Association between Rs6226 and Rs6224 Polymorphisms of the Furin Gene and Cardiovascular Diseases

Ewa Podolecka* and Ewa Zukowska-Szczechowska1

Abstract

Purpose: This study focused on the association between functional polymorphisms of the furin gene and cardiovascular disease.

Methods: Genotyping was conducted on 504 subjects with a mean age of 56.7 ± 9.5 years. Allelic discrimination assay was used to detect rs6226 and rs6224 polymorphisms. Among with echocardiography and BP, vascular compliance of large (C1) and small (C2) arteries was also measured.

Results: Women with the CC genotype of the rs6226 polymorphism had higher systolic blood pressure (SBP) compared to the CG genotype (p=0.0199). Men with CC and CG genotypes of the rs6226 polymorphism had lower small artery elasticity indices (C2) compared to GG genotype (p=0.0185). Ischemic heart disease was more common in women with CC when compared to GG genotype of the rs6226 polymorphism (p=0.00080) and with TT compared to GG and GT genotypes of the rs6224 polymorphism (p=0.0059). Myocardial infarction was more common among men with CC compared to GG genotype of the rs6226 polymorphism (p=0.0250) and with GT and TT compared to GG genotype of rs6224 polymorphism (p=0.0053). A weak link was found between rs6226 and rs6224 polymorphisms (rs=0.4724).

Conclusions: The rs6226 and rs6224 furin polymorphisms are associated with cardiovascular diseases, but appear to exert independent functional effects on their pathogenesis.

Keywords: Furin; Functional polymorphisms; Metalloproteinases; Extracellular matrix; Cardiovascular risk factors

Abbreviations

BMI: Body Mass Index; C1-Large Artery Elasticity Index; C2-Small Artery Elasticity Index; DBP: Diastolic Blood Pressure; IVSDd: Intraventricular Septum Diastolic Diameter; LVEDV: Left Ventricle End-Diastolic Volume; LVEF-Left Ventricle Ejection Fraction; LVM: Left Ventricle Mass; LVMi: Left Ventricle Mass Index; MMPs: Metalloproteinases; MMP-1: Matrix Metalloproteinase-1; MMP-2: Matrix Metalloproteinase-2; MMP-9: Matrix Metalloproteinase-9; MT-1-MMP: Pro-Metalloproteinase 1; pro-MMP-2: Pro-Metalloproteinase 2; PWDd: Posterior Wall Diastolic Diameter; SBP: Systolic Blood Pressure; WHR: Waist/Hip Ratio

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Background

Furin is an enzyme of the serine proteinase family that catalyses the maturation of a group of pro-protein substrates. The protein is encoded by the furin gene located on the q arm of chromosome 15, locus 26.1 [1-3]. Furin is synthesized in a non-active form, as a pro-protein, which changes into the active form during autocatalysis. The furin gene transcript has been detected in all tested human cells and tissues [4-7] and plays an important role in the pathogenesis of numerous bacterial, viral, and neoplastic diseases. It is also believed to modulate the development and progression of atherosclerosis via the activation of specific matrix metalloproteinases (MMPs) [8].

Furin activates pro-metalloproteinase 1 (MT-1-MMP) in the Golgi apparatus during proteolysis. Later, enzymatically active MMP-1 is released outside the cell and triggers pro-metalloproteinase-2 (pro-MMP-2) activation [9,10].

MMP-1 and MMP-2 play a significant role in the migration and proliferation of smooth muscle cells; they participate in vascular wall remodelling and in the development and progression of atherosclerotic lesions [11]. As a result of vascular wall remodelling, vascular compliance decreases. Increased arterial wall stiffness is an established risk factor of cardiovascular diseases [12,13] and increases pulse wave velocity in the cardiovascular system. Measurement of vascular compliance is a non-invasive method to assess arterial wall stiffness [14].

The aim of our study was to determine the relationship between the rs6226 and rs6224 polymorphisms of the furin gene and risk factors for cardiovascular disease including blood pressure, vascular compliance, and parameters of left ventricular hypertrophy. We also sought to determine the relationship between individual polymorphisms and the incidence of ischemic heart disease and myocardial infarction, and further investigated a potential link between these 2 polymorphisms.

Methods

The study group consisted of 504 unrelated men and women (mean age 56.7 ± 9.5 years). There were 320 men (mean age 57.3 ± 9.2 years) and 184 women (mean age 55.8 ± 10.0 years) in the study group (Tables 1 and 2). Subjects were recruited in 3 reference centers for cardiovascular diseases in the south of Poland. Recruitment was conducted through probands-index patients with high cardiovascular risk: co-existence of cardiovascular disease, hypertension, or risk: co-existence of cardiovascular disease, hypertension, or
phenotypes to the candidate genes” was conducted with the consent of the Ethical Board at the Silesian Medical University (Consent No. NN-013-145/03).

Fasting serum glucose, TC-cholesterol, HDL-cholesterol, triglycerides and LDL-cholesterol were determined in all subjects. LDL cholesterol levels were determined using Friedwald formula: 
\[ \text{LDL} [\text{mmol/l}] = [\text{TC} - \text{HDL} - \left( \frac{\text{TG}}{2} \right)] \]

Genotyping was conducted using the iPLEX™ assay on Sequenom MassARRAY® system (Sequenom Inc., San Diego, US) according to the manufacturer’s protocol. Allelic discrimination assay was performed to detect the rs6226 and rs6224 polymorphisms of the furin gene.

The cardiac echo involved the measurement of parameters characterizing the left ventricle, such as posterior wall diastolic diameter (PWDd), inter-ventricular septum diastolic diameter (IVSDD), left ventricular end-diastolic volume (LVEDV) and left ventricular ejection fraction (LVEF%). The left ventricular mass (LVM) was calculated using Devereux formula:
\[ \text{LVM} [\text{g}] = 1.04 \left( \frac{[\text{IVSDD} + \text{PWDd} + \text{LVEDV}]}{10} \right) - 13.6. \]

The vascular compliance of large (C1) and small (C2) arteries was performed using the HDI/Pulse Wave™ CR-2000 Research Cardiovascular Profiling Instrument. This instrument differentiates between 2 systems - the high-pressure system, i.e. the aorta with its main branches and the low-pressure system, consisting of peripheral arteries. The results of these measurements were the large artery elasticity index (C1) expressed in ml/mmHgx10 and the small artery elasticity index (C2) expressed in ml/mmHgx100, as well as the oscillometric measurement of SBP and DBP [15,16].

Table 1: Demographic and clinical characteristics of the study group.

<table>
<thead>
<tr>
<th>Phenotypic traits (n=504)</th>
<th>Mean value ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.7 ± 9.5</td>
</tr>
<tr>
<td>Body mass index (BMI) (kg/m2)</td>
<td>27.7 ± 4.1</td>
</tr>
<tr>
<td>Waist to hip ratio (WHR)</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Systolic blood pressure (SBP) (mmHg)</td>
<td>148.3 ± 22.3</td>
</tr>
<tr>
<td>Diastolic blood pressure (DBP) (mmHg)</td>
<td>85.6 ± 12.5</td>
</tr>
<tr>
<td>Large artery elasticity index (C1) (ml/mmHgx10)</td>
<td>10.3 ± 3.9</td>
</tr>
<tr>
<td>Small artery elasticity index (C2) (ml/mmHgx100)</td>
<td>2.9 ± 2.1</td>
</tr>
<tr>
<td>Posterior wall diastolic diameter (PWDd) (mm)</td>
<td>10.6 ± 1.8</td>
</tr>
<tr>
<td>Interventricular septum diastolic diameter (IVSDD) (mm)</td>
<td>10.9 ± 1.7</td>
</tr>
<tr>
<td>Left ventricular end-diastolic volume (LVEDV) (cm3)</td>
<td>137.5 ± 44.2</td>
</tr>
<tr>
<td>Left ventricular mass (LVM) (Devereux) (g)</td>
<td>251.5 ± 75.0</td>
</tr>
<tr>
<td>Left ventricular mass index (LVMI) (g/m²)</td>
<td>133.2 ± 36.1</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (LVEF) (%)</td>
<td>55.7 ± 7.9</td>
</tr>
<tr>
<td>Fasting serum glucose (mg %)</td>
<td>97.2 ± 32.4</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.3 ± 1.4</td>
</tr>
<tr>
<td>HDL - cholesterol (mmol/l)</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.8 ± 1.0</td>
</tr>
<tr>
<td>LDL - cholesterol (mmol/l)</td>
<td>3.5 ± 1.3</td>
</tr>
</tbody>
</table>

Table 2: Clinical characteristics of the study group.

<table>
<thead>
<tr>
<th>Phenotypic trait (n=504)</th>
<th>No. of subjects</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statin use</td>
<td>191</td>
<td>37.9%</td>
</tr>
<tr>
<td>Hypotensive medication use</td>
<td>379</td>
<td>75.2%</td>
</tr>
<tr>
<td>Smoking status</td>
<td>305</td>
<td>60.5%</td>
</tr>
<tr>
<td>Blood hypertension</td>
<td>392</td>
<td>77.8%</td>
</tr>
<tr>
<td>DM 2</td>
<td>72</td>
<td>14.3%</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>332</td>
<td>65.9%</td>
</tr>
<tr>
<td>Myocardial infarction (MI)</td>
<td>207</td>
<td>41.1%</td>
</tr>
</tbody>
</table>

Statistical Analysis

The values of quantitative variables were compared between subjects with the respective analyzed genotypes of furin gene polymorphism using non-parametric Kruskal–Wallis one-way analysis of variance. The distribution of qualitative variables in the analysed patient groups was compared using the χ2 test.

Multiple linear regression analysis was used in order to determine which parameters were independently related to such variables as C1 and C2; SBP and DBP; as well as LVM and LVMI. This analysis involves all possible combinations of the variables, apart from individual genotypes of both studied furin gene polymorphisms. A p value of <0.05 was assumed as the statistical significance criterion.

The Hardy-Weinberg law was used in order to determine the distribution of genotypes of rs6226 and rs6224 polymorphisms of the furin gene. In order to investigate a potential association between these 2 polymorphisms, the CubeX software was used [17]. The measurement of the association between the polymorphisms is referred to as r² coefficient, which can have a value between 0 (zero association) and 1 (100% association).

Results

Mean basal and clinical characteristics of the study group (mean age 56.7 ± 9.5 years) are shown in Tables 1 and 2, including SBP and DBP, serum glucose and lipid levels, parameters characterizing the left ventricle, and the large (C1) and small artery (C2) elasticity indices. In the study population, the large artery elasticity index (C1) was inversely proportional to the subject’s age, SBP and coronary artery disease, while the negative determinants of C2 were age, SBP, WHR, smoking and coronary artery disease (Table 3). Factors independently related to higher SBP included age, BMI, female gender and diabetes. Multifactorial analysis further revealed a linear relationship between LVM and SBP, body surface area (BSA), WHR, male gender and ischemic heart disease (Table 3).

Women with the CC genotype of the rs6226 polymorphism had higher SBP compared to the CG genotype (p=0.0199) (Table S1). Men with the CC and CG genotypes of the rs6226 polymorphism showed lower values of small artery elasticity index (C2) compared to men with the GG genotype (p= 0.0185). Women with the CC genotype variant of the rs6226 polymorphism developed ischemic heart disease more often, compared to those with the CG and GG genotypes (Table S1). In the same group of women, higher SBP was also found. Women with the TT genotype variant of the rs6224 polymorphism also developed ischemic heart disease more often, compared to those with the GG and GT genotypes (Table S2). These female subjects
often (Table S2). Therefore it can be assumed that the GT and TT variants of the rs6224 polymorphism developed myocardial infarction more often compared to those with the GG genotype (Table S1). In the same group of male patients with the GT variant of the rs6226 polymorphism there was a relationship between reduced small artery compliance and an increased incidence of ischemic heart disease. Men with the GT variant of the rs6226 polymorphism developed myocardial infarction more often compared to those with the CC genotype. Takeuchi et al. demonstrated lower values of small artery elasticity index in patients with ischemic heart disease compared to the control group. Increased small artery stiffness leads to left ventricular hypertrophy because of increased vascular resistance. Our results are in agreement with data published before [29-31]. For example, in the MESA study Heckbert et al. demonstrated a relationship between LVM and coronary artery disease (Table 3).

The r'coefficient calculated for the rs6226 and rs6224 polymorphisms was 0.4724.

**Discussion**

Atherosclerosis is a multifactorial process involving cellular, extracellular and inflammatory components. Matrix metalloproteinases (MMPs), including matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9), are involved in vascular wall remodelling and in the development and proliferation of atherosclerotic plaques. Both MMPs are activated by furin. Furin also activates - by means of intracellular proteolysis - pro-metalloproteinase-1 (MT-1-MMP), transforming it into matrix metalloproteinase-1 (MMP-1). At a later stage, enzymatically active MMP-1 is released outside the cell and acts as a trigger for pro-metalloproteinase 2 (pro-MMP-2) activation and its transformation to MMP-2. These proteases play a substantial role in migration and proliferation of smooth muscle cells, thus participating in vascular wall remodelling [8,9]. Vascular wall remodelling requires degradation and re-synthesis of extracellular matrix (ECM), which is composed of structural proteins (collagen and elastin), glycosaminoglycans, proteoglycans and adhesion ligands. MMPs are the main enzymes responsible for collagen degradation, and furin participates in vascular wall remodelling by means of MMP activation [18,19]. The inflammatory process shows a correlation with increased vascular wall stiffness. A relationship was also shown to exist between the increased levels of C-reactive protein and fibrinogen and reduced vascular compliance. As atherosclerosis progresses, vascular compliance decreases [20-23].

Hypertension predisposes patients to develop atherosclerosis, while reducing vascular compliance at the same time [24,25]. Raffetto et al. showed an increased MMP-2 expression in the aortic walls of patients with primary hypertension [26]. In our study, the large artery elasticity index (C1) was inversely proportional to the subject’s age, SBP and coronary artery disease (Table 3).

Syeda et al. demonstrated the relationship between reduced small artery compliance and the incidence of ischemic heart disease [27]. Takeuchi et al. found lower values of small artery elasticity index in patients with ischemic heart disease compared to the control group. Increased small artery stiffness was related to ischemic heart disease, regardless of patient’s age, gender and blood pressure [28]. Increased vascular wall stiffness leads to left ventricular hypertrophy because of increased vascular resistance. Our results are in agreement with data published before [29-31]. For example, in the MESA study Heckbert et al. demonstrated a relationship between LVM and SBP, BMI, diabetes and smoking [29].

In atherosclerosis, which plays a crucial role in the pathogenesis of ischemic heart disease, macrophages and smooth muscle cells migrate from the intima media to the tunica adventitia and proliferate [32-34]. Inflammatory cytokines increase MT-1-MMP activity in vascular endothelial cells, smooth muscle cells and macrophages. Furin participates in the activation of this enzyme by proteolysis. MMP-2 activated by MMP-1 is the most frequently found among all the metalloproteinases in smooth muscle cells. Its activation gives a rise to smooth muscle cell migration to the tunica adventitia. Zempo et al. demonstrated a relationship between MMP-2 activity and smooth muscle cell migration to the tunica adventitia after vascular wall injury [35]. In patients with acute coronary syndromes, elevated MMP-2 levels are observed [36-38]. This protease was identified in

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**Table 3**: Multiple linear regression model for the variables independently related to systolic blood pressure (SBP), diastolic blood pressure (DBP), large artery elasticity index (C1), small artery elasticity index (C2), left ventricular mass (LVM) and left ventricular mass index (LVMI).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variables</th>
<th>Standardized regression coefficient</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP Corrected R²=0.12 p&lt;0.001</td>
<td>Age, BMI, Female sex, Diabetes mellitus</td>
<td>β=0.23, β=0.20, β=0.14, β=0.13</td>
<td>p&lt;0.001, p&lt;0.001, p&lt;0.001, p=0.003</td>
</tr>
<tr>
<td>DBP Corrected R²=0.08; p&lt;0.001</td>
<td>BMI, Age</td>
<td>β=0.18, β=0.14</td>
<td>p=0.001, p=0.001</td>
</tr>
<tr>
<td>C1 Corrected R²=0.59 p&lt;0.001</td>
<td>SBP, BSA, Age, Coronary artery disease</td>
<td>β=−0.67, β=0.28, β=0.14, β=0.11</td>
<td>p&lt;0.001, p&lt;0.001, p&lt;0.001, p=0.001</td>
</tr>
<tr>
<td>C2 Corrected R²=0.51 p&lt;0.001</td>
<td>SBP, Body area, Coronary artery disease, Smoking, GG polymorphism of rs6226, Waist to hip ratio (WHR), Age</td>
<td>β=−0.57, β=0.34, β=−0.18, β=−0.12, β=−0.12, β=−0.11, β=−0.09</td>
<td>p&lt;0.001, p=0.001, p&lt;0.001, p&lt;0.001, p&lt;0.001, p=0.008, p=0.01</td>
</tr>
<tr>
<td>LVM Corrected R²=0.33 p&lt;0.001</td>
<td>Waist to hip ratio (WHR), Coronary artery disease, Body area, SBP, Male sex, Height</td>
<td>β=0.14, β=0.22, β=0.32, β=0.21, β=0.21, β=0.17</td>
<td>p&lt;0.001, p=0.02, p&lt;0.001, p=0.02, p&lt;0.001, p=0.03</td>
</tr>
<tr>
<td>LVM Corrected R²=0.20 p&lt;0.001</td>
<td>Coronary artery disease, Waist to hip ratio (WHR), Age, Male sex, Height, SBP</td>
<td>β=0.20, β=0.16, β=0.10, β=0.23, β=0.16, β=0.09</td>
<td>p&lt;0.001, p=0.002, p=0.003, p=0.04, p=0.008, p=0.04</td>
</tr>
</tbody>
</table>

 did not differ significantly in terms of pre-existing risk factors for cardiovascular disease (Table S2).

In our study, men with the CC variant of the rs6226 polymorphism developed myocardial infarction more often compared to those with the CG and GG genotypes (Table S1). In the same group of male subjects, lower values of small artery elasticity index (C2) were found, which confirms previous results that demonstrated a relationship between reduced small artery compliance and an increased incidence of cardiovascular disease. On the other hand, men with the GT and TT variants of the rs6224 polymorphism developed myocardial infarction more often compared to those with the GG genotype (Table S2). Male subjects with the GG, GT and TT genotypes did not differ significantly in terms of pre-existing risk factors of cardiovascular disease.

Women with the TT variant of the rs6224 polymorphism developed ischemic heart disease more often compared to those with the GG and GT genotypes, whereas men with GT and TT variants of the rs6224 polymorphism developed myocardial infarction more often (Table S2). Therefore it can be assumed that the GT and TT genotype variants of the rs6224 polymorphism are also related to higher activity of the enzyme.
References

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patients with stable coronary heart disease (from the Heart and Soul Study). Am J Cardiol 9: 1131-1135.


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