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Molecular characterization of a Han Chinese family with essential hypertension

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ABSTRACT. Mutations in the mitochondrial genome have been found to be associated with essential hypertension. Here, we report the clinical and molecular characterization of a three-generation Han Chinese family with maternally inherited hypertension. Most strikingly, this pedigree exhibited a high penetrance of hypertension. Sequence analysis of the mitochondrial genome showed the presence of a homoplasmic T16189C mutation in the D-loop and the intergenic CO2/tRNA^{Lys} 9-bp common deletion, as well as a set of polymorphisms belonging to the East Asia haplogroup B5b1. The well-known T16189C mutation, which is in the first hypervariable segment of the mitochondrial control region, is implicated to be associated with a wide range of clinical disorders. Moreover, the genetic polymorphism 9-bp common deletion is found to be associated with hepatocellular carcinoma in the Han Chinese population. Thus, the combination of T16189C mutation and the 9-bp deletion may have caused mitochondrial dysfunction and contributed to the development of essential hypertension in this Chinese family.

Key words: Hypertension; mtDNA; Mutation; Chinese family

INTRODUCTION

Essential hypertension (EH, MIM 145500) is a major public health problem and is a significant risk factor for heart attacks, stroke, and end-stage renal disease (He and Whelton, 1997; Hajjar et al., 2006). EH is commonly regarded as a multifactorial disease influenced by both genetic and environmental factors. Familial aggregation of high blood pressure, despite different environmental factors, suggests that genetic factors are involved in the etiology of hypertension (Zinner et al., 1971; Havlik and Feinleib, 1982). In fact, human hypertension is a condition associated with endothelial dysfunction and oxidative stress (Romero and Reckelhoff, 1999). Mitochondrial dysfunction has been implicated in both human and experimental hypertension. Moreover, maternal transmission of EH had been implicated in some pedigrees, indicating that mutations in mitochondrial DNA (mtDNA) are one of the molecular bases for this disorder (Watson et al., 2001; Wilson et al., 2004). It is generally believed that mutations in mtDNA may cause mitochondrial dysfunction; consequently, an inefficient metabolism caused by mitochondrial dysfunctions in skeletal and vascular smooth muscles may lead to the elevation of systolic blood pressure and, therefore, may be involved in the development of hypertension (Wisløff et al., 2005).

In order to understand the molecular mechanism underlying maternally transmitted EH, we performed a systematic and extensive mutational screening for mtDNA mutations in the Zhengzhou City of Henan Province. Here, we describe a Chinese family with high penetrance of EH. Sequence analysis of the mitochondrial genome showed the occurrence of T16189C and 9-bp deletion. We also briefly discuss the molecular pathogenesis of these mutations in hypertension.

MATERIAL AND METHODS

Subjects

As part of a genetic screening program for hypertension, a Han Chinese family (Figure 1) was ascertained at the Hypertension Clinic of the First Affiliated Hospital of Zhengzhou University. Informed consent, blood samples, and clinical evaluations were obtained from all participating family members, under the protocols approved by the Ethics Committee of Zhengzhou University. Members of this family were interviewed and evaluated to identify both personal or medical histories of hypertension and other clinical abnormalities.

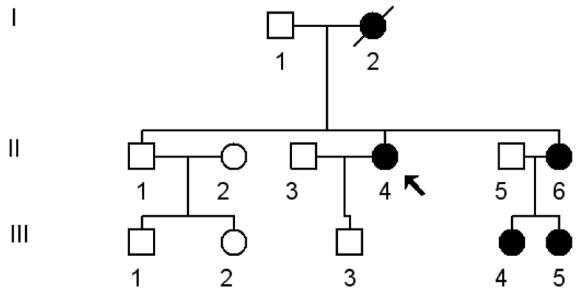

Figure 1. Han Chinese family with essential hypertension; hypertensive individuals are indicated by filled symbols, and arrow denotes the proband.

Clinical evaluation

Members of this Chinese family underwent a physical examination and laboratory assessment of cardiovascular disease risk factors. A physician measured the systolic and diastolic blood pressures of the subjects using a mercury column sphygmomanometer and a standard protocol. The first and the fifth Korotkoff sounds were taken to be indicative of systolic and diastolic blood pressure, respectively. Hypertension was diagnosed according to the recommendation of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure and the World Health Organization-International Society of Hypertension, with a systolic blood pressure of ≥140 mmHg and/or a diastolic blood pressure of ≥90 mmHg.

Mutational screening for mtDNA

Genomic DNA was isolated from whole blood cells of the participants using Puregene DNA Isolation Kits (Gentra Systems, Minneapolis, MN, USA). The entire mitochondrial genome of the proband (II-4) was PCR amplified in 24 overlapping fragments by using sets of the light-strand and heavy-strand oligonucleotide primers, as described previously (Rieder et al., 1998). Each fragment was purified and subsequently analyzed by direct sequencing in an ABI 3700 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA) using the Big Dye Terminator Cycle sequencing reaction kit. In addition, 250 healthy individuals from the same area were obtained as controls. The resultant sequence data were compared with the revised consensus Cambridge sequence (GenBank accession No. NC_012920) (Andrews et al., 1999).

Phylogenetic and haplogroup analyses

A total of 17 vertebrate mtDNA sequences were used for the inter-specific analysis. The conservation index was then calculated by comparing the human nucleotide variants with 16 other vertebrates. For haplogroup classification, the entire mtDNA sequences of the proband (II-4) were assigned to the East Asia mitochondrial phylogenetic tree, as described elsewhere (Kong et al., 2006).

RESULTS

Clinical features of the Chinese family with essential hypertension

The proband (II-4) is a 65-year-old woman who came from the Zhengzhou City of Henan Province. She went to the Department of Cardiology in the First Affiliated Hospital, Zhengzhou University for treatment of hypertension. Her blood pressure was 145/90 mmHg. Physical examination, laboratory assessment of cardiovascular disease risk factors, and routine electrocardiography showed no other clinical abnormalities, including diabetes mellitus, vision loss, deafness, and other neurological disorders. The clinical characterizations of the affected members in this family are listed in Table 1.

Mutational analysis of the mitochondrial genome

The maternally transmitted pattern of hypertension in this pedigree suggested the

involvement of the mitochondrial genome and led us to analyze the mtDNA mutations in matrilineal relatives. We performed PCR amplifications of the mitochondrial genome and subsequently sequenced the PCR fragments from the proband (II-4), as well as the matrilineal relatives (II-6, III-4, III-5). As shown in Table 2, a total of 22 variants were identified. Of these, six variants were in the D-loop, four variants were in the 12S rRNA gene, three variants were in the 16S rRNA gene, eight variants were in mitochondrial protein coding genes, and one variant was in the intergenic CO2/tRNA^{Lys} region. Most of the single nucleotide polymorphisms were well-known mutational hot spots and none of these variants could be defined as "novel" (Bandelt et al., 2009). The variants in rRNA and polypeptides were further evaluated by phylogenetic analysis and sequenced from other organisms including mouse (Bibb et al., 1981), bovine (Gadaleta et al., 1989), and *Xenopus laevis* (Roe et al., 1985). Besides the D-loop T16189C mutation and the intergenic CO2/tRNA^{Lys} 9-bp deletion, none of other variants showed evolutionary conservation nor implicated to have functional consequences.

Table 1. Clinical characterization of the members in this family with essential hypertension.

Subjects	Gender	Age of onset (year)	Age at test (year)	Systolic pressure (mmHg)	Diastolic pressure (mmHg)
II-4	Female	60	65	145	90
II-6	Female	65	68	150	85
III-4	Female	38	40	140	95
III-5	Female	36	42	130	100

Table 2. Complete mtDNA variants in this Chinese family.

Gene	Position	Replacement	Conservation (H/B/M/X)*	Previously reported
D-loop	16189	T to C		Yes
	16213	G to A		Yes
	16217	T to C		Yes
	16295	C to T		Yes
	16299	A to G		Yes
	16519	T to C		Yes
12S rRNA	709	G to A	G/A/A/-	Yes
	750	A to G	A/A/A/-	Yes
	1422	Del G		Yes
	1438	A to G	A/A/A/G	Yes
16S rRNA	2706	A to G	A/G/A/A	Yes
	3107	T to C		Yes
	3209	A to T		Yes
ND2	4769	A to G		Yes
CO1	5465	T to C		Yes
	7028	C to T		Yes
NC7	8281-8289	Del CCCCCTCTA	T/S/L/Q	Yes
ATP8	8860	A to G(Thr to Ala)	T/A/A/T	Yes
CO3	9123	G to A		Yes
ND4	11719	G to A		Yes
Cytb	14751	C to T		Yes
	14766	C to T (Thr to Ile)		Yes

*Conservation of amino acid for polypeptides of nucleotides for rRNAs in human (H), bovine (B), mouse (M), and *Xenopus laevis* (X).

DISCUSSION

In this study, we have performed clinical, genetic, and molecular characterization of a Han Chinese family with high penetrance of essential hypertension. Hypertension as a sole

clinical phenotype was only presented in matrilineal relatives, suggesting that mutations in mtDNA were the molecular bases of this disorder. Clinical and genetic evaluation of this family indicated variable severity and age onset in hypertension. In particular, the age of onset ranged from 36 to 60 years, with an average of 50 years. Moreover, matrilineal relatives in this family had an earlier age of onset of hypertension, suggesting that mitochondrial sequence variants may be a risk factor in early molecular diagnosis and detection.

Sequence analysis of the complete mitochondrial genome identified 22 polymorphisms belonging to the human mitochondrial haplogroup B5b1 (Kong et al., 2006). Of these, the homoplasmic D-loop T16189C mutation and the CO2/tRNA^{Lys} 9-bp deletion were of special interest. The T16189C mutation was in the first hypervariable segment of human mtDNA control region that contains many control elements for transcription and replication and was an important area of interaction of mtDNA with nuclear-encoded proteins (Wallace, 2015). The T-to-C transition at 16189 generated an uninterrupted homopolymeric C-tract that was highly unstable and caused heteroplastic length variation of mtDNA by replication slippage. Preliminary data suggest that the T16189C mutation resulted in a modest reduction in mtDNA copy number compared with nuclear DNA, which might have a mildly detrimental effect on respiratory chain function in beta cells. Moreover, mtDNA T16189C mutation mapped precisely to a novel point of origin of mtDNA replication (OriB), which made it likely that the mutation would alter mtDNA function (Yasukawa et al., 2005). Therefore, the critical position of the T16189C mutation in the mitochondrial genome suggested that the mutation might have a direct role in the pathogenesis of essential hypertension in this family.

The 9-bp (CCCCCTCTA) common deletion in the small non-coding segment located between the CO2 and tRNA^{Lys} gene had been used as a genetic marker to trace descent from people of East Asian origin (Yao et al., 2000). This deletion has also been suggested to be associated with several other diseases including cancer (Krishnan and Turnbull, 2010; Zhuo et al., 2010; Komandur et al., 2011). Due to its specific location, the 9-bp deletion may have the potential to alter downstream and upstream gene expression. A recent study identified that this deletion may create binding sites for hsa-miR-519c-5p and hsa-miR-526a; thus, this deletion may be involved in the pathogenesis of hepatocellular carcinoma (Jin et al., 2012). Similarly, this 9-bp deletion may also be associated with EH. Taken together, the combination of the D-loop T16189C mutation and the CO2/tRNA^{Lys} 9-bp deletion may contribute to the high penetrance of EH in this Han Chinese family.

Conflicts of interest

The authors declare no conflict of interest.

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