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Original article

The heteroplasmic 15059G>A mutation in the mitochondrial cytochrome *b* gene and essential hypertension in type 2 diabetes

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ABSTRACT

Aim: The long-term stress of high blood pressure levels increases the risk of a variety of macro- and microvascular complications of type 2 diabetes (T2D). The etiology of essential hypertension (EH) has been explored in depth, but the pathophysiology is multifactorial, complex, and poorly understood. Recent findings showed a role of inherited mutations in mitochondrial DNA (mtDNA) in maternally inherited forms of hypertension. However, an impact of somatic mtDNA mutations in the development of EH is significantly less investigated. In this study, we examined whether the level of heteroplasmy for the 15059G>A mutation in the mitochondrial cytochrome *b* gene is associated with EH in T2D.
Patients and methods: The heteroplasmy level in mtDNA isolated from blood of 189 diabetic participants randomly selected from general population (124 of whom had EH) was quantified using a real-time PCR.
Results: The 15059G>A heteroplasmy exceeding 39% was found to be significantly associated with a higher risk of EH (odds ratio 1.96; *P* (Fisher) 0.032).
Conclusion: There is the first evidence reporting association between the mtDNA 15059G>A mutation heteroplasmy and EH in T2D.

1. Introduction

Mutations in mitochondrial DNA (mtDNA) are known to cause a variety of hereditary disorders with complex phenotypes including those that have hypertension as one of their clinical outcomes (for example, such as the HUPRA syndrome comprising hyperuricemia, metabolic alkalosis, pulmonary hypertension, and progressive renal failure in infancy) [1].

The involvement of homoplasmic, inherited mtDNA mutations in etiology of familial, maternally inherited forms of hypertension (MIH) was found in several studies including the analysis of a large Han Chinese pedigree with suggestively maternally transmitted hypertension [2–7]. All homoplasmic mtDNA mutations associated with MIH caused functional defects. The 4435A>G mutation located at immediately 3′ end to the anticodon, corresponding to the conventional position 37 of $tRNA^{Met}$, was found to affect the fidelity of codon recognition, structural formation, and stabilization of functional tRNAs [7]. The 4263A>G mutation resided at the processing site for the $tRNA^{Ile}$ 5′-end precursor results in reduced efficiency of the $tRNA^{Ile}$ precursor 5′-end cleavage catalyzed by RNase P [6]. The 4401A>G mutation situated at the spacer immediately to the 5′ end of $tRNA^{Met}$ and $tRNA^{Gln}$ genes causes reduction in the steady-state levels of both mitochondrial tRNAs [3]. The 4295A>G mutation, which is located at immediately 3′ end to the anticodon, corresponding to conventional position 37 of $tRNA^{Ile}$, has functional effect similar to that of the 4435A>G mutation [2]. Finally, the mitochondrial hypertension-associated ND1 T3308C mutation that locates in two nucleotides adjacent to the 3′ end of mitochondrial $tRNA^{Leu}$ UUR leads to an alteration on the processing of the H-strand polycistronic RNA precursors or the destabilization of ND1 mRNA [4]. However, despite a high penetrance, these mutations seem to be infrequent since they were observed in only a few families. For example, the 4263A>G mutation was found in only one family and was absent in 49 other families with matrilineal hypertension [6].

Up to 60% of people with type 2 diabetes (T2D) suffer from hypertension [8]. The long-term stress of high blood pressure levels increases the risk of other diabetic complications such as stroke, coronary artery disease (CAD), diabetic retinopathy, and nephropathy. A common form of human hypertension, e.g. essential hypertension (EH), is a highly polygenic condition caused by the combination of small changes in the expression of many genes, in conjunction with a variable collection of environmental factors. In total, 14 independent chromosome loci have been

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identified so far for blood pressure traits that reached genome-wide significance including replication in independent cohorts [9]. However, altogether these variants explain only a very small fraction of the heritability of blood pressure traits [10,11].

Since chromosomal DNA variants exhibit only a modest effect in EH, one may hypothesize that, in contrast, somatic mtDNA mutations could significantly contribute to the pathogenesis of hypertension, and genetic predisposition to EH may be influenced by a ratio between mutated and wild-type mtDNA, e.g. by heteroplasmy level. A non-redundant role of mtDNA heteroplasmy was shown in human aging [12] and several age-related pathologic conditions including atherosclerosis [13], Alzheimer's disease [14], and diabetes [15]. The entire mtDNA sequencing in US pedigrees of African and European descent revealed significant changes at the mtDNA sequence of hypertensive probands thereby suggesting for a potential role of mtDNA mutations in EH [16].

To date, the analysis of a role of somatic mtDNA mutations in EH (itself and as a vascular complication of diabetes) is still in its infancy. Furthermore, current knowledge of molecular mechanisms by which these mutations contribute to the pathogenesis of EH is widely unclear. Indeed, any pilot report dealing with the consideration of the involvement of mtDNA sequence alterations in hypertension may represent significant interest for further understanding of genetic roots of EH.

A G-to-A mutation at nucleotide 15059 of the mtDNA sequence was first described in a patient with mitochondrial myopathy [17]. This substitution results in the replacement of a glycine at amino acid position 190 of mitochondrial cytochrome *b* with a stop codon leading to a truncated protein that misses 244 amino acids at the C-terminus of cytochrome *b* [18]. In this study, we found association of this mtDNA mutation with EH in 189 unrelated Russian individuals affected with T2D.

2. Patients and methods

2.1. Subjects

This study was kept in accordance with the Helsinki Declaration of 1975 as revised in 1983. The study involved 189 unrelated patients (83 males, 106 females) who underwent a regular medical check-up in the Endocrinology Research Center (Moscow). On admission, a careful history was taken with special attention to cardiovascular risk factors including a family history of cardiovascular diseases and type T2D. All participants gave their informed consent prior to their inclusion in the study, and the protocol was approved by the ethics committee of the Endocrinology Research Center.

T2D was defined according to the American Diabetes Association diagnostic criteria as either a fasting plasma glucose concentration $\geq$126 mg/dL or a postprandial plasma glucose concentration $\geq$200 mg/dL 2 h after a 75-g oral glucose, glycated hemoglobin (HbA1c) $\geq$6.5% [19] and/or treatment by glucose-lowering agents. Obesity was defined as when body mass index (BMI) $\geq$ 30 kg/m^2 [20]. EH was diagnosed according to the European Society of Hypertension and the European Society of Cardiology classifications [21]. During the patient work-up, concomitant coronary heart disease (CHD) was evaluated by coronary angiography. Coronary angiography was interpreted as pathological if there was at least 50% stenosis in a coronary vessel. Coronary vessel disease on coronary angiography was identified as single, two, or three-vessel disease. Standard 12-lead echocardiography was used for the diagnosis of left ventricular hypertrophy (LVH) [21,22]. Myocardial infarction (MI) was diagnosed according to the joint criteria of the Expert Consensus Document [23].

2.2. Biochemical measurements

After collection, blood samples were centrifuged immediately, and plasma was stored at −70 °C. Plasma concentrations of cholesterol, were measured by gas–liquid chromatography–mass spectrometry using a HP 5890 series II gas chromatograph combined with a HP 5971 mass selective detector (Agilent Technologies, Böblingen, Germany) [24]. HbA1c was measured using an ion-exchange high performance liquid chromatography (normal reference range: 4.1–6.4%). Blood serum glucose was measured using the Glucose Assay Kit (BioVision, Mountain View, CA, USA). Plasma insulin levels were determined by means of an enzymatic immunoassay (Insulin Assay Kit, CisBio Bioassays, Bedford, MA, USA). Plasma lipoprotein and profiles were analyzed by enzymatic methods [25]. High density lipoprotein (HDL) cholesterol was measured enzymatically in the supernatant after the precipitation of apolipoprotein B-containing lipoproteins [26], and low density lipoprotein (LDL) cholesterol were calculated using the Friedewald formula [27].

2.3. DNA analysis

Mitochondrial DNA was isolated with the Aquapure Genomic DNA Blood Kit (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's protocols. The heteroplasmy level of the mtDNA mutation 15059 was quantified by the Taqman allele discrimination assay on the Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) as recommended by the manufacturer. PCR started at 95 °C for 15 s, followed by 40 cycles at 95 °C for 15 s and 60 °C for 60 s. The reaction mixture (25 μl) contained template DNA (5 ng), TaqMan Universal PCR Master Mix (2× composed of DNA polymerase AmpliTaq Gold), AmpErase UNG (uracil *N*-glycosylase), dNTPs with dUTP, and appropriate $MgCl_2$ concentrations according to the Applied Biosystems data sheet, 200 nmol/L of each TaqMan probe 5′-FAM-gcctatattacggat-catttctc-BHQ1-3′ and 5′-VIC-gcctatattacagatcatttctc-BHQ1-3′, and 200 nmol/L of each primer 5′-agacgtaaattatggctgaa-3 as forward and 5′-cctcagaatgatatttggc-3′ as reverse. The PCR products were quantified with an automatic sequence detection system (ABI Prism 7500; Applied Biosystems) at each step of amplification using the SDS software (version 1.0; Applied Biosystems). Primers were synthesized by Eurogene (Moscow, Russia).

2.4. Statistical analysis

Data were analyzed using a software package SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Clinical and biochemical parameters of groups of patients were compared using the two-sample *t*-test. The normality of the 15059G>A heteroplasmy frequency distribution in the study population was visually appreciated from normal probability plots and objectively evaluated by the Shapiro–Wilk *W*-test [28]. Quartiles with their confidence intervals (CI) were computed from each distribution according to Aczel [29] and Conover [30] and analyzed by *t*-test. Odds ratios (OR) and their 95% CI were calculated using the calculator for confidence intervals of odds ratio [31]. Two-tailed Fisher's exact test was used in the analyzis of 2 × 2 contingency tables. *P*-values of less then 0.05 were considered significant.

3. Results

Baseline characteristics of patients are presented in Table 1. Of 189 diabetic participants, 124 (66%) and 45 (24%) had clinically manifested EH and CHD, respectively. Compared to 65 normotensive subjects, hypertensive patients were significantly older (66.6 ± 9.2 yrs vs. 62.0 ± 9.1 yrs, $P < 0.001$), had higher systolic