Original Article

The Association between the C5263T Mutation in the Mitochondrial *ND2* Gene and Coronary Heart Disease among Young Chinese Han People*

HAN Guo Xin[1,§], XIA Lei[2,§], LI Shuo Shuo[1,§], JIN Qin Hua[3], SONG Yang[1], SHEN Hong[1], WANG Li Li[1], KONG Ling Jie[1], LI Tan Shi[1], and ZHU Hai Yan[1,#]

1. Emergency Department, General Hospital of Chinese PLA, Beijing 100853, China; 2. Medical Support Department, General Hospital of Chinese PLA, Beijing 100853, China; 3. Cardiology Department of Chinese PLA, Beijing 100853, China

Abstract

Objective This study aimed to investigate the genetic background of mitochondrial genes in young patients with Coronary heart disease (CHD) to provide a foundation for the early prevention of young patients with CHD.

Methods 115 cases of young (≤ 45 years) CHD Chinese Han patients (case group), 100 cases of older (> 45 years) Chinese Han CHD patients (experimental group) hospitalized and 100 cases of healthy people through physical examination (control group) at the General Hospital of PLA between January 2014 and December 2015 were selected. General information, clinical assessment, pedigree analysis, and mitochondrial full sequence scanning were performed**.** The pedigrees of one patient harbouring the C5263T mutation were recruited. Mitochondrial functional analysis including cellular reactive oxygen species (ROS) levels and mitochondrial membrane potential (MMP) were performed on pedigrees with the C5263T mutation (mutation group) and without the mutation (non-mutation group).

Results The differences in biochemical tests ($P > 0.05$) between the case group and experimental group were not significant. The C5263T single-nucleotide mutation of the mitochondrial *ND2* gene was observed in 2 young CHD patients in the case group. The premature CHD of these 2 patients followed a pattern of maternal inheritance. The mutation group (I1, II2) had higher ROS levels (4750.82 ± 1045.55 *vs.* 3888.58 ± 487.60, $P = 0.022$) and lower MMP levels ($P = 0.045$) than the non-mutation group (II1, III1, III2).

Conclusion We speculated that the mitochondrial C5263T mutation might be associated with the occurrence CHD in Chinese Han young people.

Key words: Mitochondrion; Coronary heart disease; Young

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INTRODUCTION

As early as 1962, the relationship between mitochondria and human disease was concerned[1]. In the meantime, a number of molecular epidemiological studies have been published on the associations between mtSNPs and neurological, psychiatric[2], or metabolic diseases especially cardiovascular diseases. In our previous study, we reported the associations between

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§These authors contributed equally to this work.

#Correspondence should be addressed to Dr ZHU Hai Yan, PhD, associate professor, Tel: 86-10-66937224, Fax: 86-10-66939306, E-mail: zhuhy301@aliyun.com

Biographical notes of the first authors: HAN Guo Xin, male, master, majoring in cardiovascular emergency; XIA Lei, female, PhD, professor, majoring in hospital management and logistics; LI Shuo Shuo, female, master, majoring in cardiovascular emergency.

mitochondrial gene mutations and essential hypertension in Chinese Han population[3-5]. Norikoetareported significant associations between mtSNPS and myocardial infarction[6], atherosclerotic cerebral infarction[7], metabolic syndrome[8] and longevity among the Japanese[9-10] and significant associations between mitochondrial haplogroup T and coronary artery disease, as well as diabetic retinopathy in Middle European Caucasians were indicated[11]. However, the relationship between mitochondrial gene mutations and coronary heart disease in the Chinese Han population, especially in young people, has not been reported. In our study, C5263T single-nucleotide mutation of the mitochondrial *ND2* gene was observed in the Chinese Han family of coronary heart disease attacked at young age. 5263 is a nucleotide position in the gene encoding the *ND2* protein and ND2 protein encoded by the *ND2* gene is a subunit of Complex I. The C5263T mutation refered to the substitution of the cytosine at nucleotide 5263 by a thymidine. The resultant change of the encoded alanine to valine might alter the ND2 protein Complex I to induce mitochondrial calcium overload and increase production of reactive oxygen species (ROS), thus causing myocardial apoptosis, sustained myocardial damage, and electrophysiological disorders[12]. Therefore, we hypothesized that C5263T mutation might be involved in the occurrence and development of premature CHD in young people.

METHODS

Patient Inclusion Criteria

The inclusion criteria[13-14] were as follows: typical clinical presentations of paroxysmal retrosternal pain, including a tightness, squeezing, or pressure sensation and a burning sensation that could radiate to the left arm, jaw, neck, back, shoulder, or left forearm ulnar side, that was intermittent or persistent and was sustained for > 10-20 min, with suspected acute coronary syndrome; angiographic results indicating that at least one coronary artery had a decrease in diameter of ≥ 50% as observed from more than 2 different angles; and a clinical diagnosis of myocardial infarction. Based on age, patients were divided into the case group (≤ 45 years) and the experimental group (age of on-set great than 45 years).

Exclusion Criteria

Patients who had recent surgery, trauma, acute infection, and other combined chronic diseases and malignant tumours were excluded. All included patients signed informed consent forms.

Study Methods

Collection of Clinical Data General information, including the patient's age, gender, and body mass index (BMI), was collected. Cubital vein samples of patients were collected to detect creatine kinase (CK), cardiac troponin T (cTnT), B-type natriuretic peptide (BNP), total cholesterol (TC), triglyceride (TG), fibrinogen (Fib), high-sensitivity C-reactive protein (hs-CRP), low density lipoprotein-cholesterol (LDL-C), glycated haemoglobin, high density lipoprotein-cholesterol (HDL-C), and D-dimer (DD). Biochemical tests were performed using an automatic biochemical analyser (Hitachi 7600DDP, Japan) in the Department of Biochemistry in the General Hospital of the Chinese People's Liberation Army. Coagulation tests were performed using an STA automatic coagulation analyser (STA-Evolution, France) in the Clinical Laboratory Department of the General Hospital of the Chinese People's Liberation Army.

Coronal Angiography and Evaluation Criteria Coronal angiography was performed using the Judkins method through the right femoral artery or the right radial artery route. Multi-position projection and multi-site radiography were performed. The imaging diagnosis was confirmed by 2 cardiovascular intervention specialists. According to the evaluation criteria, CHD was diagnosed when the left main coronary artery and 3 epicardial coronary arteries and their large branches had maximum stenosis diameters of ≥ 50%.

Mitochondrial Genome Sequencing Mitochondrial DNA in whole blood was extracted using a reagent kit (Promega Wizard, A1120, USA). Primers were designed to amplify all 24 DNA fragments in mitochondria. PCR products were purified using the QIAEXII purification reagent kit (Qiagen) and processed using the BigDye Terminator Cycle sequencing reaction reagent kit. Sequencing and analysis were directly performed using the ABI3700DNA automatic sequencing instrument. Comparison and analysis of DNA sequencing results and corresponding protein sequences were performed using the SeqWeb program GAP (GCG). Comparison of homology was performed using BLAST from the National Center for Biotechnology Information (NCBI). All sequencing results were compared to the 2013 edition of the Cambridge Reference Sequence[15].

Pedigree Analysis According to the regulations of the Ethics Committee of the General Hospital of the Chinese People's Liberation Army, signed informed consent was obtained from all subjects. Family