one of which is called ATP synthase, which itself has ATP synthase 6 (MT-ATP6) as one of its building blocks.⁵

According to Charles Darwin, humans with suitable phenotype and genotype can survive a particular environment better than the other.⁶ Genetic drift can cause genetic material to change during the evolution process, possibly leading to genetic polymorphism especially about climate adaptation. Furthermore, MT-ATP6 has a higher level of polymorphism and variation than other genes that construct ATP synthase.⁷ Therefore; this adaptation could lead to a variation of MT-ATP6 gene polymorphism over the world.⁷ Coincidentally, there is an ongoing controversy about whether or not climate can cause genetic variation, especially for MT-ATP6 gene.⁹,¹⁰ As such, this study aims to find this variation of MT-ATP6 gene between tropical, sub-tropical, and polar climate.

Methods

A sample of DNA mitochondria (mtDNA) was collected from GenBank® which is a genetic sequence database of publicly available DNA sequences collects all genome data from every research that can be freely accessed. We collected 3,210 DNA mitochondria (mtDNA) as secondary data, taken from 27 populations distributed across the tropical, sub-tropical, and polar area.¹¹ Inclusion criteria used in this research were human mtDNA taken from the normal population (not having metabolic abnormality) and the presence of MT-ATP6 region (8,527-9,207) in the sequences. The exclusion criterions were human mtDNA haplotype that has a frequency less than 1% or mtDNA sample without a country, making climate-based grouping impossible.
Collected samples were then aligned and trimmed by using software Unipro UGENE. Each sample was then translated into amino acid sequences using specific mitochondrial DNA Codon and grouped according to the climate: tropic, sub-tropic, and polar population. After that, samples were grouped according to haplotype and calculated to find the frequency of each haplotype in every climate population. If the result were less than 1%, the haplotype was considered as mutation and excluded. Next, we did the analysis using software Arlequin to calculate the genetic diversity. Besides, we also did another analysis using haplogroup M, which is the most common haplogroup in the world. Mitochondrial DNA data were then grouped according to haplogroup using MitoTool. Every haplotype that belongs to haplogroup M found in this analysis which using 3 countries that represent each climate populations (tropic, sub-tropic, and polar) were calculated to find the frequency of each haplotype. The countries used were India which represents tropic, Japan for sub-tropic, and Russia for polar. These countries were chosen because each was traversed by haplogroup M during migration out of Africa and these countries had the largest number in the sample population. After that, we calculated each haplotype inside haplogroup M and compared them. The result of each analysis was described.

As gratitude to the previous researcher which data was used in this study, we list the entire data source in Table 6, Table 7, and Table 8.

**Results**

In this study, we used 3,210 mtDNA sequences from 27 populations and found 21 haplotype variations (Table 1). Prefix “h” indicates the haplotype, and we can define the polymorphism location by comparing each haplotype using h1 as the reference sequence. Each haplotype was grouped into 3 climate group: tropic, sub-tropic, and polar (Table 2). In tropic population, h1 had the highest frequency, and this was also the case with the sub-tropic population. Unlike the two populations, the polar population had h6 as the haplotype with the highest frequency, even though its worldwide frequency is much lower than h1 or h2.

Based on the number of sample and frequency of haplotype found, we also calculated the haplotype diversity of each climate population and compared it to each other. Haplotype diversity shows the diversity of population with the value from zero to one. Zero indicates that every individual in the population is virtually identical and one indicates that there are no similar individuals in the population.

From the analysis, we found that the three populations had similar haplotype diversity (Table 3). Although similar in diversity value, each population had their haplotype variation as shown in Table 2.

We also investigated this variation using haplogroup approach, so we could compare haplotypes found in each population that belongs to haplogroup M (Table 4). From the table, we found that there are variations in haplotype that all belong to haplogroup M in each population, especially for the sub-tropic and polar population.

In another approach, we used haplogroup M that was well distributed in tropic, sub-tropic, and polar population and looked into their haplotype variation (Table 5). We used 3 different countries to represent their climate population: India, Japan, and Russia, all three of which were traversed in haplogroup M route of migration. These countries also had sufficient sample size to represent each climate population.

**Table 1. Population Sample**

| Tropic Population | n  | Sub-tropic Population | n  | Polar Population | n  |
|-------------------|----|-----------------------|----|------------------|----|
| Zambia            | 32 | Spain                 | 76 | Russian          | 602|
| Namibia           | 141| Japan                 | 672| Siberian         | 106|
| Botswana          | 290| Franco-Cantabrian      | 39 | Greenland        | 15 |
| Angola            | 22 | Tunisia               | 43 | Sweden           | 14 |
| Philippines       | 260| North American        | 75 | Finland          | 192|
| Papua New Guinea  | 298|                       |    |                  |    |
| Sudan             | 20 |                       |    |                  |    |
| Chad              | 11 |                       |    |                  |    |
| Ethiopia          | 17 |                       |    |                  |    |
| Yemen             | 8  |                       |    |                  |    |
| Somalia           | 20 |                       |    |                  |    |
| India             | 72 |                       |    |                  |    |
| Oman              | 3  |                       |    |                  |    |
| Indonesia         | 9  |                       |    |                  |    |
| Cook Island       | 14 |                       |    |                  |    |
| Solomon Island    | 205|                       |    |                  |    |
| Polynesia         | 294|                       |    |                  |    |
| Sum               | 1716|                      | 905|                  | 929 |

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Table 2. World Population Haplotype Variation Table

| Haplotype | MT-ATP6 Polypeptide Sequence |
|-----------|-------------------------------|
| h1        | A T P A F L T A M I H M S A I I S |
| h2        | T . . . . . . . . . . . . . . . . |
| h3        | T . . . . . . . . . . . . . . . . |
| h4        | T . . . . . . . . . . . . . . . . |
| h5        | T . . . . . . . . . . . . . . . . |
| h6        | T . . . . . . . . . . . . . . . . |
| h7        | T . . . . . . . . . . . . . . . . |
| h8        | T T . . . . . . . . . . . . . . . |
| h9        | T . . . . . . . . . . . . . . . . |
| h10       | T . . . . . . . . . . . . . . . . |
| h11       | T . . . . . . . . . . . . . . . . |
| h12       | T . . . . . . . . . . . . . . . . |
| h13       | T . . . . . . . . . . . . . . . . |
| h14       | T . . . . . . . . . . . . . . . . |
| h15       | A . . . . . . . . . . . . . . . . |
| h16       | T . . . . . . . . . . . . . . . . |
| h17       | S . . . . . . . . . . . . . . . . |
| h18       | A . . . . . . . . . . . . . . . . |
| h19       | S . . . . . . . . . . . . . . . . |
| h20       | F . . . . . . . . . . . . . . . . |
| h21       | V . . . . . . . . . . . . . . . . |

Table 3. MT-ATP6 Haplotype Frequency in Population Studied

| Haplotype | Tropic | Sub-tropic | Polar | Total |
|-----------|--------|------------|-------|-------|
| f         | %      | f         | %     | f     | %     | F     | %     |
| h1        | 715    | 46.73     | 379   | 46.00 | 104   | 12.15 | 1198  | 37.32 |
| h2        | 566    | 36.99     | 318   | 38.59 | 95    | 11.10 | 979   | 30.50 |
| h3        | 30     | 1.96      | 24    | 2.91  | -     | -     | 54    | 1.68  |
| h4        | 16     | 1.05      | 12    | 1.46  | -     | -     | 28    | 0.87  |
| h5        | -      | -         | 11    | 1.33  | 24    | 2.80  | 35    | 1.09  |
| h6        | -      | -         | 7     | 0.85  | 510   | 59.58 | 517   | 16.11 |
| h7        | -      | -         | 10    | 1.21  | 5     | 0.58  | 15    | 0.47  |
| h8        | -      | -         | 6     | 0.73  | 12    | 1.40  | 18    | 0.56  |
| h9        | -      | -         | 2     | 0.24  | 25    | 2.92  | 27    | 0.84  |
| h10       | -      | -         | 3     | 0.36  | 39    | 4.56  | 42    | 1.31  |
| h11       | -      | -         | 2     | 0.24  | 16    | 1.87  | 18    | 0.56  |
| h12       | 21     | 1.37      | -     | -     | 1     | 0.12  | 22    | 0.69  |
| h13       | 44     | 2.88      | -     | -     | -     | -     | 44    | 1.37  |
| h14       | 71     | 4.64      | -     | -     | -     | -     | 71    | 2.21  |
| h15       | 18     | 1.18      | -     | -     | -     | -     | 18    | 0.56  |
| h16       | 20     | 1.31      | -     | -     | -     | -     | 20    | 0.62  |
| h17       | 29     | 1.90      | -     | -     | -     | -     | 29    | 0.90  |
| h18       | -      | -         | 43    | 5.22  | -     | -     | 43    | 1.34  |
| h19       | -      | -         | 7     | 0.85  | -     | -     | 7     | 0.22  |
| h20       | -      | -         | 15    | 1.75  | 15    | 0.47  |
| h21       | -      | -         | 10    | 1.17  | 10    | 0.31  |
Table 4. Frequency of MT-ATP6 Haplotype Variation Found in Population Studied and Their Haplotype Diversity

| Population | n  | k  | h                  |
|------------|----|----|--------------------|
|            | (Sample size) | (Haplotype) | (Diversity) |
| Tropic     | 1530 | 10 | 0.6408 ± 0.0079    |
| Sub-tropic | 824  | 13 | 0.6359 ± 0.0103    |
| Polar      | 856  | 12 | 0.6139 ± 0.0175    |

* Other mean every haplotype that found in haplogroup M but was excluded due to the frequency are less than 1%

Table 5. Haplotype Variation in Haplogroup M

| Climate  | Country | Haplotype | f | %  |
|----------|---------|-----------|---|----|
| Tropic   | India   | h1        | 63| 88 |
|          | (n=72)  | other*    | 9 | 12 |
| Sub-tropic | Japan | h1        | 86| 81.11|
|          | (n=106) | h2        | 1 | 0.94|
|          |         | h7        | 10| 9.43|
|          |         | other*    | 9 | 8.49|
| Polar    | Russia  | h1        | 5 | 28 |
|          | (n=18)  | h6        | 9 | 50 |
|          |         | h7        | 4 | 22 |

Discussion

In this study, we discovered 21 haplotype variations out from 27 populations. Despite having the highest number of population in this study, the tropic population had the least number of haplotype among three populations. The dominant haplotype in this study was h1 and h2 which was both found in every climate population with h1 as the most frequent haplotype in tropic and sub-tropic. Differing from the previous populations; the polar population had h6 as the dominant haplotype. H6 was only found in polar and sub-tropic with low frequency. We also analysed h6 in polar population and found that h6 was distributed among every population included in polar population, 313 sequences in Russia, 138 sequences in Finland, 53 sequences in Siberian, and six sequences in Sweden.

Besides, we also found exclusive haplotypes that were only found in one of the population, such as h13, h14, h15, h16, and h17 that were only found in the tropic. There are several theories that can explain this condition. First, during the migration out of Africa, humans with particular haplotype came and stayed in one area and did not go to another climatic region as described by Out of Africa theory.1 Second, this variation could be explained by genetic drift theory as a human adaptation process during evolution. When humans migrate into a territory with a different climate, humans can adapt to the changes and survive, maintaining or even increasing in numbers. They who cannot adapt, however, are unable to survive and their numbers drop.2, 8

From the diversity calculation among three populations shown in Table 3, we found that tropic, sub-tropic and polar populations have similar diversity level, but there were haplotype variations in each climate group. According to Out of Africa theory, after the migration from one area to new area, humans spread out to new areas, leading to variety of genotype and phenotype with nearly same diversity level.1

In haplogroup analysis, we found that in the tropic, there was the only h1 that belonged to haplogroup M, but in sub-tropic and polar areas, there were several haplotypes belonging to haplogroup M beside h1. There was an indication that MT-ATP6 gene mutated and generated a polymorphism inside haplogroup M due to adaptation process, especially in sub-tropic and polar areas.

Limitations of this study include the limited variety of population used in each climate group because secondary data were only gathered from GenBank. This might limit the variation of MT-AT6 gene that could potentially be found in this study and hence not every haplotype in the world was represented here. Thus, we suggest a new study with a larger population with more variation so it can give a more accurate reflection on the spread of MT-ATP6: whether it is affected by migration or by genetic drift as an effort of adaptation.
Table 6. Source of Tropic Population Data Sample Retrieve from GenBank

| Population | n  | Title                                                                 | Year  | Author                                                                 |
|------------|----|----------------------------------------------------------------------|-------|------------------------------------------------------------------------|
| Zambia     | 32 | Ancient substructure in early mtDNA lineages of southern Africa     | 2013  | Barbieri C, Vicente M, Rocha J, Mpoloka SW, Stoneking M, Pakendorf B. |
| Namibia    | 141| Complete mtDNA genomes of Filipino ethnolinguistic groups a melting pot of recent and ancient lineages in the Asia-Pacific region | 2013  | Tabbada KA, Salvador JM, Calacal GC, Sagum MS, Datar FA, Padilla SG, De Ungria MC, Stoneking M. |
| Botswana   | 290| Melanesian mtDNA Complexity                                          | 2007  | Stoldt M, Koki G, Horvat G, Zhadanov S, Schurr TG, Merriwether DA.    |
| Angola     | 22 |                                                                       |       |                                                                        |
| Philippian | 260|                                                                       |       |                                                                        |
| Papua New  | 16 |                                                                       |       |                                                                        |
| Guinea     |    |                                                                       |       |                                                                        |
| Sudan      | 20 |                                                                       |       |                                                                        |
| Chad       | 11 |                                                                       |       |                                                                        |
| Ethiopia   | 17 | The Expansion of mtDNA Haplogroup L3 within and out of Africa        | 2012  | Soares P, Alshamali F, Pereira JB, Fernandes V, Silva NM, Alfonso C, Costa MD, Musilova E, Macaulay V, Richards MB, Cerny V, Pereira L. |
| Yemen      | 2  |                                                                       |       |                                                                        |
| Somalia    | 20 |                                                                       |       |                                                                        |
| India      | 72 | The earliest settlers' antiquity and evolutionary history of Indian populations evidence from M2 mtDNA lineage | 2008  | Kumar S, Padmanabham PB, Ravuri RR, Uttaravalli K, Koneru P, Mukherjee PA, Das B, Kotal M, Xaviour D, Saheb SY, Rao VR. |
| Oman       | 3  | Pleistocene-Holocene boundary in Southern Arabia from the perspective of human mtDNA variation | 2012  | Al-Abri A, Podgorna E, Rose JL, Pereira L, Mulligan CJ, Silva NM, Bayouni R, Soares P, Cerny V. |
| Yemen      | 6  |                                                                       |       |                                                                        |
| Indonesia  | 9  | Mitochondrial genome variation in 2 individual from Bandung           | 2011  | Ngli Y, Noer AS, Syukriani YF, Natalia D, Ahmad AS, Syah YM.          |
| Cook Island| 14 | Maternal History of Oceania from Complete mtDNA Genomes: contrasting Ancient Diversity with Recent Homogenization Due to the Austronesian Expansion | 2014  | Duggan AT, Evans B, Friedlaender FR, Friedlaender JS, Koki G, Merriwether DA, Kayser M, Stoneking M. |
| Papua New  | 282|                                                                       |       |                                                                        |
| Guinea     |    |                                                                       |       |                                                                        |
| Solomon Island| 205|                                                                       |       |                                                                        |
| Polynesian | 294|                                                                       |       |                                                                        |
| Total      | 1716 |                                                                     |       |                                                                        |

Table 7. Source of Sub-Tropic Population Data Sample Retrieve from GenBank

| Population | n  | Title                                                                 | Year  | Author                                                                 |
|------------|----|----------------------------------------------------------------------|-------|------------------------------------------------------------------------|
| Spain      | 76 | The Expanded mtDNA Phylogeny of the Franco-Cantabrian Region Upholds the Pre-Neolithic Genetic Substrate of Basques. | 2013  | Cardoso S, Valverde L, Alfonso-Sanchez MA, Palencia-Madrid L, Elcoroaristizabal X, Algorta J, Catario S, Arteta D, Herrera RJ, Zarrabetita MT, Pena JA, de Pencorbo MM. |
| Japan      | 672| Mitochondrial genome variation in eastern Asia and the peopling of Japan | 2004  | Tanaka M, Cabrera VM, Gonzalez AM, Larruga JM, Takeyasu T, Fuku N, Guo LJ, Hirose R, Fujita Y, Kurata M, Shinoda K, Umetru K, Yamada Y, Oshida Y, Sato Y, Hattori N, Mizuno Y, Arai Y, Hirose N, Ohta S, Ogawa O, Tanaka Y, Kawamori R, Shamoto-Nagai M, Maruyama W, Shimokata H, Suzuki R, Shimodaira H. |
| Franco-Cantabrian | 39 | Genetic continuity in the Franco-Cantabrian region new clues from a mtDNA lineage | 2012  | Gomez-Carballa A, Olivieri A, Behar D, Achilli A, Torroni A. |
| Tunisia    | 43 | Data from complete mtDNA sequencing of Tunisian centenarians testing haplogroup association and the golden mean to longevity | 2009  | Costa MD, Cherni L, Fernandes V, Freitas F, Ammar El Gaaied AB, Pereira L. |
| North America | 75 | Drawing the history of the Hutterite population on a genetic landscape inference from Y-chromosome and mtDNA genotypes | 2011  | Pichler I, Fuchsberger C, Platzer C, Caliskan M, Marroni F, Peter PP, Ober C. |
| Total      | 905 |                                                                     |       |                                                                        |
Table 8. Source of Polar Population Data Sample Retrieve from GenBank

| Population | n  | Title                                                                 | Year | Author                                                                 |
|------------|----|----------------------------------------------------------------------|------|------------------------------------------------------------------------|
| Russia:    | 20 | Investigating the Prehistory of Tungusic Peoples of Siberia and the Amur-Ussuri Region with Complete mtDNA Genome Sequences and Y-chromosomal Markers | 2013 | Duggan AT, Whitten M, Wiebe V, Crawford M, Butthof A, Spitsyn V, Makarov S, Novgorodov I, Osakovsky V, Pakendorf B. |
| Russia:    | 168|                                                                      |      |                                                                        |
| Russia:    | 39 |                                                                      |      |                                                                        |
| Russia:    | 130| Y-chromosomal Markers                                               |      |                                                                        |
| Russia:    | 137|                                                                      |      |                                                                        |
| Russia:    | 31 |                                                                      |      |                                                                        |
| Siberia    | 22 | Mitochondrial DNA diversity in indigenous populations of the southern extent of Siberia, and the origins of Native American haplogroups | 2005 | Starikovskaya EB, Sukernik RI, Derbeneva OA, Volodko NV, Ruiz-Pesini E, Torroni A, Brown MD, Lott MT, Hosseini SH, Huoponen K, Wallace DC. |
| Siberia    | 84 | Mitochondrial genome diversity in arctic Siberians, with particular reference to the evolutionary history of Beringia and Pleistocene peopling of the Americas. | 2008 | Volodko NV, Starikovskaya EB, Mazunin IO, Eiltsov NP, Naidenko PV, Wallace DC, Sukernik RI. |
| Greenland  | 15 | Paleo-Eskimo mtDNA genome reveals matrilineal discontinuity in Greenland | 2008 | Gilbert MT, Kivisild T, Gronnow B, Andersen PK, Metspalu E, Reidla M, Tammi E, Axelesson E, Goetherstrom A, Campos PF, Rasmussen M, Metspalu M, Higham TF, Schwenninger JL, Nathan R, De Hoog CJ, Koch A, Moller LN, Andreasen C, Meldgaard M, Vilemms R, Bendiex C, Willerslev E. |
| Russia     | 4  | A recent genetic link between Sami and the Volga-Ural region of Russia | 2007 | Ingman M, Gyllensten U.                                                |
| Sweden     | 14 |                                                                      |      |                                                                        |
| Russia     | 73 | Mitogenomic diversity in tatars from the volga-ural region of Russia | 2010 | Malymarchuk B, Derenko M, Denisova G, Kravtsova O.                      |
| Finland    | 192| Lack of Polymorphisms in a Segment of MTND5 Gene in Haplogroup J.    | 2003 | Moilanen JS, Finnila S, Majamaa K.                                     |

Total 929

Conclusions

We concluded that there was a genetic variation of MT-ATP6 as a polymorphism of DNA mitochondria among the population living in the tropic, sub-tropic, and polar climates. The processes that cause this variation could not be fully explained, but there are two conditions that could explain this variation. First, human migrated from Africa to a specific area can cause several variations among human MT-ATP6 haplotype over the world. Second, due to adaptation, human genetic are mutated, so the gene is vary according to the climate as indicated by this study. The benefit of this study is mainly theoretical: the result of this study can be used as a basis to pursue further bioinformatics studies about MT-ATP6 variation.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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