Int J Clin Exp Pathol 2016;9(6):6015-6022
www.ijcep.com /ISSN:1936-2625/IJCEP0022534

Original Article

A sequence analysis of mitochondrial DNA hypervariable segment I among Han ethnic patients with HIV/AIDS in Guizhou, China

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Received December 22, 2015; Accepted March 7, 2016; Epub June 1, 2016; Published June 15, 2016

Abstract: This study aims to investigate the gene polymorphisms in mitochondrial DNA (mtDNA) hypervariable segment I (HVS I) among Han ethnic patients with HIV/AIDS in Guizhou, China, and to explore the effects of mitochondrial gene polymorphisms on HIV infection. Blood samples were collected from 110 Han ethnic patients with HIV/AIDS and 110 healthy Han ethnic newborn babies and anti-coagulated by EDTA. Then, mitochondrial DNA was extracted, and the nucleic acid fragments of 643 bp containing non-encoding HVS I was amplified by PCR. After the purification of PCR products, sequencing was performed, and the sequencing results were compared with revised Cambridge Reference Sequence to analyze mutant sites and mutation frequency in each site. Base mutation frequency distribution in single nucleotide site in mtDNA was compared between two groups. Results: In the nucleic acid fragment of 341 bp in the D-Loop HVS I (nucleotide sites, 16,024~16,365) among those patients with HIV/AIDS and the healthy controls, 380 base mutations occurred in the case group, with an average base mutation rate of 1.01%, and 241 base mutations in the control group, with an average base mutation rate of 0.64%, indicating that base mutation rate in the case group was obviously higher than that in the control group. These results suggest that mtDNA gene mutations, namely quality changes in mitochondria, exist in HIV/AIDS patients; however, whether HIV infection leads to mtDNA gene mutations in patients or the population with mtDNA gene mutations is susceptible to HIV infection requires further study to confirm.

Keywords: Mitochondrial DNA hypervariable segment I, Han ethnic, HIV/AIDS, sequence analysis

Introduction

Acquired immune deficiency syndrome (AIDS) is a chronic infectious disease caused by human immunodeficiency virus (HIV) infection. Shortly after HIV entering the human body, it destroys the immune system, which results in patients being susceptible to various opportunistic infections even secondary tumors, etc., leading to death. Clinical application of highly active antiretroviral therapy (HAART) extends survival time of patients and improves the life quality. However, HAART cannot completely eliminate HIV and patients need to receive long-term or lifelong medication, although the underlying treatment limits the virus replication in many patients. Currently, the disease is widely spreading in Africa, Asia, Latin America, etc., resulting in a worldwide epidemic, which seriously affects the development of society and economy as well as threats the health of human being [1-4]. During the 30 years from 1985 when the first case of HIV/AIDS was detected in China, the epidemic situation has covered all provinces of the country [3, 4].

Mitochondria are the unique organelle with genetic information except the nuclei of eukaryotes, and mainly functions to concert the energy produced by oxidative phosphorylation in the body to adenosine triphosphate (ATP), acting as a place for storing and supplying energy substances in vivo. Mitochondrial DNA (mtDNA) is the unique genetic material except nuclei; Study

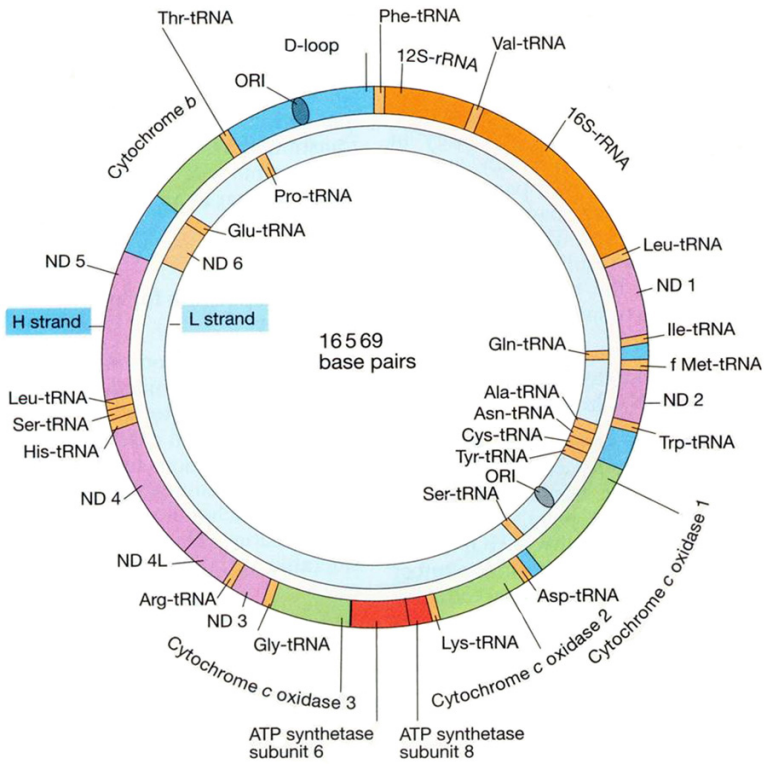

Figure 1. Human mitochondrial gene structure.

has finished the sequencing of human mtDNA, and grouped mtDNA into encoding region and non-encoding region (control region) [5]. mtDNA is a closed circular double-stranded molecule containing 16,569 base pairs (bp), with one heavy chain (H chain) located in outer ring and one light chain (L chain) in inner ring, both of which have encoding function (**Figure 1**) (http://tupian.baike.com/a1_27_82_01300536071309139023828680435_jpg.html&prd=so_tupian). A total of 37 structure genes are encoded in the encoding region of mtDNA; among them, 13 structure genes encode polypeptides which constitute oxidative phosphorylation enzymes and partial subunits of respiratory chain complex, while other 24 structure genes encode 2 ribosomal RNAs (rRNA: 12S rRNA and 16S rRNA)and 22 transfer RNAs (tRNA) [6, 7]. Non-encoding region, also called control region or hypervariable region, consists of base sequences with a size of 1,122 bp between 1-576 bp and 16,024-16,569 bp, forming a starting point of replication, a D-loop region and 2 starting points of transcription, and is a region with the rapidest evolutionary rate of mtDNA and the highest frequency of polymorphism. D-loop region, also called D-ring region or displacement loop region, has small selective pressure, encodes no protein and is a mutational hot spot during evolutionary process. 3 hypervariable regions near the starting point of replication in the mtDNA control region are as follows: hypervariable segment I (HVS I), hypervariable segment II (HVS II), and hypervariable segment III (HVS III), consisting of base sequences between 16,024-16,365 bp, 73-340 bp, and 438-576 bp, respectively. Except for D-loop region, genes of the other regions in mtDNA contain no intron and rarely present recombination; therefore, mutations in mtDNA always affect the vital functional regions in genome. mtDNA, free in the matrix, is lacking to the effective protection from histone and has an imperfect self-repair system after damage, leading to its susceptibility to mutations and obvious realization of polymorphisms [8, 9]. The present study, through sequencing in mtDNA HVS I among HIV/AIDS patients, attempts to investigate the gene polymorphisms in mtDNA HVS I, to explore the effects of mitochondrial gene polymorphisms on HIV infection, to make clear whether predisposing genes exist in AIDS, and to clarify the pathogenesis of AIDS from the aspect of molecular biology, and thereby providing novel evidence for the more effective prevention and treatment of AIDS.

Materials and methods

All experimental operations of the present study were conducted in the central laboratory of Zunyi Medical College and the Center for Disease Control and Prevention in Zunyi. Subjects were selected according to informed consent, ethical principles and security principles: 1. case group, blood samples of 110 Han ethnic patients with HIV/AIDS were provided by the Center for Disease Control and Prevention in Zunyi, Guizhou; inclusion criteria were as follows: HIV antibody-positive patients were con-

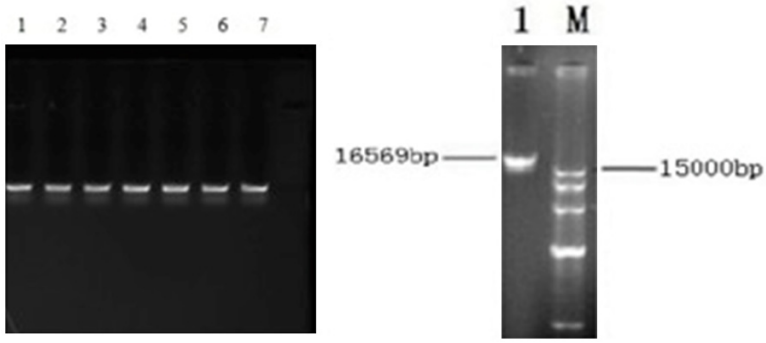

Figure 2. Verification of 7 mtDNA templates selected randomly by electrophoresis, M is DNA Marker DL15000, band 1 is an mtDNA template selected randomly, with a size of 16,569 bp.

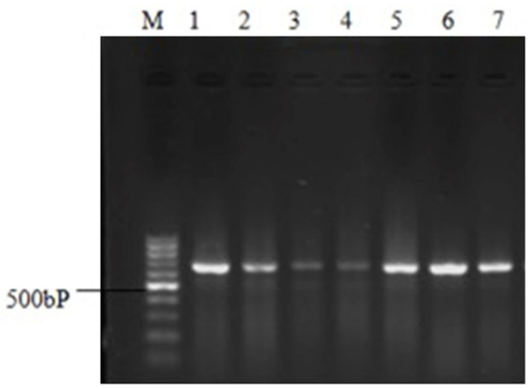

Figure 3. M is DNA Marker DL1000, bands 1, 2, 3, 4, 5, 6 and 7 are mtDNA bands selected randomly with a size of 643 bp.

firmed by the Center for Disease Control and Prevention in Guizhou using western blot (WB) method, and age was over 18 years old; 2. control group, blood samples of 110 healthy newborn babies in Guizhou were provided by the Affiliated Hospital of Zunyi Medical College and the Affiliated Hospital of Guiyang College of Traditional Chinese Medicine. Blood samples (5 ml) were collected from each subject in the case group and the control group, anti-coagulated by EDTA, and stored at -80°C. All above subjects were from the Chinese Han population in Guizhou. Whole blood genome was extracted by using the Ezup genomic DNA extraction column extraction kit (Sangon Biotech Co., Ltd., Shanghai, China). Primers were designed according to revised Cambridge Reference Sequence (rCRS) by Shanghai Sangon Biotech Co., Ltd. The sequence between 15,879~15,898 in L chain was selected as upstream primer (R15879 5'-AATGGGCCTGTCCTTGTAGT-3'), and the sequence between 16,553~16,530 in H chain as downstream primer (F16553 5'-AATTTGGGGAACGTGTGGGCTATTT-3'); base number relative to the 5'-terminal region was 643 bp. The PCR amplification in HVS I was performed by using a PCR amplification kit (Sangon Biotech Co., Ltd., Shanghai, China). PCR amplification products of the target fragments were verified by using 1% agarose gel electrophoresis, and the confirmed PCR products were purified by a PCR purification kit (Sangon Biotech Co., Ltd., Shanghai, China). The purified PCR products were sent to Shanghai Sangon Biotech Co., Ltd. for bidirectional sequencing, and the obtained DNA sequences were initially compared in http://www.ncbi.nlm.nih.gov/pubmed for sequence source identification. Sequence diagrams were read with Chromas software and compared with derived sequences to revise nucleotide sequences. The forward and reverse sequences of the same nucleotide sequence were revised applying DNAStar software, and compared with rCRS after assembly. The obtained information on nucleotide variation was compared with the database in www.mitomap.org, and the new-found mutation sites were further confirmed. The average base mutation rate = the total number of mutation bases/the total number of detected bases × 100%.

Results

The integrity of the whole blood genome extracted with the Ezup genomic DNA extraction column extraction kit was verified by randomly selecting 7 mtDNA templates (**Figure 2**).

Electrophoresis results for the PCR amplification products of the target fragments in mtDNA D. Loop HVS I demonstrated that all amplified target nucleic acid fragments presented clear bands with a size of 643 bp (**Figure 3**).

Sequencing results and bidirectional sequence diagrams for the PCR amplification products of the target fragments in mtDNA D. Loop HVS I showed that most PCR amplification products presented relatively clear base peaks by purifi-

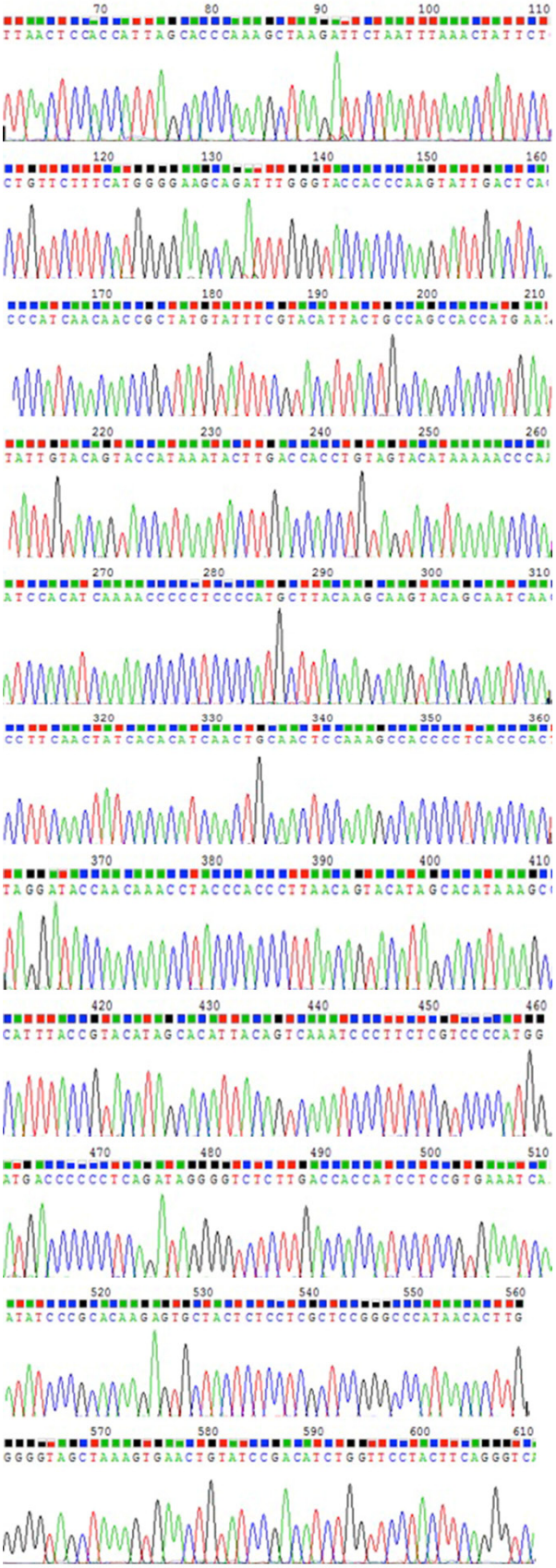

Figure 4. The plot of PCR product containing mtDNA D. Loop HVS I by single direction sequencing.

cation and sequencing. Comparison in http://blast.ncbi.nlm.nih.gov/ revealed that the homology with human mtDNA reached more than 98% with a highest similarity, confirming that the amplified PCR products were in human mtDNA HVS I. The present study conducted bidirectional sequencing in the PCR products of HVS I of all samples, and the original peak chart for sequencing and the sequence map after splicing of one sample were selected randomly, as seen in the following figure (**Figures 4**, **5**).

Sequencing in mtDNA D. Loop HVS I for variation situation and site analysis was shown in **Tables 1**, **2**. In the detected nucleic acid fragment of 341 bp in the D. Loop HVS I (nucleotide sites, 16,024~16,365), 380 base variations were detected, with an average base variation rate of 1.01% (380/[(16,365 - 16,024) × 110] × 100%); while 241 base variations were observed in the control group, with an average base variation rate of 0.64% (241/[(16,365 - 16,024) × 110] × 100%). Comparison revealed an evidently higher base variation rate in the case group than the control group, showing significantly statistical significance ($P<0.01$). At the nucleotide site of 16,223, 53 cases with C-T variation were found in the case group, with an incidence of 48.2% (53/110), and 43 cases with C-T variation in the control group, with an incidence of 39.1% (43/110), which implied that the variation frequency in the case group was higher than that in the control group, but showing no obviously statistical significance ($P>0.05$). At the nucleotide site of 16,362, there were 36 cases with T-C variation in the case group, with an incidence of 32.7% (36/110), and 25 cases with T-C variation in the control group, with an incidence of 22.7% (25/110), demonstrating that the variation frequency in the case group was higher than that in the control group, without obviously difference ($P>0.05$). Statistics (the χ^2 test was conducted by using SPSS17.0 software) was performed in the base variation frequency of the nucleotide sites (16,024~16,365) of the samples in both case group and control group, which revealed increases in the base variation frequency of all sites, but no apparent statistical difference ($P>0.05$). The results were seen in **Table 1**.

Figure 5. The spliced sequence of PCR product containing mtDNA D. Loop HVS I by bidirectional DNA sequencing.

In the detected nucleic acid fragment of 341 bp in the D. Loop HVS I (nucleotide sites, 16,024~16,365) in the case group, single nucleotide polymorphism locus and mutation sites were seen in **Table 2**.

Discussion

Over the past 30 years, scholars have done a lot of researches on the etiology, pathogenesis, route of transmission, diagnosis, treatment and prevention of AIDS and the relationship between HIV and organism; moreover, some progress has been made. The current use of HAART facilitates the significant decrease of morbidity and mortality rates of AIDS, and rebuilds the immune functions of patients, whose survival time and life quality have been improved correspondingly. HIV infection in developed countries has been gradually becoming a controllable chronic disease, but HAART treatment is unable to restrict the late-phase viral replication while cannot entirely clear the virus in patients [10, 11]. Therefore, there is still a long hard way to go in the prevention and control of AIDS, and considerable in-depth researches are needed as well.

In recent years, studies have found that the pace of the progression from HIV-infection to AIDS varies possibly depending on individual DNA differences; in other words, the genotype of mtDNA may be associated with the pace of AIDS development. For instance, among the white population, the pace of AIDS progression in the patients with specific mutations of haplotype groups J and U5a was two times faster than those without the two haplotype groups; while in those with a specific mutation of haplotype group H3, the disease progression was 2 times slower than those without this haplotype group. Therefore, it is supposed that haplotype groups J and U5a can lead to the loss of mitochondrial energy, and immunocytes with less mitochondrial energy are more susceptible to HIV attack resulting in the death of immunocyte, which accelerates the progression of the disease [12]. These findings provide strong theoretical support for the study on the association between mtDNA polymorphisms and the susceptibility and development of AIDS.

Apart from the nucleus, human mtDNA is the only genetic material and characterized by strict maternal inheritance, lack of recombination, huge variation in groups, and high mutation rate, etc. [13, 14]. Mitochondria, free in the active oxygen environment of the matrix, are lacking to an effective damage/repair system and more prone to mutation damage than nuclear genes, and thereby influencing cellular functions and resulting in various diseases [15-17]. mtDNA is a closed circular double-stranded molecule with a full length of 16,569 bp, and grouped into encoding region and non-encoding region in which, D. loop region is a mutational hot spot, especially HVS I, with quite obvious polymorphisms; therefore, sequencing in HVS I can obtain a number of genetic information. The polymorphisms of mtDNA include heterogeneity, length polymorphisms, sequence polymorphisms and single nucleotide polymorphisms (SNPs), among which, SNPs are a kind of polymorphism that currently receive much attention, and is caused by single-base mutation. Mitochondria are the main organelle of energy generation in human cells. Either HIV infection itself or anti retroviral drugs may affect the mitochondrial function [18, 19]. The polymorphism study of mitochondrial genes favors the understanding of the relationship between drug efficacy and AIDS disease progression, which may offer support for the compatibility of clinical medication and improve therapy regimen.

In this study, through the PCR amplification and sequencing in the HVS I of the D. loop region of the blood samples from patients with HIV/AIDS, and the comparison analysis with rCRS, it was revealed that among the patients with HIV/

Table 1. Mutation frequency of SNP locus in mt DNA D. Loop HVS I

mt-SNP locus	Mutation type	Case group (n = 110)		Control group (n = 110)		χ^2	P[a]	F
16086	T/C	3	2.7%	0	0	2.987	0.084	
16092	T/C	7	6.4%	4	3.6%	0.861	0.353	
16093	T/C	6	10.7%	3	5.4%	1.980	0.159	
16111	C/T	2	1.8%	0	0	2.018	0.155	
16114	C/T	5	4.5%	4	3.6%	0.116	0.734	
16129	G/A	24	21.8%	19	17.3%	0.723	0.395	
16136	T/C	3	2.7%	0	0	2.987	0.084	
16140	T/C	2	1.8%	0	0	2.018	0.155	
16162	A/G	4	7.1%	1	1.8%	3.173	0.075	
16164	A/G	3	2.7%	0	0	2.987	0.084	
16172	T/C	13	11.8%	6	5.4%	2.283	0.093	
16174	C/T	1	0.9%	0	0			1.0
16175	A/G	2	1.8%	0	0	2.018	0.155	
16179	C/T	1	0.9%	0	0			1.0
16182	A/C	23	20.9%	16	14.5%	1.527	0.217	
16183	A/C	34	28.6%	25	22.7%	1.876	0.171	
16184	C/T	9	8.2%	8	7.3%	0.064	0.801	
16185	C/T	8	7.3%	5	4.5%	0.736	0.391	
16189	T/C	45	40.9%	35	31.8%	1.964	0.161	
16189	delT	1	0.9%	0	0			1.0
16192	C/T	1	0.9%	0	0			1.0
16223	C/T	53	48.2%	43	39.1%	1.848	0.174	
16234	C/T	3	2.7%	0	0	2.987	0.084	
16257	C/A	5	3.6%	3	2.7%	0.519	0.471	
16260	C/T	3	2.7%	0	0	2.987	0.084	
16261	C/T	3	2.7%	0	0	2.987	0.084	
16266	C/T	2	1.8%	0	0	2.018	0.155	
16274	G/A	3	2.7%	2	1.8%	0.205	0.651	
16287	C/T	1	0.9%	0	0			1.0
16290	C/T	3	2.7%	0	0	2.987	0.084	
16291	C/T	1	0.9%	0	0			1.0
16293	A/G	2	1.8%	0	0	2.018	0.155	
16297	T/C	12	10.9%	9	8.2%	0.474	0.491	
16298	T/C	14	12.7%	9	8.2%	1.214	0.27	
16300	A/G	2	1.8%	0	0	2.018	0.155	
16302	A/G	2	1.8%	0	0	2.018	0.155	
16304	T/C	15	12.5%	11	10%	0.698	0.404	
16309	A/G	2	1.8%	0	0	2.018	0.155	
16311	T/C	3	5.4%	0	0	2.987	0.084	
16316	A/G	2	1.8%	0	0	2.018	0.155	
16319	G/A	3	5.4%	0	0	2.987	0.084	
16320	C/T	9	8.2%	8	7.3%	0.074	0.786	
16324	T/C	3	2.7%	0	0	2.987	0.084	
16327	C/T	3	2.7%	0	0	2.987	0.084	
16355	C/T	7	6.4%	5	4.5%	0.353	0.553	
16362	T/C	36	32.7%	25	22.7%	2.745	0.098	

AIDS, in the detected nucleic acid fragment of 341 bp in the D. Loop HVS I (nucleotide sites, 16,024~16,365), 380 base variations were detected, with an average base variation rate of 1.01% (380/[(16,365 - 16,024) × 110] × 100%); while 241 base variations were observed in the control group, with an average base variation rate of 0.64% (241/[(16,365 - 16,024) × 110] × 100%), revealing an evidently higher base variation rate in the case group than the control group, with significantly statistical significance ($P<0.01$); at the nucleotide site of 16,223, 53 cases with C-T variation were found in the case group, with an incidence of 48.2% (53/110), and 43 cases with C-T variation in the control group, with an incidence of 39.1% (43/110), which implied that the variation frequency in the case group was higher than that in the control group, but showing no obviously statistical significance ($P>0.05$); at the nucleotide site of 16,362, there were 36 cases with T-C variation in the case group, with an incidence of 32.7% (36/110), and 25 cases with T-C variation in the control group, with an incidence of 22.7% (25/110), demonstrating that the variation frequency in the case group was higher than that in the control group, without obviously difference ($P>0.05$).

Through investigating the peripheral blood mononuclear cells of 25 HIV-infected patients who received no antiretroviral drug therapy and 25 normal persons, data discovered that mtDNA content in the HIV-infected patients declined dramatically [20], moreover, mtDNA content in the immune-activated CD4+ T and CD8+ T cells demonstrated a more evident decrease compared to non-activated cells, the content of respiratory chain complexes III and IV reduced, glycerol 3-phosphate dehydrogenase was lowered, activities of mitochondrial respiratory chain complex III were inhibited, and peroxidized lipid membrane increased, the conclusion drew from which is that: HI infection is associated with the oxidative dam-

Table 2. Single nucleotide polymorphism locus and mutation sites

	SNP locus	Gene pool	Case group	Control group
Mutationsites	16086	T	C	T
	16111	C	T	C
	16136	T	C	T
	16140	T	C	T
	16164	A	G	A
	16174	C	T	C
	16175	A	G	A
	16179	C	T	C
	16192	C	T	C
	16234	C	T	C
	16260	C	T	C
	16261	C	T	C
	16266	C	T	C
	16287	C	T	C
	16291	C	T	C
	16293	A	G	A
	16300	A	G	A
	16302	A	G	A
	16309	A	G	A
	16311	T	C	T
	16316	A	G	A
	16319	G	A	G
	16324	T	C	T
	16327	C	T	C
SNP locus	16092	T	C	C
	16093	T	C	C
	16114	C	T	T
	16129	G	G	G
	16162	A	G	G
	16172	T	C	C
	16182	A	C	C
	16183	A	C	C
	16184	C	T	T
	16185	C	T	T
	16189	T	C	C
	16223	C	T	T
	16257	C	A	A
	16274	G	A	A
	16290	C	T	T
	16297	T	C	C
	16298	T	C	C
	16304	T	C	C
	16320	C	T	T
	16355	C	T	T
	16362	T	C	C

age of peripheral blood mononuclear cells and decreased mtDNA content, and HIV infection may lead to the reduction of mtDNA content in the organism, especially in immune-activated cells. After a 5-year follow-up research in 36 HIV-1-infected patients who underwent no antiretroviral drug therapy, it has been demonstrated that mtDNA content declined evidently after a 5-year seroconversion, contributing to the conclusion that HIV-1 infection influences mtDNA content, especially the mtDNA content in immune-activated cells [21]. These research results all implicated that HIV infection could affect cellular mtDNA content, which is the most significant in the immune-activated cells.

Our experimental findings demonstrate that: in the nucleic acid fragment of 341 bp that belongs to the D. Loop HVS I (nucleotide sites, 16,024~16,365) in the detected HIV/AIDS patients, the occurrence of base mutation and its mutation frequency were significantly higher than those in the control group; gene mutations in mtDNA, namely quality changes in mitochondria, existed in patients with HIV/AIDS. Whether HIV infection leads to mtDNA gene mutations in patients or the population with mtDNA gene mutations is susceptible to HIV infection requires further study to confirm.

Acknowledgements

This study was supported by stadholder funding of Guizhou Province (funding number: 040-109) and the funding title is “effect of mtDNA polymorphism on immune reconstitution of AIDS intervened by HAART therapy”.

Disclosure of conflict of interest

None.

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