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The role of mitochondrial DNA mutations in a Han Chinese population on sepsis pathogenesis

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Abstract. – OBJECTIVE: Individual susceptibility to sepsis has received increasing attention in recent years, and the study of genetic variations has become a hotspot regarding sepsis pathogenesis. We, therefore, investigated the association between mitochondrial genotype and sepsis susceptibility.

PATIENTS AND METHODS: One hundred patients admitted with sepsis and registered by five intensive care units (ICUs) in the People's Liberation Army Hospital and the Beijing Aerospace Center Hospital between January 2015 and January 2016 were enrolled as a case group, and 100 healthy persons were recruited as a control group. Patients' general information was obtained, and clinical evaluations and mitochondrial sequence screening were performed.

RESULTS: A total of 718 single nucleotide polymorphisms (SNPs) were detected in 708 loci in 100 patients. There were 1754 mutations in 456 loci in the coding region and 567 mutations were found in the RNA region. A total of 34 loci (from 40 cases) were novel mutations. A10398G (52.52%), C5178A (24.24%), C150T (17.17%), G3010A (17.17%), and T16189C (16.16%) were the most frequently observed conserved non-synonymous mutations that were differently expressed between the case and control groups ($p<0.05$). A5863T and C3270 deletion mutations were located on the genes encoding tRNATyr and tRNALeu, respectively. Small changes in the tRNA gene were likely to result in protein level changes.

CONCLUSIONS: We suggest that mitochondrial SNPs may be associated with the pathogenesis of sepsis.

Key Words:
Mitochondrial DNA, Sepsis, SNPs.

Introduction

Sepsis is a clinical syndrome, which is caused by an infectious agent causing excessive and uncontrolled systemic inflammation leading to multiple organ dysfunction or even failure. With the development of modern medicine, especially the progress in microbiology and immunology, studies on the pathogenic mechanisms of sepsis are constantly increasing in depth. The emergence of antibiotics and organ support technology play important beneficial roles for the treatment of sepsis[1]. Nevertheless, sepsis morbidity and mortality rates remain as high as 20-30%[2,3]. The clinical presentation of sepsis patients is very diverse. Even with the same infection, the occurrence, development, and prognosis of the disease differ among patients. An increasing number of scholars have begun to realize that the pathological changes associated with sepsis have individual differences[4] and that different genetic backgrounds are one of the most important factors underlying these individual differences among patients[5]. There is little research on the role of mitochondrial genetics in the development of sepsis. The aim of this study was to identify the association between mitochondrial mutations and genetic susceptibility to sepsis.

Patients and Methods

Patients

One hundred patients admitted with sepsis and registered by five intensive care units (ICUs) in the People's Liberation Army Hospital and the Beijing Aerospace Center Hospital between January 2015 and January 2016 were enrolled as a case group, and 100 healthy persons were recruited as a control group. Sepsis diagnoses were defined according to the sepsis-3 diagnostic criteria[6]. Exclusion criteria: pregnant women, patients with active malignant tumors, undergoing autoimmune disease hormone therapy, and chemotherapy patients. HIV-infected patients were excluded. All subjects provided written informed consent,

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and the study was approved by the Ethical Committee of the People's Liberation Army Hospital.

Patient General Information

The age and gender of all subjects were recorded.

Mitochondrial Gene Analysis

Whole blood mitochondrial DNA was extracted using a reagent kit (Promega Wizard, A1120, Madison, WI, USA). Primers were designed to amplify all 24 DNA fragments in mitochondria. PCR products were purified using the QIAEXII purification reagent kit (Qiagen, Hilden, Germany). Sequencing and analysis were directly performed using the Applied Biosystems ABI3700DNA automatic sequencing instrument (Foster City, CA, USA). Comparison and analysis of DNA sequencing results and corresponding protein sequences was performed using the SeqWeb program GAP (GCG). Comparison of homology was performed using BLAST from the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA). All sequencing results were compared to the 2013 edition of the Cambridge Reference Sequence (comparison source: MitoMap (http://www.mitomap.org).

Statistical Analysis

All statistical analyses were performed using SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA). All quantitative data were presented as the mean ± standard deviation and analyzed using the t test or the Wilcoxon rank sum test. Qualitative data are described using the number of cases and percentages and analyzed using the χ^2 test. $p<0.05$ was considered statistically significant.

Results

There was no significant difference in gender and age between the two groups ($p>0.05$) (Table I). The two groups were generally unbiased. A total of 3.179 point mutations were detected in 72 of 100 patients in the case group. There were 1.754 point mutations detected in 456 sites in the coding region of the polypeptide. A total of 567 point mutations in 98 sites were found in the RNA region. There were 193 mutations in 41 previously reported sites. A total of 34 novel loci were found in 40 cases (Table II), of which A10398G (52.52%), C5178A (24.24%), C150T (17.17%), G3010A (17.17%), and T16189C (16.16%) were the most frequently observed conserved non-synonymous mutations (Figure 1) showing a significantly different distribution ($p<0.05$) between the case and control group (Table III).

Discussion

As the organelle responsible for oxygen metabolism and energy metabolism in cells, mitochondria are the sites of electron transfer and respiratory movement in the majority of cells. Mitochondria also are involved in the maintenance of calcium balance, cellular signaling pathways, and transcription regulation[7-9]. Additionally, mitochondria have functions in cell growth and cell cycle regulation and are involved in apoptosis signaling pathways[10]. As early as 1962, the association between mitochondria and human diseases drew attention and was studied[11]. Nowadays, nearly 600 types of mitochondrial gene mutations have been discovered to be related to diseases[12]. Sepsis is a disease with genetic-associated susceptibility; the close association between its pathological progression and structural/functional changes in mitochondria has received attention and been studied[13,14]. Researches using platelets, which do not contain nuclear genes, suggested that mitochondrial genes could be used as independent factors to participate in and affect the pathological progression of sepsis[15]. However, the specific pathogenic mechanism was unknown. The mitochondrial genome is divided into coding and control regions. The coding region in turn is divided into coding (13 polypeptides) and RNA (2rRNA and 22tRNA) coding regions. In this study, whole sequence scanning of mtDNA was performed on 100 pa-

Table I. General patient information.

General information	Case group	Control group	*p*
Gender	Male (n=70)	Male (n=70)	0.293
Age	56.99±19.74	54.99±15.96	0.376

Table II. Novel mtDNA mutations found in the case group.

Mutation sites	Mutation type	Number of cases	Gene region
344	T344C	1	MT-HV2
383	T383A	1	MT-DLOOP
389	G389A	2	MT-OHR,MT-3H,MT-ATT,MT-CR
427	C427A	1	MT-OHR,MT-LSP,MT-TFL
439	A439C	1	MT-OHR,MT-LSP,MT-TFL,MT-HV3
601	G601A	1	tRNA phenylalanine
641	A641AA	2	tRNA phenylalanine
785	C785CC	1	12S ribosomal RNA
1868	G1868T	1	16S ribosomal RNA
1874	A1874AT	1	16S ribosomal RNA
2019	G2019GG	1	16S ribosomal RNA
3188	T3188A	1	16S ribosomal RNA
3270	C3270:	1	tRNA leucine 1
4648	T4648TT	1	MT-ND2
5282	A5282T	1	MT-ND2
5403	A5403CC	1	MT-ND2
5863	A5863T	1	tRNA tyrosine
6363	G6363T	1	MT-CO1
6459	T6459C	1	MT-CO1
7780	A7780G**	1	MT-CO2
8609	C8609T	1	MT-ATP6
8612	T8612C	1	MT-ATP6
10138	T10138TCA	1	MT-ND3
10677	G10677A	1	MT-ND4L
11333	A11333C	1	MT-ND4
11355	A11355G	1	MT-ND4
11528	A11528G	1	MT-ND4
11606	A11606G	1	MT-ND4
13263	A13263G	3	MT-ND5
15683	C15683CC	1	MT-CytB
15764	G15764A	2	MT-CytB
15768	A15768C	1	MT-CytB
15867	A15867AA	1	MT-CytB
16434	G16434A	1	MT-ATT,MT-CR,MT-7SDNA

tients with sepsis, and the results found a total of 718 SNPs in 708 loci. Differences in the mitochondrial genome exist between different races[16]. In this study, all subjects were Han Chinese, and the obtained mitochondrial gene sequencing data were representative of the Han Chinese mitochondrial gene and mutation distribution characteristics. Population differences and pedigree research has a certain reference value. A novel A5863T mutation located on the tRNATyr gene (Figure 2) was found in this study. There is only one tRNA isoacceptor for tyrosine encoded in the mitochondrial genome, and A5863T is likely to impair the formation of the correct secondary and tertiary structures of the encoded tRNA, which may, thereby, produce a nonfunctional tRNATyr, leading to the possibility of sepsis. The 3270 locus is located on the tRNALeu gene, which is a hotspot gene of mitochondrial pathogenesis. It is related to various human diseases, and 11 tRNALeu gene mutations associated disease have been found. Indeed, small changes in

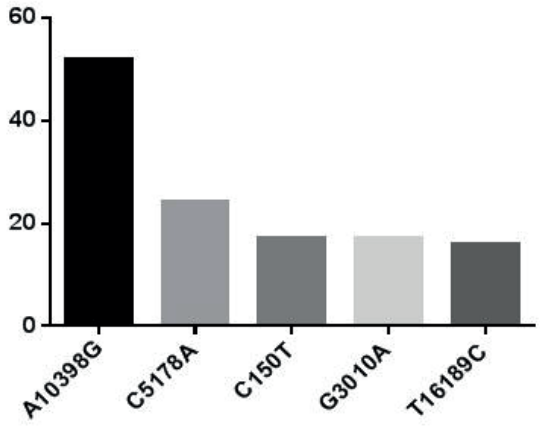

Figure 1. Distribution of candidate genes.

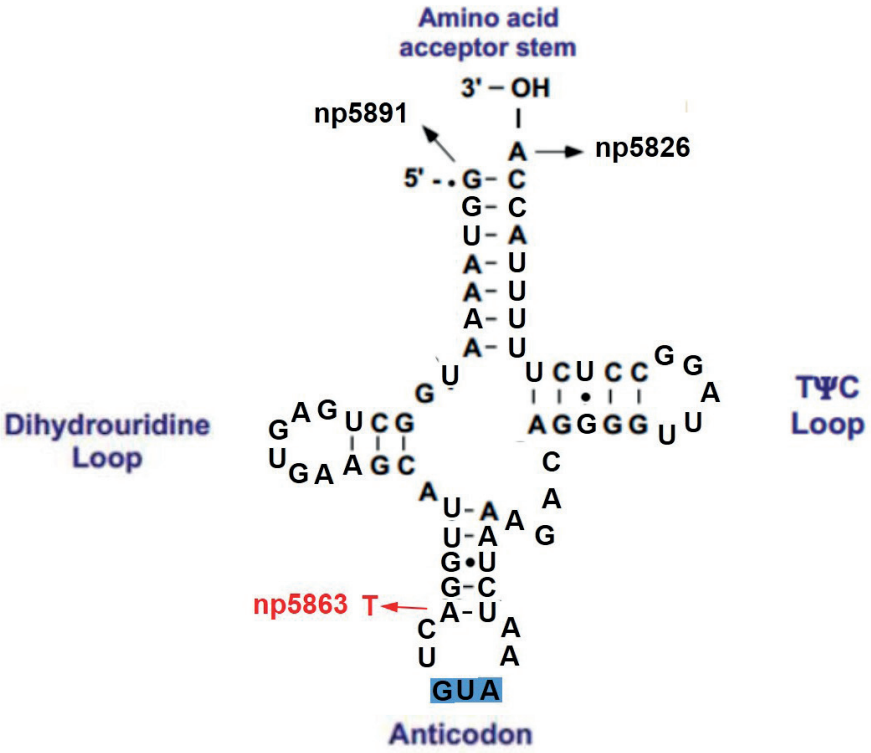

Figure 2. Secondary structure of mitochondrial tRNATyr.

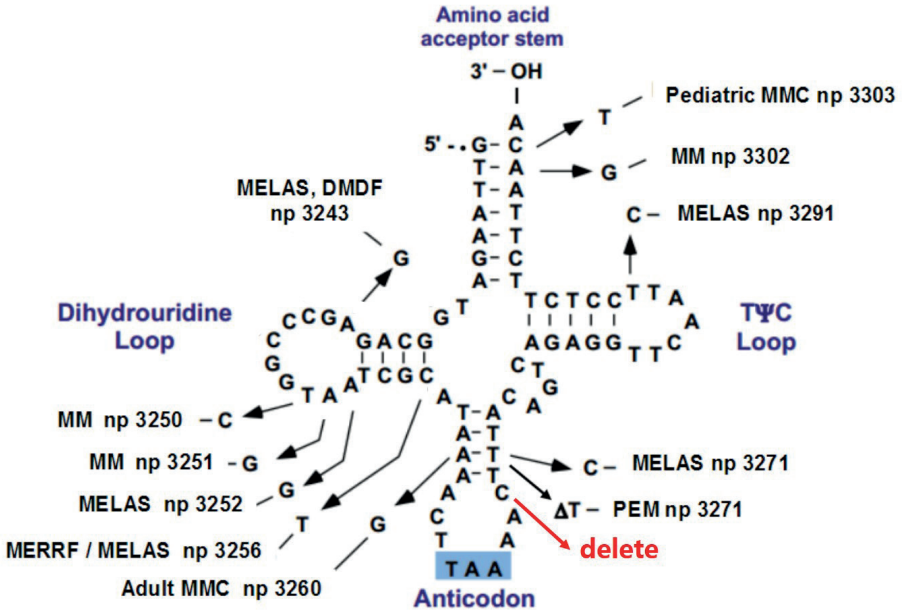

Figure 3. Secondary structure of mitochondrial tRNALeu.

the tRNALeu gene have resulted in protein level changes. In this study, a C3270 deletion mutation was found at position A36 of the anti-codon loop (Figure 3), and this structural change affected 3 ‘end cleavage, tailing, and aminoacylation, thus affected amino acid translation. Mitochondrial tRNALeu gene mutations in the pathogenesis of sepsis cannot be ignored, and the C3270 deletion

Table III. Candidate genes of the case and control groups.

Mutation type	Case group (n)	Control group (n)	p
A10398G	52	2	0.023*
C5178A	24	1	0.001*
C150T	17	0	0.001*
G3010A	17	1	0.001*
T16189C	16	1	0.001*

*$p<0.05$

mutation may become a new target for diagnosis and treatment of sepsis. Under sepsis conditions, infection and excessive inflammation disrupt the normal physiological mitochondria pathways. Oxygen utilization is decreased and production capacity is insufficient, resulting in the production of large amounts of reactive oxygen species (ROS) and extensive apoptosis. A meta-analysis of studies from other countries showed that the mitochondrial respiratory chain Complex I is the major site for ROS production[17,18]. Among the candidate gene loci, the 5178 locus is in the ND2 gene, which encodes a subunit of complex I. The C5178A mutation may cause ND2 protein complex I changes, resulting in increased mitochondrial ROS, calcium overload, and opening of the mitochondrial permeability transition pore (mPTP)[19-21]. This, in turn, leads to decreased mitochondrial membrane potential and the release of various proteins, including cytochrome c, which can further induce apoptosis and promote the development of sepsis and multiple organ failure[22]. Significant mutations with high frequencies in the case group were identified as candidate gene loci. All five-candidate sites were previously reported to be disease-related, but all mutations were not previously associated with sepsis. The pathology of sepsis is complex and involves numerous immune responses and factors. Genetic susceptibility to sepsis is less likely to be determined by only a few or one gene, and is currently thought to be due to the synergistic or additive effects of multiple genes[23]. In recent years, the relationship between mitochondrial gene mutations and sepsis has mainly focused on sepsis immune regulation or the product coding gene region, as well as the key factors in the sepsis process, such as the lipid of immune recognition phase of lipopolysaccharide-binding proteins, cell-surface antigen CD14, and Toll-like receptors[24]. The coding and regulatory genes of key inflammatory factors, such as TNFα, IL-1, and IL-6, are important targets for the study of single nucleotide mutation sites contributing to initial sepsis outbreaks. In the present study, the distribution of these candidate genes in the control group was significantly different from that in the case group. This confirmed that these candidate genes were associated with sepsis. Studies have shown that mtDNA can passively leak into the serum during trauma and severe necrosis of tissues and organs. In early sepsis, serum mitochondrial DNA content increased significantly and this trend persisted for a longer period, potentially serving as an early warning signal[25]. mtDNA release into the blood is not passive but a procedural process[26], which may be related to the mitochondrial mutations of the candidate. Zhang et al[27] found that mitochondrial DNA can induce and promote inflammatory responses, and some animal experiments also confirmed that mtDNA can lead to acute lung injury. Mitochondrial DNA may act as a "Trojan horse" to the body as a potential risk. The mechanism by which mitochondrial DNA is activated by mitochondrial DNA is similar to the immune response elicited by exogenous antigens. Under normal circumstances, mitochondrial DNA is isolated from mitochondria, but in the case of infection or trauma, mitochondrial DNA actively or passively enters the intracellular environment, inducing and promoting an inflammatory response, leading to the uncontrolled amplification of inflammation and sepsis.

Conclusions

The role of mitochondrial DNA mutations in sepsis pathogenesis and the association between mitochondrial inheritance and genetic predisposition to sepsis are worthy of further investigation and validation, which is of interest for individualized and differentiated treatment of sepsis.

Conflict of interest

The authors declare no conflicts of interest.

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