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Is hyperhomocysteinemia a causal factor for heart failure? The impact of the functional variants of *MTHFR* and *PON1* on ischemic and non-ischemic etiology

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ABSTRACT

Background: Hyperhomocysteinemia was found to be uniformly associated with the development of heart failure (HF) and HF mortality; however, it is uncertain whether this relation is causative or not. We used Mendelian randomization to examine the associations of the methylene tetrahydrofolate gene (*MTHFR*) and paraoxonase 1 gene (*PON1*) variants as a proxy for lifelong exposure to high Hcy and Hcy-thiolactone concentrations with the development of HF in men aged ≤60 years and the occurrence of adverse effects at one–year follow–up.
Methods: The study enrolled 172 men with HF: 117 with ischemic etiology (iHF) related to coronary artery disease (CAD) and 55 with non-ischemic etiology (niHF) related to dilated cardiomyopathy (DCM). The reference group of 329 CAD patients without HF and the control group of 384 men were also analyzed.
Results: Hyperhomocysteinemia (OR = 2.0, $P < 0.05$) and the *MTHFR 677TT/1298AA*, *677CC/1298CC* genotypes (OR = 1.6, $P = 0.03$) were associated with HF regardless of its etiology, especially among normotensives (OR = 4.6, $P = 0.001$ and OR = 2.3, $P = 0.003$, respectively). In niHF, the *PON1 162AA* (OR = 2.3, $P = 0.03$) and *575AG + GG* (OR = 0.46, $P = 0.01$) genotypes also influenced the risk. The interaction between HDLC < 1 mmol/L and the *PON1 575GG* genotype was found to influence the risk of iHF (OR = 7.2, $P = 0.009$). Hyperhomocysteinemia improved the classification of niHF patients as 'high-risk' by 10.1%. Ejection fraction <30% and DCM increased the probability of HF death or re-hospitalization within one year.
Conclusion: Our results provide evidence that hyperhomocysteinemia is a causal factor for niHF in DCM, while dysfunctional HDL could contribute to the pathogenesis of iHF.

1. Introduction

Chronic heart failure (HF) is an increasingly common cause of death in developed countries. The factors that underlie this epidemiological observation are complex, including the increase in the average age of life expectancy on the one hand and the prolongation of the lives of cardiac patients by modern therapy on the other [1]. In patients with coronary artery disease (CAD), damage of the myocardium induced by myocardial infarction very often leads to the development of systolic HF. Another common cause of HF is high blood pressure. In 5–20% of patients, HF is initiated by cardiomyopathy, usually idiopathic dilated cardiomyopathy (DCM), in which the development of left ventricular dysfunction occurs in the absence of abnormal loading conditions (valve disease, CAD, or arterial hypertension) [2]. Several clinical studies point to high levels of homocysteine (Hcy) as another important risk factor for HF [3–8] (data reviewed in Table I, online only). Adding hyperhomocysteinemia (HHcy) to the existing clinical risk scores may significantly improve the assessment of the risk of future cardiovascular disease, including HF [9]. Hcy is a non-proteinogenic sulfur amino acid whose metabolism is at the intersection of two metabolic pathways: remethylation and transsulfuration; however, transsulfuration is nonexistent in cardiovascular tissues [3]. The most frequent causes of mild-to-moderate HHcy are folic acid (FA) or vitamin B12 deficiency and the presence of a functional variant in the gene encoding methylenetetrahydrofolate reductase (MTHFR), the key enzyme involved in Hcy remethylation [3]. Two genetic polymorphisms of the *MTHFR* gene: *677C>T* (rs1801133, Ala222Val) and *1298A>C* (rs1801131, Glu429Ala) can influence plasma Hcy levels [10,11]. Individuals with

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the *677TT* and *677CT* genotypes have 30% and 65% of the expected enzyme activity; in turn, in homozygotes of the *1298C* allele, the enzyme's activity is decreased to 60% [11].

Hcy can also be metabolized to the thioester Hcy-thiolactone in an error-editing reaction in protein biosynthesis [12]. This highly reactive metabolite of Hcy contributes to its adverse effects on the cardiovascular system and is hydrolyzed back to Hcy by paraoxonase 1 (PON1). PON1 is a calcium-dependent esterase located on high-density lipoproteins, which is involved in the antioxidant protection conferred by these lipoproteins on low-density lipoprotein oxidation [13]. Polymorphisms in the coding region of the *PON1* gene: *162T>A* (rs854560, Leu55Met) and *575A>G* (rs662, Arg192Gln) account for an approximately 10-fold inter-individual variation in Hcy thiolactonase activity, with higher activity demonstrated for carriers of the *162T* and *575G* alleles [12,14]. In contrast, isoenzymes encoded by alleles *162A* and *575A* have higher antioxidant capacity towards oxidized low-density lipoprotein [15]. Another functional variant, *−108C>T* (rs705379), located within the promoter region, influences the expression of the *PON1* gene and explains nearly 25% of the inter-individual variability in the plasma concentration of this enzyme [16]. In previous studies, the *MTHFR* and *PON1* polymorphisms were considered as potential risk factors for cardiovascular disease [14,17,18], but few publications were focused on HF [5,19,20].

The evidence for the causal relation between HHcy and the development of HF originates mainly from experimental studies [21] (data reviewed in Table I, online only), as the validity of clinical research focused on Hcy can be affected by numerous unmeasured confounding factors associated with both the disease and exposure. The approach of Mendelian randomization, using common genetic polymorphisms that affect protein function and modify exposure to a particular factor as proxies that are less susceptible to confounding or reverse causality, may strengthen the ability of these studies to draw causal inferences [22].

The aim of the present study was to evaluate the relationships between the *MTHFR* (*677C>T*, *1298A>C*) and *PON1* (*−108C>T*, *162T>A*, and *575A>G*) genotypes, the serum concentrations of Hcy and FA, and the development of systolic HF before the age of 60. Male patients with ischemic HF (iHF) related to CAD and those with non-ischemic HF (niHF) related to DCM were considered. The role of arterial hypertension and lipoprotein levels as modifier factors was also assessed. Finally, the impact of the studied factors on the course of HF within the period of one year was analyzed. Because of Mendelian randomization, we expected to find that the *MTHFR 677TT* and *1298CC* genotypes (as a proxy of lifelong high blood Hcy concentration) and the *PON1 162AA* and *PON1 575AA* genotypes (as a proxy of lifelong augmented Hcy-thiolactone exposure) would be associated with an increased risk of HF development and/or its fatal course if high blood Hcy concentrations were a causal factor for HF occurrence and/or progression.

2. Patients and methods

2.1. Study population

The study included 446 men with CAD, 55 men with systolic HF related to DCM, and 384 control men without symptoms of heart disease. The study subjects were selected from patients scheduled for coronary angiography at two institutions from western Poland in the years 2003–2013. The controls were age- and sex-matched volunteers selected simultaneously, who received negative electrocardiographic treadmill test results according to the Bruce protocol. The cardiac structure and function in all subjects were assessed by electrocardiography and echocardiography.

CAD was defined by positive coronary angiography, while the inclusion criteria for DCM-related niHF were: myocardial dilatation and left ventricular ejection fraction ≤ 40% (evaluated by echocardiography), and the absence of: CAD (evaluated by coronary angiography), uncontrolled hypertension (blood pressure > 160/110 mm Hg confirmed by 3 repeated measurements), hemodynamically significant valvular heart disease, and markers of active myocarditis. The additional exclusion criteria for the study of Hcy/FA were: blood creatinine level > 124 μmol/L, the use of vitamin supplements (B_{12}, B_6, FA) or drugs (fibrates, methotrexate, metformin, theophylline) affecting the Hcy level within the period of 6 months prior to enrollment, and history of systemic lupus, cancer, or congenital storage diseases. HHcy was recognized at total Hcy levels in serum ≥ 15 μmol/L.

Based on the criteria presented by Perk et al., the clinical risk factors were categorized as follows: smoking (current or within last 10 years); diabetes (fasting glucose level > 7 mmol/L, HbA1c > 6.5%, or current treatment with insulin or oral hypoglycemic agents); and hypertension (blood pressure > 140/90 or current treatment with antihypertensive agents) [23]. The studied subjects were treated pharmacologically with statins, antiplatelet drugs, and other drugs: antihypertensive or antidiabetic, depending on their clinical condition. In accordance with the work by D'Agostino et al., the Framingham Risk Score (FRS) was calculated based on the following parameters: sex, age, systolic blood pressure, hypertension treatment, current smoking, diabetes, and TC and HDLC levels [24]. A group of 125 patients with HF (84 with CAD, 41 with DCM) completed the one-year observation of adverse cardiovascular events.

The study protocol was approved by the local Bioethics Committee of the Poznan University of Medical Sciences, and the subjects provided their informed consent to participate in the study. All experiments were carried out in compliance with the relevant laws and guidelines in accordance with the ethical standards of the Declaration of Helsinki.

2.2. Biochemical analyses and genotyping

Standardized laboratory protocols were used to determine the blood levels of total high-density lipoprotein and low-density cholesterol, triglycerides, glucose, and uric acid (UA). The total Hcy and FA levels were assessed by competitive immunoassays using commercially available diagnostic tests for the IMMULITE 2000 analyzer (MONLAB, Spain). Genomic DNA was extracted from circulating blood lymphocytes by using an acid guanidinium thiocyanate and phenol/chloroform extraction. The *MTHFR* and *PON1* polymorphisms were determined by PCR-RFLP according to a previously described method [10,11,16,25].

2.3. Statistical analysis

The quantitative variables were compared using ANOVA and a *t*-test or Kruskal–Wallis and Mann–Whitney *U* tests, as appropriate. Qualitative parameters were compared using a χ^2 test or Fisher's exact test. Genotype frequencies were tested for Hardy-Weinberg equilibrium using a χ^2 test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl), while linkage disequilibrium between the *MTHFR* and *PON1* polymorphisms and haplotype frequencies were evaluated using Haploview software [26]. The values of odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated for the effects of the genotype and HHcy, which were further adjusted for the FRS values by logistic regression. Gene-environmental interactions were assessed with the two-by-four table according to Botto and Khoury [27]. Independent predictors of Hcy concentration were selected by multivariable regression analysis, in which Hcy and FA levels were studied after logarithmic transformation (as ln-Hcy and ln-FA, respectively). Clinical factors evaluated in this analysis included: age, smoking status, arterial hypertension, diabetes, BMI values, blood lipid and lipoprotein levels, and genotypes. Pearson's coefficients (r) were calculated for correlations between: plasma Hcy and UA; plasma Hcy and the studied polymorphisms (model of dose of variant allele); and the blood levels of lipids and lipoproteins and the studied polymorphisms (model of dose of variant allele). The levels of Hcy and UA were studied after logarithmic transformation (as ln-Hcy and ln-UA, respectively).