The association between 3020INSC mutation in NOD2 gene and arterial stiffness in hypertensives

Summary

Background The innate immune system elicits an inflammatory process which is believed to be involved in the development of vascular damage in hypertension. The NOD2 gene (CARD15) is involved in the control of the innate immune system. The aim of this study was to assess the occurrence of the 3020insC mutation in the NOD2 gene in relation to vascular damage in hypertensives.

Materials and methods In 466 hypertensives, genotyping for 3020insC mutation, cholesterol, serum creatinine, and echocardiography were carried out. Systolic (SBP) and diastolic (DBP) blood pressure and pulse pressure (PP) were taken at 2–3 month intervals for one year and treated according to ESC/ESH guidelines.

Results The 3020insC mutation was found in 7.08% of hypertensives and was associated with earlier onset of hypertension 48.2 ± 12.1 vs. 42.8 ± 10.9, p < 0.01 (age of pts). After one year of treatment, patients with the mutation had significantly higher SBP (138.48 ± 18.35 vs. 131.74 ± 14.97, p < 0.04) and PP (57.27 ± 14.04 vs. 52.70 ± 12.96, p < 0.04) than patients without the mutation, whereas the number of drugs was the same in both groups.

Conclusions The results suggest that the 3020insC mutation is associated with earlier onset of hypertension and increased arterial stiffness

key words: arterial stiffness, innate immunity, NOD2 mutation


Introduction

The structural and functional changes of vascular vessels characterize arterial hypertension. Remodelling of vessels results in thickening of the artery wall, increased pulse pressure, and atherosclerosis [1, 2]. Many factors are believed to be involved in the development of vascular damage in hypertension, and recently, innate and adaptive immune mechanisms are recognized as a cause of atherosclerosis and remodelling of vessels [3–6]. The innate immune system elicits inflammatory response to various pathogens via various receptors [7–9]. The NOD2 gene (also known as CARD15), discovered in 2001, is believed to be involved in the control of the innate immune system activating nuclear factor kB and causing the inflammatory reaction and apoptosis [10–16]. Apoptosis and inflammation play crucial roles in the process of the remodelling of arteries in hypertension [17]. Several mutations have been identified in the NOD2 gene, which are linked to inflammation and apoptosis. One of them, frameshift mutation 3020insC (1007fs), occurs in the European population relatively often and has been proven to be associated with the inflammatory process [18–20]. In 2005 the NOD2 gene was discovered in human endothelium [21, 22]. Recently, it was shown that allelic variants of NOD2 were associated with preeclampsia syndrome with susceptibility to severe hypertension [23]. In the face of the relation between NOD2 mutation and blood pressure control in preeclampsia, it appears very likely that mutation in the NOD2 gene is associated with vascular vessel function and structure. We tested only the 1007fs mutation in the NOD2 gene because our previous study showed that frequency NOD2 gene variants (1007fs,G908R and R702W and P268S polymor-
phism) in the Polish population of patients with rectal cancer was found in the 1007Ts mutation and there is an association between frequency of cancer and age [23–25].

The aim of the study was to see whether the occurrence of the 3020insC mutation in the \textit{NOD2} gene is associated with the clinical manifestation of cardiovascular damage in hypertensives.

**Materials and methods**

The study included 466 hypertensives treated in the Cardiology Outpatient Clinic in Leczyzna Citomed in Toruń. The study was in accordance with the Declaration of Helsinki and was approved by the Commission of Bioethics at Collegium Medicum of Nicolaus Copernicus University in Toruń. Informed consent was obtained from all subjects.

The enrolment examination included medical history, current therapy, previously diagnosed cardiovascular diseases, physical examination, and BMI. Fasting blood was sampled in tubes with EDTA for genotyping, total cholesterol, and creatinine and stored at –80°C. eGFR was estimated according to MDRD formula. Total cholesterol was determined enzymatically, and creatinine according to Jaffe’s method. Left ventricular mass (LVM) was estimated using a Vivid 3 GE echocardiograph with a 2.5 MHz ultrasound probe according to Penn convention and Devereux formula and expressed as left ventricular mass index (LVMI) calculated as LVM/body surface area [16].

Blood pressure measurements (BP) were taken at 2–3 month intervals over one year in accordance with ESC/ESH 2003 guidelines. The mean of two consecutive measurements were reported. The target blood pressure values were < 140 mm Hg systolic (SBP) and < 90 mm Hg diastolic (DBP) for nondiabetics. Pulse pressure (PP) was calculated according to the equation PP = SDP-DBP. All patients were subjected to antihypertensive therapy according to ESC/ESH guidelines.

**Genotyping**

The 3020insC mutation was detected using PCR reaction [4]. For the PCR reaction, four different primers were used: “3020sens”, “3020anty”, “3020wild”, and “3020mut”, forming three different pairs. The “3020wild” and “3020mut” primers have a wild sequence and a 1007Ts mutation equivalent, respectively, on their 3’ ends. The thermal conditions of the PCR reaction and the concentrations of the substrates were adjusted so the highest specificity could be obtained. If the 3020insC mutation appeared, the “3020mut” primer bound to the DNA on the template while the “3020insC” primer did not. Afterwards, the product of the reaction was subjected to electrophoresis. An exemplary photograph of electrophoresis with marked 3020insC mutation is shown in figure 1.

**Statistical analysis**

Statistical analysis was performed with SPSS 12.0 PL software and Microsoft Excel calculation sheet. The patients included in the study were divided into two groups: one group consisted of patients with identified 3020insC mutation in \textit{NOD2} (\textit{NOD2}(+)), and the second group comprised patients with negative results (\textit{NOD2}(−)). For each variable, mean value and standard deviation were calculated using t-Student or Mann-Whitney test. Shapiro-Wilk test was used to estimate normality of distribution. \( p < 0.05 \) was considered significant. The ANCOVA test was applied to compare mean values between the groups. Linear regression analysis was used to evaluate the correlation between variables.

**Results**

3020insC mutation in the \textit{NOD2}/\textit{CARD15} gene was found in 33 hypertensives (7.08%) (16 females and 17 males). The \textit{NOD2}(+) was associated with earlier onset of hypertension (tab. I), and higher systolic blood pressure and pulse pressure in the face of the same number of taken antihypertensive drugs (tab. II, fig. 2). LVMI and eGFR mean values did
not differ significantly in respect to the appearance of the 3020insC mutation (tab. I). In multivariate analysis gender, SBP and PP were all predictive of LVMI (p = 0.00; p = 0.03; p = 0.04, respectively), while GFR was influenced only by gender (p = 0.02). None of these parameters appeared to be influenced by the presence of the 3020insC mutation.

**Table I. Clinical characteristics of patients in **NOD2(−)** and **NOD2(+)** groups (mean ± SD)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th><strong>NOD2(−)</strong></th>
<th><strong>NOD2(+)</strong></th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>433</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.4 ± 9.5</td>
<td>56.4 ± 10.4</td>
<td>NS</td>
</tr>
<tr>
<td>Onset of hypertension (patient age)</td>
<td>48.2 ± 12.1</td>
<td>42.8 ± 10.9</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>29.5 ± 4.3</td>
<td>30.00 ± 4.8</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol [mg/dl]</td>
<td>223.1 ± 47.6</td>
<td>216.9 ± 48.1</td>
<td>NS</td>
</tr>
<tr>
<td>GFR [ml/min/1.73 m²]</td>
<td>86.9 ± 23.5</td>
<td>94.1 ± 27.1</td>
<td>NS</td>
</tr>
<tr>
<td>LVMI [g/m²]</td>
<td>117.3 ± 24.5</td>
<td>124.2 ± 28.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Discussion**

This cross-sectional study suggests that the 3020insC mutation in the **NOD2/CARD15** gene predisposes to earlier onset of hypertension and worse blood pressure control. It is known that the frequency of these mutations is related to some inflammato-
The 3020insC mutation in the NOD2 gene occurs among hypertensives. It suggests also that the 3020insC mutation may play a role in arterial remodelling in hypertensive patients.

References

Conclusions
The 3020insC mutation in the NOD2 gene occurs among hypertensives. It suggests also that the 3020insC mutation may play a role in arterial remodelling in hypertensive patients.