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Effect of mitochondrial tRNA^{Lys} mutation on the clinical and biochemical characteristics of Chinese essential hypertensive subjects

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ABSTRACT

Mitochondrial dysfunction has been potentially implicated in both human and experimental hypertension. We performed the mutational analysis of tRNA^{Lys} gene by PCR amplification and subsequent sequence analysis of the PCR fragments from 990 Chinese essential hypertensive subjects. We also made a comparative analysis of the collected data of the essential hypertension subjects who carried tRNA^{Lys} mutation and those who did not carry the mutation using the methods of 1:1 case-control study. We totally found 7 mutation sites in 10 subjects. The onset ages of the individuals carrying the mutation were earlier than those who did not bear them. The level of blood urea nitrogen in hypertension subjects who carried tRNA^{Lys} mutation was higher than the hypertension subjects who did not carried tRNA^{Lys} mutation, while the serum potassium was significantly lower. The level of platelet count in hypertension subjects who carried tRNA^{Lys} mutation was lower. The level of ventricular septal thickness in hypertension subjects who carried tRNA^{Lys} mutation was higher and the level of left ventricular end diastolic diameter in hypertension subjects was significantly lower. Mitochondrial tRNA^{Lys} mutations might result in the change of their structure and function, and then damaged the blood metabolism, the balance of the blood electrolyte, the steady-state of the blood cells and the heart structure and function, which were involved in the progress of the essential hypertension. Part of the essential hypertension patients clinically presented the characters of maternal inheritance, which might be associated with the tRNA^{Lys} mutation.

1. Introduction

Essential hypertension is one of the most common and complex genetic diseases, with the genetic contribution being about 30–50%. Studies have shown that individuals who have a family history of hypertension are more incline to have hypertension than those who do not have the family history. Sometimes the genetics of hypertension showed a pattern of maternally inherited [1–3]. Like many other polygenic diseases, the occurrence of the essential hypertension is the result of the interactions between different disease associated genes and environmental factors. Different genes not only play special roles but also crosstalk with other genes. Mediated from different level, such as molecules, cells, tissues and organs, the blood pressure finally increases when then the crosstalk adds up to a certain degree. Previous studies of our laboratory revealed that the frequency and quantity of mutation in the mitochondrial DNA is higher in hypertensive subjects than that of in the normal subjects. Mutations mainly distributed in the non-coding D loop region, especially in tRNA genes.

Recently, several mtDNA point mutations have been identified to be associated with cardiovascular disease including the essential hypertension [4–7]. Investigators have provided further evidence of a maternal effect on hypertension status and systolic blood pressure [8]. Mitochondrial heritability for systolic blood pressure was about 5%, and mitochondrial effects may account for about 35% of hypertensive pedigrees. Their findings are consistent with an involvement of mitochondrial DNA mutations in hypertension.

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With an effort to understand a role of mitochondrial genome in pathogenesis of cardiovascular disease in Chinese population, we have initiated a systematic and extended mutational screening of mtDNA in a large cohort of hypertension subjects [9–12]. We aimed to observe the relationship between the mitochondrial tRNA mutation and the essential hypertension by examining the mutation of $tRNA^{Lys}$. We also wanted to explore the inherited signs and clinical characters of maternally inherited essential hypertension.

2. Materials and methods

2.1. Subjects

We evaluated 990 individuals with hypertension, and all participants were out-patients or in-patients at the Institute of Geriatric Cardiology, Chinese People's Liberation Army. Hypertension was defined as a systolic blood pressure of $\geqslant$140 mmHg and/or a diastolic blood pressure of $\geqslant$90 mmHg on repeated measurements or receiving antihypertensive medication. Secondary hypertension was excluded by history and physical examination. All subjects were apparently healthy, based on the report of their medical history. A positive family history of essential hypertension was defined as the occurrence of hypertension in one or both biologic parents. The ethics committee of our institution approved the study protocol. All of the subjects enrolled in this study were Chinese, and all of the subjects gave informed consent to participate in this study. Exclusion criteria: participants who were diagnosed as secondary hypertension (primary aldosteronism, renal arterial sterosis, aortic coarctation, etc.), congenital heart diseases, organic valve diseases. The 242 control DNA samples were obtained from a panel of unaffected individuals from Chinese ancestry. All hypertensive individuals underwent a physical examination, laboratory assessment of cardiovascular disease risk factors, and routine electrocardiography. A physician measured the systolic and diastolic blood pressures of subjects using a mercury column sphygmomanometer and a standard protocol. The first and the firth Korotkoff sounds were taken as indicative of systolic and diastolic blood pressure, respectively. The average of three such systolic and diastolic blood pressure reading was taken as the examination blood pressure. Hypertension was defined according to the recommendation of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (JNC VI) and the World Health Organization-International Society of Hypertension as a systolic blood pressure of 140 mmHg or higher and/or a diastolic blood pressure of 90 mmHg or greater.

2.2. Study protocol

The subjects were studies and the plasma samples were collected. Casual blood pressure was measured in supine position over a 15 min resting period. Thereafter, blood samples were drawn from the antecubital vein for the measurement of blood glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, serum electrolyte, whole blood cell count, hemoglobin and platelets. Cardiac output was indexed by body surface area.

2.3. Laboratory assay

In all subjects plasma samples were analyzed immediately. Lipid levels were measured by the Hiachi 912 analyser. Whole blood cell count, hamnoglobin and platelets were eatimated by Sysnex XE2100.

2.4. Echocardiographic examination

Imaging and Doppler echocardiography were performed in all of the participants in this study. Comprehensive two-dimensional echocardiography was performed using a cardiac ultrasound unit (Sonos 5500; Hewlett Packard, Andover, MA). Echocardiographic parameters were measured by the consensus of two experienced investigators who were blinded to the metabolic data of the subjects. LV mass was estimated using the formula validated by Devereux and Reichek: LV mass (g) = $1.04 \times \{(IVSTd + PWTd + LVDd)3 - LVDd3\} - 13.6$. The LV mass index was obtained traditionally by dividing the LV mass by body surface area (LVMI, g/m^2).

2.5. Mitochondrial DNA analysis

DNA was isolated from whole blood using Promega Wizard®
Genomic DNA Purification Kit (Madison, WI). The primers for fragment is as follows: DNA was extracted from peripheral blood leukocytes using standard techniques. PCR amplification of the mitochondrial $tRNA^{Lys}$ gene was carried out, using the following primers: forward, 5′-ACGAGTACACCGACTACGGC-3′; reverse, 5′-TGGGTGGTTGGTGTAAATGA-3′. The cycling program for PCR consisted of a denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, and then a final extension step at 72 °C for 5 min. Amplifications were performed in Perkin-Elmer 9600 thermocyclers, using "optical" reaction plates and caps (PE-Biosystems, Warrington, UK). The PCR products were directly sequenced on both strands. Sequencing was done using an ABI PRISM Dye Terminator Cycle sequencing kit (Perkin-Elmer, Applied Biosystem, Foster City, Calif., USA) according to the manufacturer's instructions.

2.6. Statistical analysis

Statistical analyses were performed with SPSS 10.0. The sex ratio among hypertensive subjects, history of diabetes and family history of hypertension were compared by χ^2 test. Values for continuous variables are presented as adjusted mean ± standard error. $P < 0.05$ was deemed statistically significant.

3. Results

3.1. General description of mitochondrial $tRNA^{Lys}$ mutation in essential hypertensive subjects

As shown in Table 1, we totally found 7 mutation sites in 10 subjects from the mutation analysis of mitochondrial $tRNA^{Lys}$ of 990 essential hypertensive subjects. Only the mutation site of A8348G was previously reported to be associated with the occurrence of the cardiomyopathy, other 6 mutation sites were the first reported by us [13].

Table 1
Mutation site of mitochondrial $tRNA^{Lys}$ in essential hypertensive individuals.

Site of mutation	Number of mutations
U8311C	1
G8334A	1
U8337C	1
A8343G	3
A8346G	1
A8347G	2
A8348G	1

3.2. General characteristics of mitochondrial tRNA^{Lys} mutation in essential hypertensive subjects

According to the matching conditions, we selected 10 essential hypertensive patients without mutation of mitochondrial tRNA as the control group.

Medical history revealed that essential hypertensive patients with or without tRNA^{Lys} mutations have denied the long-term, regular application of diuretics, lipid drug as well as other possible medicine which affect the blood lipid, renal function, electrolyte, blood glucose and the routine blood test.

The general data of essential hypertensive patients with or without tRNA^{Lys} mutations were analyzed, the results were shown in Table 2.

As shown by Table 2, a total of 10 hypertensive patients appear tRNA^{Lys} mutation, and the ratio of men to women was 1:1. The mean age of onset of hypertension was significantly lower for the patients with tRNA^{Lys} than that of without tRNA^{Lys} mutation ($P < 0.05$).

3.3. Blood biochemical characteristics of the hypertensive patients with or without mitochondrial tRNA^{Lys} mutation

We made a comparative analysis of the biochemistry data of the essential hypertension subjects who carried tRNA^{Lys} mutation and those who did not carry the mutation using the methods of 1:1 case-control study. As shown in Table 3, the level of blood urea nitrogen in hypertension subjects who carried tRNA^{Lys} mutation was higher than the hypertension subjects who did not carried tRNA^{Lys} mutation ($P < 0.05$). The serum potassium was significantly lower in hypertension subjects who carried tRNA^{Lys} mutation than those who did not carried the mutation ($P < 0.05$). There is no statistical difference between two groups in the level of blood lipid, blood glucose, blood sodium, blood magnesium and other blood biochemistry.

3.4. Routine blood test of the hypertensive patients with or without mitochondrial tRNA^{Lys} mutation

We made a comparative analysis of the routine blood test of the essential hypertension subjects who carried tRNA^{Lys} mutation and those who did not carry the mutation using the methods of 1:1 case-control study.

As shown in Table 4, the level of platelet count in hypertension subjects who carried tRNA^{Lys} mutation was lower than those who did not carried tRNA^{Lys} mutation ($P < 0.05$). There is no statistical difference between two groups in WBC, RBC and HB.

3.5. Echocardiographic characteristics of the hypertensive patients with or without mitochondrial tRNA^{Lys} mutation

We made a comparative analysis of echocardiography of the essential hypertension subjects who carried tRNA^{Lys} mutation and those who did not carry the mutation using the methods of 1:1 case-control study. As shown in Table 5, the level of ventricular septal thickness in hypertension subjects who carried tRNA^{Lys} mutation was higher than those who did not carried tRNA^{Lys} mutation ($P < 0.05$). The level of left ventricular end diastolic diameter in hypertension subjects who carried tRNA^{Lys} mutation was significantly lower than those who did not carried tRNA^{Lys} mutation ($P < 0.05$). There is no statistical difference between two groups in left ventricular mass index, left ventricular end diastolic posterior wall thickness, left ventricular end systolic diameter, left atrial, cardiac index and other aspects.

Table 3
Comparison of the blood biochemistry in patients with or without tRNA^{Lys} mutation.

	Mutation	Control	*P* value
TG (mmol/L)	1.55 ± 0.75	1.64 ± 0.70	0.37
CHO (mmol/L)	4.66 ± 0.83	4.99 ± 0.85	0.25
HDL (mmol/L)	1.30 ± 0.19	1.11 ± 0.26	0.07
LDL (mmol/L)	2.60 ± 0.99	3.39 ± 0.92	0.09
GLU (mmol/L)	5.29 ± 0.64	5.08 ± 0.51	0.18
BUN (mmol/L)	6.52 ± 1.27	4.99 ± 0.82	0.002*
Cr (mmol/L)	75.3 ± 18.2	64.7 ± 10.5	0.066
K (mmol/L)	4.02 ± 0.44	4.65 ± 0.48	0.015*
Na (mmol/L)	140.4 ± 2.8	140.2 ± 2.9	0.41
Mg (mmol/L)	0.89 ± 0.05	0.91 ± 0.10	0.22

TG represents triglyceride;CHO represents cholesterol; HDL represents high density lipoprotein; LDL represents low density lipoprotein; GLU represents blood glucose; BUN represents blood urea nitrogen; Cr represents creatinine.
* $P < 0.05$.

Table 4
Comparison of the routine blood test in patients with or without tRNA^{Lys} mutation.

	Mutation	Control	*P* value
WBC ($\times 10^9$/L)	6.85 ± 1.33	86.5 ± 21.0	0.09
RBC ($\times 10^9$/L)	4.53 ± 0.46	4.54 ± 0.41	0.46
HB (g/dL)	142.6 ± 12.7	149.2 ± 25.9	0.26
PLT ($\times 10^9$/L)	186.1 ± 52.2	221.9 ± 46.3	0.048*

WBC represents white blood cell; RBC represents red blood cell; HB represents hemoglobin; PLT represents platelet.
* $P < 0.05$.

4. Discussion

Mitochondrial tRNA genes are all single copy genes; therefore any mutation of the tRNA gene will eventually lead to the instability of the mitochondrial translation system, and any point mutation of a certain tRNA gene would be deadly vital for its activity [14–16]. Systematic study of the relationship between the disease and tRNA mutation not only helps us to understand the mechanism of the occurrence of the mutation related disease, but also improves the diagnosis, precaution and treatment of the disease.

The human mitochondrial tRNA^{Lys} is an example of a simplified structure of the mitochondrial tRNA. D loop of this mitochondrial tRNA has only three base pairs, which could occur as a result of some structural instability [17].

Table 2
Comparison of the general data of patients with or without tRNA^{Lys} mutation.

	Case	Sex (male/female)	Age (years)	Onset age (years)	BMI (kg/m^2)
Mutation	10	5/5	60.7 ± 14.3	48.4 ± 15.7	24.7 ± 2.6
Control	10	5/5	62 ± 12.9	55.8 ± 13.3	25.6 ± 2.5
P value	–	–	0.39	0.047*	0.175

BMI represents body mass index.
* $P < 0.05$.

Table 5
Comparison of the echocardiography in patients with or without $tRNA^{Lys}$ mutation.

	Mutation	Control	*P* value
LVMI (g/m^2)	93.9 ± 21.5	86.5 ± 21.0	0.11
IVSD (mm)	11.0 ± 1.3	9.8 ± 1.3	0.011*
LVPWd (mm)	9.9 ± 1.2	9.6 ± 2.2	0.31
LVIDd (mm)	45.1 ± 3.1	45.6 ± 4.6	0.39
LVIDs (mm)	29.0 ± 2.5	32.1 ± 5.6	0.046*
LA (mm)	34.2 ± 4.4	34.5 ± 5.3	0.45
SV (ml/beat)	62.6 ± 11.3	59.8 ± 10.3	0.29
EF (%)	66.1 ± 6.5	64.2 ± 7.2	0.25
CO (L/min)	4.17 ± 0.87	4.39 ± 0.73	0.27
CI ($L/min/m^2$)	2.36 ± 0.47	2.45 ± 0.36	0.33

LVMI represents left ventricular mass index; IVSD represents interventricular septal thickness in diastole; LVPWd represents left ventricular posterior wall thickness in diastole; LVIDd represents left ventricular internal dimension in diastole; LVIDs represents left ventricular internal dimension in systole; LA represents left atrium; SV represents strove volume; EF represents ejection fraction; CO represents cardiac output; CI represents cardiac index.
* $P < 0.05$.

In the present study, we performed the mutational analysis of $tRNA^{Lys}$ gene by PCR amplification and subsequent sequence analysis of the PCR fragments and we found a total of 7 site mutations. Compared with the essential hypertensive individuals without $tRNA^{Lys}$ mutation, primary hypertensive patients with $tRNA^{Lys}$ mutations shared common features of the onset time of hypertension significantly ahead of schedule. The individuals carried a $tRNA^{Lys}$ mutation were more easy to develop hypertension stimulated by environmental factors. We also noticed that differences in body mass index between the essential hypertensive individuals with or without mitochondrial $tRNA^{Lys}$ mutation, which excluded the effect of BMI factors on the development of hypertension.

It is well known that the blood glucose, blood lipid, creatinine, urea nitrogen and other biochemical abnormalities closely related to the occurrence and development of primary hypertension. These factors can be the abnormal factors involved in the occurrence and development of essential hypertension, and they were also the results of the development of hypertension, which led to the aggravation of deterioration of the target organ damage.

In order to clearify whether the mitochondrial $tRNA^{Lys}$ affect the biochemical indicators of the individuals, we compared and analyzed the blood glucose, blood lipid, serum creatinine, urea nitrogen and other biochemical abnormalities of the individuals with or without $tRNA^{Lys}$ mutation. We found that the level of blood urea nitrogen in hypertension subjects who carried $tRNA^{Lys}$ mutation was higher than the hypertension subjects who did not carried $tRNA^{Lys}$ mutation ($P < 0.05$). The serum potassium was significantly lower in hypertension subjects who carried $tRNA^{Lys}$ mutation than those who did not carried the mutation ($P < 0.05$). There is no statistical difference between two groups in the level of blood lipid, blood glucose, blood sodium, blood magnesium and other blood biochemistry.

Research has demonstrated that changes of blood cells closely related to the development and progression of essential hypertension [18–21]. The present experimental showed that the level of platelet count in hypertension subjects who carried $tRNA^{Lys}$ mutation was lower than those who did not carried $tRNA^{Lys}$ mutation ($P < 0.05$). There is no statistical difference between two groups in WBC, RBC and HB. Our results demonstrated that $tRNA^{Lys}$ mutations decreased the platelet number, but the possible mechanism is not clear, need further study.

Through the analysis of the cardiac ultrasound results, we found that the level of ventricular septal thickness in hypertension subjects who carried $tRNA^{Lys}$ mutation was higher than those who did not carried $tRNA^{Lys}$ mutation. The level of left ventricular end diastolic diameter in hypertension subjects who carried $tRNA^{Lys}$ mutation was significantly lower than those who did not carried $tRNA^{Lys}$ mutation, which showed that the mutation of $tRNA^{Lys}$ affected the structure and function of heart.

In summary, the present study indicated that mitochondrial tRNA mutation may be involved in the pathology process of hypertension, and we also found there existed mitochondrial genetic characteristic in Chinese essential hypertension individuals. The present research provided a solid theoretic foundation for furthering research to explore occurrence, development of the essential hypertension.

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References

[1] H. Guo, X.Y. Zhuang, A.M. Zhang, W. Zhang, Y. Yuan, L. Guo, D. Yu, J. Liu, D.K. Yang, Y.G. Yao, Presence of mutation m.14484T>C in a Chinese family with maternally inherited essential hypertension but no expression of LHON, Biochim. Biophys. Acta 2012 (1822) 1535–1543.
[2] Q. Qiu, R. Li, P. Jiang, L. Xue, Y. Lu, Y. Song, J. Han, Z. Lu, S. Zhi, J.Q. Mo, M.X. Guan, Mitochondrial tRNA mutations are associated with maternally inherited hypertension in two Han Chinese pedigrees, Hum. Mutat. 33 (2012) 1285–1293.
[3] S. Wang, R. Li, A. Fettermann, Z. Li, Y. Qian, Y. Liu, X. Wang, A. Zhou, J.Q. Mo, L. Yang, P. Jiang, A. Taschner, W. Rossmanith, M.X. Guan, Maternally inherited essential hypertension is associated with the novel 4263A>G mutation in the mitochondrial tRNAIle gene in a large Han Chinese family, Circ. Res. 108 (2011) 862–870.
[4] Z. Jia, X. Wang, Y. Qin, L. Xue, P. Jiang, Y. Meng, S. Shi, Y. Wang, J. Qin, M.X. Guan, Coronary heart disease is associated with a mutation in mitochondrial tRNA, Hum. Mol. Genet. 22 (2013) 4064–4073.
[5] C.Q. Lu, J.Y. Zhang, A.G. Xu, J.J. Zhang, D.D. Li, X. Fu, J. Guo, Q.F. Xu, Mitochondrial tRNAMet mutation is associated with clinical and biochemical characteristics in primary hypertension, Mol. Med. Rep. 7 (2013) 1924–1928.
[6] A.J. Marian, Mitochondrial genetics and human systemic hypertension, Circ. Res. 108 (2011) 784–786.
[7] Z. Li, Y. Liu, L. Yang, S. Wang, M.X. Guan, Maternally inherited hypertension is associated with the mitochondrial tRNA(Ile) A4295G mutation in a Chinese family, Biochem. Biophys. Res. Commun. 36 (2008) 906–911.
[8] Q. Yang, S.K. Kim, F. Sun, J. Cui, M.G. Larson, R.S. Vasan, D. Levy, F. Schwartz, Maternal influence on blood pressure suggests involvement of mitochondrial DNA in the pathogenesis of hypertension: the Framingham Heart Study, J. Hypertens. 25 (2007) 2067–2073.
[9] Y. Liu, Y. Li, J. Gao, C. Zhu, Y. Lan, J. Yang, Z. Li, M. Guan, Y. Chen, Molecular characterization of a Chinese family carrying a novel C4329A mutation in mitochondrial tRNAIle and tRNAGln genes, BMC Med. Genet. 23 (2014) 15–84.
[10] R. Li, Y. Liu, Z. Li, L. Yang, S. Wang, M.X. Guan, Failures in mitochondrial tRNAMet and tRNAGln metabolism caused by the novel 4401A>G mutation are involved in essential hypertension in a Han Chinese Family, Hypertension 54 (2009) 329–337.
[11] Y. Liu, R. Li, Z. Li, X.J. Wang, L. Yang, S. Wang, M.X. Guan, Mitochondrial transfer RNAMet 4435A>G mutation is associated with maternally inherited hypertension in a Chinese pedigree, Hypertension 53 (2009) 1083–1090.
[12] Y. Liu, Z. Li, L. Yang, S. Wang, M.X. Guan, The mitochondrial ND1 T3308C mutation in a Chinese family with the secondary hypertension, Biochem. Biophys. Res. Commun. 368 (2008) 18–22.
[13] I. Ueki, Y. Koga, N. Povalko, Y. Akita, J. Nishioka, S. Yatsuga, R. Fukiyama, T. Matsuishi, Mitochondrial tRNA gene mutations in patients having mitochondrial disease with lactic acidosis, Mitochondrion 6 (2006) 29–36.
[14] T. Suzuki, A. Nagao, T. Suzuki, Human mitochondrial tRNAs: biogenesis, function, structural aspects, and diseases, Annu. Rev. Genet. 45 (2011) 299–329.
[15] S. Konovalova, H. Tyynismaa, Mitochondrial aminoacyl-tRNA synthetases in human disease, Mol. Genet. Metab. 108 (2013) 206–211.
[16] L. Levinger, M. Mörl, C. Florentz, Mitochondrial tRNA 3' end metabolism and human disease, Nucleic. Acids. Res. 32 (2004). 5430- 5341.
[17] G. Leszczynska, P. Leonczak, K. Wozniak, A. Malkiewicz, Chemical synthesis of the 5-taurinomethyl(-2-thio)uridine modified anticodon arm of the humanmitochondrial tRNA(Leu(UUR)) and tRNA(Lys), RNA 20 (2014) 938–947.

[18] Y. Wen, High red blood cell distribution width is closely associated with risk of carotid artery atherosclerosis in patients with hypertension, Exp. Clin. Cardiol. 15 (2010) 7–40.
[19] S. Wu, H. Lin, C. Zhang, Q. Zhang, D. Zhang, Y. Zhang, W. Meng, Z. Zhu, F. Tang, F. Xue, Y. Liu, Association between erythrocyte parameters and metabolic syndrome in urban Han Chinese: a longitudinal cohort study, BMC Public Health 13 (2013) 989.
[20] E. Gkaliagkousi, G. Passacquale, S. Douma, C. Zamboulis, A. Ferro, Platelet activation in essential hypertension: implications for antiplatelet treatment, Am. J. Hypertens. 23 (2010) 229–236.
[21] G. Schillaci, M. Pirro, G. Pucci, T. Ronti, G. Vaudo, M.R. Mannarino, C. Porcellati, E. Mannarino, Prognostic value of elevated white blood cell count in hypertension, Am. J. Hypertens. 20 (2007) 364–369.