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Research Article

Genetic predisposition in patients with hypertension and normal ejection fraction to oxidative stress

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Abstract

The role of oxidative stress (OXS) due to myocardial nitric oxide synthase (NOS) uncoupling related to oxidative depletion of its cofactor tetrahydrobiopterin (BH_4) emerged in the pathogenesis of heart failure with preserved ejection fraction. We determined the prevalence of six single nucleotide polymorphisms (SNPs) of genes encoding enzymes related to OXS, BH_4 metabolism, and NOS function in ≥60-year-old 94 patients with hypertension and 18 age-matched controls with normal ejection fraction. Using echocardiography, 56/94 (60%) patients with hypertension had left ventricular (LV) diastolic dysfunction (HTDD+ group) and 38/94 (40%) patients had normal LV diastolic function (HTDD− group). Four SNPs (rs841, rs3783641, rs10483639, and rs807267) of guanosine triphosphate cyclohydrolase-1, the rate-limiting enzyme in BH_4 synthesis, one (rs4880) of manganese superoxide dismutase, and one (rs1799983) of endothelial *NOS* genes were genotyped using real-time polymerase chain reaction method and Taqman probes. Protein carbonylation, BH_4, and total biopterin levels were measured from plasma samples. No between-groups difference in minor allele frequency of SNPs was found. We calculated a genetic score indicating risk for OXS based on the minor allele frequencies of the SNPs. A high genetic risk for OXS was significantly associated with HTDD+ even after adjustment for confounding variables (odds ratio [95% confidence interval]:4.79 [1.12–20.54]; $P = .035$). In both patient groups protein carbonylation ($P < .05$ for both), plasma BH_4 ($P < .01$ for both) and in the HTDD+ group total biopterin ($P < .05$) increased versus controls. In conclusion, in patients with hypertension and normal ejection fraction, a potential precursor of heart failure with preserved ejection fraction, a partly genetically determined increased OXS, seems to be associated with the presence of LV diastolic dysfunction. J Am Soc Hypertens 2016;10(2):124–132.

Keywords: Heart failure with preserved ejection fraction; hypertension; oxidative stress.

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Introduction

Hypertension is the most common underlying cause of heart failure with preserved ejection fraction (HFPEF), which partly or entirely accounts for 78%–88% of HFPEF cases.[1–3] The transition of hypertensive heart disease to HFPEF is characterized by progressive left ventricular (LV) hypertrophy and deterioration of LV diastolic and atrial function.[4] Oxidative stress (OXS) mainly due to myocardial nitric oxide synthase (NOS) uncoupling as a consequence of the depletion of the NOS cofactor tetrahydrobiopterin (BH_4) may play a decisive role in this transition process.[5–8]

Both essential hypertension and LV diastolic dysfunction, the latter is considered the main pathophysiologic mechanism of HFPEF, are partly determined genetically by a large number of genes, each exerting only a small effect.[9,10] Thirty[11] or 30%–60%[12] of blood pressure variation can also be attributed to genetic influences, and environmental exposures account for the remaining 40%–70%.

In this study, we sought to investigate gene polymorphisms related to both hypertension or endothelial dysfunction and OXS or BH_4 metabolism to assess whether there is a genetic predisposition to OXS in patients with hypertension and normal ejection fraction (EF), and if yes, how it is associated with LV diastolic dysfunction in these patients. To this end, we determined the prevalence of six single nucleotide polymorphisms (SNPs) of genes encoding enzymes related to OXS, BH_4 metabolism, and NOS function. The prevalence of four SNPs (rs841 C>T, rs3783641 A>T, rs10483639 C>G, and rs8007267 G>A) of guanosine triphosphate cyclohydrolase-1 (GTPCH-1), the rate-limiting enzyme in BH_4 synthesis, one (rs4880 T>C) of manganese superoxide dismutase (MnSOD), and one (rs1799983 G>T) of endothelial NOS (*NOS3*) genes were determined.

The studied four GTPCH-1 SNPs are strongly linked to each other, and later it was referred as the ''pain-protective'' GTPCH-1 haplotype.[13] The minor alleles of these SNPs are associated with reduced pain sensitivity, mildly increased blood pressure, and heart rate due to decreased BH_4 production (and consequential decreased nitric oxide–mediated endothelial function and increased vascular superoxide anion radical [O_2^-] production), which mainly manifests in pathophysiological situations when BH_4 production would be normally increased due to upregulation of GTPCH-1, for example at inflammatory sites or in injured neurons[13] or in blood vessels exposed to high blood pressure, high cholesterol, or other cellular stress factors.[13]

MnSOD encoded by *SOD2* gene is an enzyme catalyzing dismutation of O_2^- to hydrogen peroxide and is an important substituent of cellular enzymatic antioxidant defense against OXS.[14] MnSOD proved to be essential for the survival of aerobic organisms, demonstrated by the extremely short survival of MnSOD knockout mice, which died shortly after birth with dilated cardiomyopathy and neurodegeneration.[14] The extensively investigated rs4880 SNP (T > C change at nucleotide level) causes a substitution of valine (GTT) with alanine (GCT) at codon 16. The alanine variant of MnSOD has an α-helical mitochondrial targeting domain, whereas the valine variant of MnSOD has a β-pleated sheet conformation.[14–16] This conformational difference results in a more efficient transport of alanine variant of MnSOD into mitochondria than the valine variant,[14–16] which results in a more efficient protection against OXS. The homozygote Ala/Ala genotype has a 30%–40% higher MnSOD activity than its Val/Val counterpart.[14–16] The alanine variant is associated with decreased risk for coronary artery disease, myocardial infarction, and atherosclerosis, whereas the Val/Val genotype is an independent genetic risk factor for coronary artery disease and vasospastic angina.[15–17]

Endothelial nitric oxide synthase (NOS3 = eNOS) synthesizes nitric oxide from L-arginine, which is a key mediator of endothelial function and reduces OXS by scavenging O_2^-. The 894 G/T substitution within exon 7 in the rs1799983 G>T SNP of *NOS3* gene leads to a 298 Glu/Asp substitution in the mature protein. It has been reported that the 298Asp variant has an enhanced susceptibility to intracellular proteolytic cleavage compared with the 298Glu variant and has been associated with a lower eNOS activity and reduced generation of nitric oxide.[18,19] However, other studies have not confirmed these associations.[20] The minor allele variant of the rs1799983 G>T SNP of *NOS3* gene may be associated with endothelial dysfunction and increased risk for coronary artery disease.[21–23]

Materials and Methods

Patients

The study was conducted from December 2007 to July 2012 at the 3rd Department of Medicine, Semmelweis University, Budapest. The study complied with the Declaration of Helsinki and was approved by the Institutional Committee on Human Research. All participants signed an informed consent. We designed to prospectively enroll 100 hypertensive patients with normal left ventricular ejection fraction (LVEF) (>50%) and 40 normotensive, healthy controls ≥60 years old over 3 years, but even during an extended period, we could enroll only 94 hypertensive patients and 18 age-matched controls. Each patient was followed up for at least 1 year and 44 patients for 3 years (the average follow-up period was 23.3 ± 12.5 months). Each patient underwent a physical examination, an electrocardiogram, a detailed echocardiography, a carotid ultrasound, and a chest X-ray at annual follow-up examinations. This study is a part of a multipurpose study conducted in the