Mitochondrial DNA Haplogroup A may confer a genetic susceptibility to AIDS group from Southwest China

Hua-Wei Wang, Yu Xu, Ying-Lei Miao, Hua-You Luo, and Kun-Hua Wang

Yunnan Institute of Digestive Disease, the First Affiliated Hospital of Kunming Medical University, Yunnan Province, China

Abstract
The acquired immunodeficiency syndrome (AIDS) in humans was one of the chronic infections caused by human immunodeficiency virus (HIV), and the interactions between viral infection and mitochondrial energetic implicated that mitochondrial DNA (mtDNA) variation(s) may effect genetic susceptibility to AIDS. Thus, to illustrate the maternal genetic structure and further identify whether mtDNA variation(s) can effect HIV infection among southwest Chinese AIDS group, the whole mtDNA control region sequences of 70 AIDS patients and 480 health individuals from southwest China were analyzed here. Our results indicated the plausible recent genetic admixture results of AIDS group; comparison of matrilineal components between AIDS and matched Han groups showed that mtDNA haplogroup A (p = 0.048, OR = 3.006, 95% CI = 1.109–8.145) has a significant higher difference between the two groups; further comparison illustrated that mtDNA mutations 16,209 (p = 0.046, OR = 2.607, 95% CI = 0.988–6.876) and 16,319 (p = 0.009, OR = 2.965, 95% CI = 1.278–6.876) have significant differences between AIDS and matched control groups, and both of which were the defining variations of mtDNA haplogroup A, they further confirmed that mtDNA haplogroup A may confer genetic susceptibility to AIDS. Our results suggested that haplogroup A may confer a genetic susceptibility to AIDS group from Southwest China.

Introduction
The acquired immunodeficiency syndrome (AIDS) in humans was one of the chronic infections caused by human immunodeficiency virus (HIV) (Shioda & Nakayama, 2006). The mortality of people with the infection of HIV without treated was more than 90% (O’Brien & Nelson, 2004). Highly active antiretroviral treatment (HAART) has significantly reduced the morbidity associated with HIV infection and AIDS (Mocroft et al., 2003), however, the HAART treatment can also inhibit human mitochondrial DNA (mtDNA) polymerase-γ which may lead to mtDNA depletion and mitochondrial dysfunction (Martin et al., 2003) and the interactions between viral infection and mitochondrial energetics implied that mtDNA variations may play crucial role in viral disease progression (Hendrickson et al., 2008).

Mitochondria played crucial role in cellular energy metabolism, free radical production, and the apoptotic pathways. Nevertheless, lacking of protective histones, an extremely inefficient DNA mismatch repair mechanism, as well as high level of reactive oxygen species (ROS) caused its higher mutation rate (Beal, 1996; DiMauro & Schon, 2001; Lightowlers et al., 1997; Wallace, 2005; Wallace et al., 1999). The specific combination of the mutations formed mtDNA haplogroup, which was widely used to trace the origin and prehistory of modern human (Cann et al., 1984, 1987) for having the regional specific mtDNA haplogroups, such as mtDNA haplogroup L, was mainly distributed to African groups (Soares et al., 2012), haplogroups H, UV, WX1, JT, and K were prevalent among European groups (Richards et al., 2000), M7, M8, M9, N9, and R9 were the major mtDNA haplogroups among East Asian groups (Hill et al., 2006; Kivisild et al., 2002), and M2, M3, M4, M5, M6, N5, R5, R6, R30, R31 were prevalent in South Asian (Palanchamy et al., 2004; Sun et al., 2006). For the crucial role of mitochondrion on regulating cellular energy metabolism, producing free radicals, and initiating and executing the apoptotic pathways, thus the mtDNA variation(s) or combination of different mtDNA mutation (mtDNA haplogroup) were deduced to be associated with different diseases. MtDNA haplogroup F1 can significantly increase the risk of nasopharyngeal carcinoma (Hu et al., 2014); haplogroup JT was associated with the higher survival rates for severe septic patients (Lorente et al., 2013); haplogroup R was a strong independent predictor of sperm motility decreased chance of asthenozoospermia (Feng et al., 2013). One of the plausible explanations for the association between mtDNA haplogroup was the subtle functional effects on affecting ATP production, ROS, heat generation, and apoptosis for mtDNA variation represented by different haplogroups (Gomez-Duran et al., 2010). However, the regional distributing pattern of different mtDNA haplogroups among different continents implied that there should be different maternal haplogroup on affecting HIV/AIDS susceptible from different regions.

In current study, to illustrate the maternal genetic structure and further identify whether any mtDNA variation(s) can effect HIV infection among southern of East Asia AIDS groups, the whole mtDNA control region of 70 AIDS patients were sequenced and
further analyzed by incorporating with 168 matched control individuals of 3 Han Chinese groups retrieved from 480 individuals belonging to 11 ethnic/Han Chinese populations.

Material and methods

Samples

Blood samples of 70 unrelated HIV seropositives patients were collected from southwest of China, which received informed consent before sample collection. All procedures were supervised and approved by the human tissue research committee of our hospital.

DNA amplification, sequencing

Genome DNA was isolated using the genomic DNA extraction kit (Axygen, Union City, CA). DNA was stored at −80°C. The whole mtDNA control region was amplified with one primer pair and purified as described in previous work (Wang et al., 2012). The purified PCR product was sequenced with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on a 3730 DNA sequencer according to the manufacturer’s manual.

Data analysis

The sequence variations were recorded by comparing with the revised Cambridge Reference Sequence (rCRS) (Andrews et al., 1999). The 70 AIDS patients and 480 health individuals were assigned to specific haplogroup, respectively according to the most recently updated mtDNA phylogeny (van Oven & Kayser, 2009) based on the specific mtDNA variations of each individual. The principle component analysis (PCA) was conducted by taking the haplogroup frequency as input factor to show the overall clustering pattern of AIDS and ethnic/Han Chinese populations from the similar geographic region, and the populations showing closer genetic affinity with AIDS group were selected as matched control groups. To assess the significance of differences observed for haplogroup distribution frequencies and mtDNA mutations frequencies (ranging from 16,024 bp to 16,365 bp) between AIDS and controls, the Pearson’s chi-square test with a one degree of freedom was used, and the Fisher’s exact test (two tailed) was applied to those cases with cell counts below five with the SPSS software package (version 16.0, SPSS company, Chicago, IL).

Results and discussion

As shown in Table S1 (supporting material online), all the 70 sequences of AIDS patients were allocated to known mtDNA haplogroup of East Asian (Kivisild et al., 2002; Kong et al., 2006), Southeast Asian (Hill et al., 2006, 2007; Peng et al., 2010) with a few lineages of unassigned M* and N* status. The southern of East Asian prevalent haplogroups, such as haplogroups B, M7, M9 and R9 (including F) (Hill et al., 2006, 2007) accounted 55.71% (39/70) of all the invested AIDS patients, which was much higher than that of northern of East Asian prevalent haplogroups A, C, D, G and N9 (Kong et al., 2003). In detail, haplogroups B, F and M7 accounted more than 51.43% of the maternal components, and pattern for haplogroups prevalent in northern East Asia group (including A, C, D, and G) was similar with that of Tibetan groups, which may reflect rather recent genetic contributions from northern of East Asia. Thus, our results indicated that the AIDS group was admixed with southern and northern of eastern Asian groups.

To get more insights into the genetic affinities between AIDS group and 11 ethnic/Han Chinese groups from the adjacent regions, the principal component map was constructed (Figure S1, supporting material online) based on the first two principal components (accounting for 65.99% of the total variations), which indicated that AIDS group has the similarity of matrilineal genetic structures with 3 Han Chinese groups, however, which was separated with other ethnic groups from the similar geographic regions (Figure S1). Our PC map further supported that the AIDS group was the recent admixture history with components from southern and northern of East Asian populations, and the former accounted much higher ratios, this pattern was different from that of ethnic groups, and the current mtDNA variation may reveal their ethnohistory to some extent.

Previous works have revealed that many human diseases were associated with mtDNA haplogroup (Achilli et al., 2011; Fernandez-Caggiano et al., 2013; Hendrickson et al., 2008). More evidences have suggested that the essential role of mitochondria in the innate immunity response to infection (Arnoult et al., 2011; McWhirter et al., 2005), and AIDS in humans was an infection caused by HIV, thus, we hypothesized that mtDNA haplogroup may confer a genetic susceptibility to AIDS. Three Han Chinese were pooled as control group, because these populations have the similarity of matrilineal genetic structures with AIDS group. The χ2 test was performed for mtDNA haplogroups with frequency higher than 5% in the AIDS and control groups. As shown in Table 1, haplogroup A (p = 0.048, OR = 3.006, 95% CI = 1.109–8.145) showed significant difference between AIDS and Han Chinese groups (p < 0.05), which indicated that individuals of haplogroup A may has an increasing risk of AIDS. To test whether any mtDNA variation(s) may contribute to the increasing risk of AIDS, the χ2 test were performed for 120 mtDNA mutations of hypervariable region HVSI (HVSI, spanning nucleotide position 16,024–16,365, was covered by both AIDS and control groups) for AIDS patients and control samples with frequency higher than 5%. As shown in Table 2, our results indicated that mtDNA variations 16,209 (p = 0.046, OR = 2.607, 95% CI = 0.988–6.876) and 16,319 (p = 0.009, OR = 2.965, 95% CI = 1.472–5.938) were associated with mtDNA haplogroup (Achilli et al., 2011; Fernandez-Caggiano et al., 2013; Hendrickson et al., 2008).

Table 1. Haplogroup frequencies and Pearson’s chi-square test in AIDS and control groups from southwest China.

| Mutation | AIDS | Control | χ² | p value | OR | 95% CI |
|----------|------|---------|----|---------|----|--------|
| 16,092   | 1    | 9       | 1.044 | 0.307 | 0.256 | 0.032–2.060 |
| 16,129   | 17   | 47      | 0.342 | 0.558 | 0.826 | 0.435–1.569 |
| 16,140   | 4    | 14      | 0.485 | 0.486 | 0.667 | 0.212–2.101 |
| 16,162   | 3    | 9       | 0.000 | 0.985 | 0.791 | 0.208–3.013 |
| 16,172   | 6    | 23      | 1.210 | 0.271 | 0.591 | 0.230–1.521 |
| 16,182C  | 7    | 23      | 0.611 | 0.434 | 0.700 | 0.286–1.716 |
| 16,183C  | 17   | 45      | 0.160 | 0.689 | 0.877 | 0.460–1.670 |
| 16,189   | 26   | 56      | 0.318 | 0.573 | 1.182 | 0.661–2.114 |
| 16,192   | 4    | 14      | 0.485 | 0.486 | 0.667 | 0.212–2.101 |
| 16,217   | 8    | 22      | 0.125 | 0.724 | 0.856 | 0.362–2.028 |
| 16,223   | 43   | 92      | 0.196 | 0.658 | 1.138 | 0.643–2.133 |
| 16,227   | 2    | 3       | 0.445 | 0.505 | 2.463 | 0.485–12.510 |
| 16,249   | 5    | 4       | 1.910 | 0.167 | 3.154 | 0.821–12.120 |
| 16,261   | 9    | 14      | 1.158 | 0.282 | 1.623 | 0.668–3.946 |
| 16,274   | 1    | 11      | 1.741 | 0.187 | 0.207 | 0.076–1.634 |
| 16,278   | 4    | 7       | 0.032 | 0.858 | 1.394 | 0.395–4.921 |
| 16,290   | 9    | 9       | 3.976 | 0.046 | 2.607 | 0.988–8.676 |
| 16,297   | 10   | 16      | 1.151 | 0.283 | 1.583 | 0.680–3.685 |
| 16,298   | 7    | 14      | 0.171 | 0.680 | 1.222 | 0.471–3.171 |
| 16,304   | 11   | 31      | 0.255 | 0.614 | 1.824 | 0.388–3.749 |
| 16,311   | 11   | 16      | 1.883 | 0.170 | 1.771 | 0.777–4.039 |
| 16,319   | 13   | 12      | 6.865 | 0.000 | 2.965 | 1.278–6.876 |
| 16,327   | 5    | 6       | 0.734 | 0.391 | 2.077 | 0.612–7.043 |
| 16,355   | 4    | 3       | 1.472 | 0.225 | 3.333 | 0.726–15.300 |
| 16,362   | 26   | 54      | 0.554 | 0.457 | 1.247 | 0.696–2.235 |

*p value was calculated by Pearson’s chi-square test; Fisher’s exact test was used when haplogroup occurred in less than five individuals.
Table 2. mtDNA variations and Pearson’s chi-square test in AIDS group and controls from Southwest China.

| Haplogroups | AIDS       | Control  | \( \chi^2 \) | p value* | OR          | 95% CI          |
|-------------|------------|----------|-------------|----------|-------------|-----------------|
| D           | 2          | 13       | 1.189       | 0.275   | 0.357       | 0.079–1.627    |
| M/*N/*      | 2          | 16       | 3.036       | 0.081   | 0.285       | 0.064–1.274    |
| B5          | 4          | 13       | 0.059       | 0.808   | 0.737       | 0.232–2.342    |
| M8*         | 4          | 12       | 0.007       | 0.933   | 0.803       | 0.250–2.581    |
| C           | 4          | 6        | 0.179       | 0.672   | 1.667       | 0.456–6.097    |
| G           | 4          | 9        | 0.000       | 1.000   | 1.091       | 0.325–3.668    |
| G2          | 4          | 6        | 0.179       | 0.672   | 1.667       | 0.456–6.097    |
| D4          | 5          | 10       | 0.007       | 0.933   | 1.238       | 0.408–3.764    |
| F1          | 7          | 22       | 0.385       | 0.535   | 0.753       | 0.306–1.851    |
| B4          | 8          | 20       | 0.003       | 0.953   | 0.974       | 0.407–2.329    |
| A*          | 9          | 8        | 3.897       | 0.048   | 3.006       | 1.109–8.145    |
| D*          | 10         | 31       | 0.520       | 0.471   | 0.753       | 0.347–1.633    |
| M7b         | 10         | 7        | 0.032       | 1.510   | 0.753       | 0.347–1.633    |
| B*          | 12         | 35       | 0.010       | 0.946   | 0.964       | 0.460–1.981    |
| F*          | 12         | 25       | 0.243       | 0.622   | 1.208       | 0.569–2.564    |
| M7*         | 12         | 21       | 0.994       | 0.319   | 1.478       | 0.683–3.190    |

Haplogroup occurred in less than four individuals in the entire case or control population and those unassigned M*, N* and R* mtDNAs were pooled together. We presented these nested haplogroups according to their phylogenetic status, e.g. B contains B4, B5, B6 and B*.

* p value was calculated by Pearson’s chi-square test; Fisher’s exact test was used when haplogroup occurred in less than five individuals.

CI = 1.278–6.876 showed statistically significant difference between AIDS and Han Chinese (p<0.05), which implied that the individual with the two mutations may have much higher susceptibility to AIDS in southwest Chinese groups. Interestingly, the mtDNA mutations 16,209 and 16,319 were two diagnostic mutations of mtDNA haplogroup A. In other world, both the statistic analyses based on mtDNA haplogroup and mtDNA control region sequence variations were pointed that individuals of haplogroup A may confer a genetic susceptibility to AIDS from Southwest China. And the mutations of haplogroup A may alter mitochondrial genome transcription (Dement et al., 2007), affect mtDNA replication and lead to electron transport chain alteration, and which would result in the release of highly ROS (Gille & Joenje, 1992) and the increasing of the ROS may cause the susceptible of HIV.

In summary, by studying the mtDNA of 70 AIDS patients from southwest China and that of 480 individuals of 11 ethnic/Han Chinese groups, our results indicated that AIDS group may represent the recent genetic admixture results of southern and northern of East Asian maternal components, which were represent the recent genetic admixture results of southern and southwest China and that of 480 individuals of 11 ethnic/Han Chinese groups, our results indicated that AIDS group may represent the recent genetic admixture results of southern and northern of East Asian maternal components, which were represent the recent genetic admixture results of southern and southwest China.

References

Achilli A, Olivieri A, Pala M, Hooshiar Kashani B, Carossa V, Perego UA, Gandini F, et al. (2011). Mitochondrial DNA backgrounds might modulate diabetes complications rather than T2DM as a whole. PLoS ONE 6:e21029.

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. (1999). Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23:147.

Arnout D, Soares F, Tattoli I, Girardin SE. (2011). Mitochondria in innate immunity. EMBO Rep 12:901–10.

Beal MF. (1996). Mitochondria, free radicals, and neurodegeneration. Curr Opin Neurobiol 6:661–6.

Cann RL, Brown WM, Wilson AC. (1984). Polymorphic sites and the mechanism of evolution in human mitochondrial-DNA. Genetics 106: 479–99.

Cann RL, Stoneking M, Wilson AC. (1987). Mitochondrial DNA and human evolution. Nature 325:31–6.

Dement GA, Maloney SC, Reeves R. (2007). Nuclear HMG1 nonhistone chromatin proteins directly influence mitochondrial transcription, maintenance, and function. Exp Cell Res 313:77–87.

Dimauro S, Schon EA. (2001). Mitochondrial DNA mutations in human disease. Am J Med Genet 106:18–26.

Feng GF, Zhang J, Feng LM, Shen NX, Li LJ, Zhu YM. (2013). Mitochondrial DNA haplogroup associated with sperm motility in the Han population. Asian J Androl 15:630–3.

Fernandez-Caggiano M, Baralobre-Barreiro J, Rego-Perez I, Crespo-Leiro MG, Paniagua MJ, Grille Z, Blanco FJ, Domenech N. (2015). Mitochondrial DNA haplogroup H as a risk factor for idiopathic dilated cardiomyopathy in Spanish population. Mitochondrion 13:263–8.

Gille JJ, Joenje H. (1992). Cell culture models for oxidative stress: Superoxide and hydrogen peroxide versus normobaric hypoxia. Mutat Res 275:405–14.

Gomez-Duran A, Pacheu-Grau D, Lopez-Gallardo E, Diez-Sanchez C, Montoya J, Lopez-Perez MJ, Ruiz-Pesini E. (2010). Unmasking the causes of multifactorial disorders: OXPHOS differences between mitochondrial haplogroups. Hum Mol Genet 19:3343–53.

Hendrickson SL, Hutcheson HB, Ruiz-Pesini E, Poole JC, Lautenberger J, Sezgin E, Kingsley L, Goedert JJ, et al. (2008). Mitochondrial DNA haplogroups influence AIDS progression. AIDS 22:2429–39.

Hill C, Soares P, Mormina M, Macaulay V, Clarke D, Blumbach PB, Vizuete-Forster M, et al. (2007). A mitochondrial stratigraphy for island Southeast Asia. Am J Hum Genet 80:29–43.

Hill C, Soares P, Mormina M, Macaulay V, Meehan W, Blackburn J, Clarke D, et al. (2006). Phylogeography and ethno genesis of aboriginal Southeast Asians. Mol Biol Evol 23:2480–91.

Hu SP, Du JP, Li DR, Yao YG. (2014). Mitochondrial DNA haplogroup confers genetic susceptibility to nasopharyngeal carcinoma in Chaoshanese from Guangdong, China. PLoS ONE 9:e87795.

Kivisild T, Volk H, Parik J, Wang Y, Papiha SS, Bandelt HJ, Vilkus R. (2002). The emerging limbs and twigs of the East Asian mtDNA tree. Mol Biol Evol 19:173–51.

Kong QP, Bandelt HJ, Sun C, Yao YG, Salas A, Achilli A, Wang CY, et al. (2006). Updating the East Asian mtDNA phylogeny: A prerequisite for the identification of pathogenic mutations. Hum Mol Genet 15:2076–86.

Kong QP, Yao YG, Liu M, Shen SP, Chen C, Zhu CL, Palanichamy MG, Zhang YP. (2003). Mitochondrial DNA sequence polymorphisms of five ethnic populations from northern China. Hum Genet 113: 391–405.

Lightowlers RN, Chinnery PF, Turnbull DM, Howell N. (1997). Mammalian mitochondrial genetics: Heredity, heteroplasmy and disease. Trends Genet 13:450–5.

Lorente L, Iceta R, Mart NMM, Lpez-Gallardo E, Sol-Viol NJ, Blanquer KL, Bardeta L, et al. (2013). Severe septic patients with mitochondrial DNA haplogroup JT show higher survival rates: A prospective, multicenter, observational study. PLoS ONE 8:e73320.

Martin AM, Hammond E, Nolan D, Pace C, Den Boer M, Taylor L, Moore H, et al. (2003). Accumulation of mitochondrial DNA mutations in human immunodeficiency virus-infected patients treated of Academician Jie-Shou Li (No: LJS-201302). Authors declare that they do not have any financial, consulting, and personal relationships with other people or organizations that could influence the author’s work.
with nucleoside-analogue reverse-transcriptase inhibitors. Am J Hum Genet 72:549–60.

Mcwhirter SM, Tenoever BR, Maniatis T. (2005). Connecting mitochondria and innate immunity. Cell 122:645–7.

Mocroft A, Ledergerber B, Katlama C, Kirk O, Reiss P, D’arminio Monforte A, Knysz B, et al. (2003). Decline in the AIDS and death rates in the EuroSIDA study: An observational study. Lancet 362:22–9.

O’Brien SJ, Nelson GW. (2004). Human genes that limit AIDS. Nat Genet 36:565–74.

Palanichamy MG, Sun C, Agrawal S, Bandelt HJ, Kong QP, Khan F, Wang CY, et al. (2004). Phylogeny of mitochondrial DNA macro-haplogroup N in India, based on complete sequencing: Implications for the peopling of South Asia. Am J Hum Genet 75:966–78.

Peng MS, Quang HH, Dang KP, Trieu AV, Wang HW, Yao YG, Kong QP, Zhang YP. (2010). Tracing the Austronesian footprint in Mainland Southeast Asia: A perspective from mitochondrial DNA. Mol Biol Evol 27:2417–30.

Richards M, Macaulay V, Hickey E, Vega E, Sykes B, Guida V, Rengo C, et al. (2000). Tracing European founder lineages in the near Eastern mtDNA pool. Am J Hum Genet 67:1251–76.

Shioda T, Nakayama EE. (2006). Human genetic polymorphisms affecting HIV-1 diseases. Int J Hematol 84:12–17.

Soares P, Alshamali F, Pereira JB, Fernandes V, Silva NM, Afonso C, Costa MD, et al. (2012). The Expansion of mtDNA Haplogroup L3 within and out of Africa. Mol Biol Evol 29:915–27.

Sun C, Kong QP, Palanichamy MG, Agrawal S, Bandelt HJ, Yao YG, Khan F, et al. (2006). The dazzling array of basal branches in the mtDNA macrohaplogroup M from India as inferred from complete genomes. Mol Biol Evol 23:683–90.

van Oven M, Kayser M. (2009). Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat 30: e386–94.

Wallace DC. (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: A dawn for evolutionary medicine. Annu Rev Genet 39:359–407.

Wallace DC, Brown MD, Lott MT. (1999). Mitochondrial DNA variation in human evolution and disease. Gene 238:211–30.

Wang HW, Li YC, Sun F, Zhao M, Mitra B, Chaudhuri TK, Regmi P, et al. (2012). Revisiting the role of the Himalayas in peopling Nepal: Insights from mitochondrial genomes. J Hum Genet 57:228–34.

Supplementary material available online