Association of A1166C Polymorphism in AT₁ Receptor Gene with Baroreflex Sensitivity

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Summary
The aim of this study was to evaluate the association of A1166C polymorphism in angiotensin II type 1 receptor (AT₁R) gene with baroreflex sensitivity (BRS in ms/mm Hg; BRSf in mHz/mm Hg) in man. BRS and BRSf were determined by a spectral method in 135 subjects (19-26 years) at a frequency of 0.1 Hz. Genotypes were detected by means of polymerase chain reaction and restriction analysis using enzyme DdeI. We compared BRS and BRSf among genotypes of this polymorphism. The frequency of genotypes of AT₁R A1166C polymorphism was: 45.9 % (AA, n=62), 45.9 % (AC, n=62), 8.2 % (CC, n=11). Differences in BRS (p<0.05) and BRSf (p<0.01) among genotypes of this single nucleotide polymorphism were found (Kruskal-Wallis: BRS - AA: 7.9±3.3, AC: 8.6±3.6, CC: 5.9±2.3 ms/mm Hg; BRSf - AA: 12.0±4.0, AC: 12.0±5.0, CC: 8.0±3.0 mHz/mm Hg). Compared to carriers of other genotypes (AA+AC) the homozygotes with the less frequent allele (CC) showed significantly lower BRSf (Mann-Whitney: BRSf - AA+AC: 12.0±4.0, CC: 8.0±3.0 mHz/mm Hg; p<0.01) and borderline lower BRS (BRS - AA+AC: 8.2±3.5, CC: 5.9±2.5 ms/mm Hg; p=0.07). We found a significant association of A1166C polymorphism in AT₁ receptor gene with baroreflex sensitivity. Homozygosity for the less frequent allele was associated with decreased baroreflex sensitivity.

Key words
Baroreflex sensitivity • Angiotensin II type 1 receptor • Polymorphism • Spectral analysis

Introduction

Arterial baroreflex is a powerful mechanism of fundamental importance for blood pressure homeostasis. It takes part in both long-term and short-term blood pressure regulation. Though a complex response of the controlling mechanisms mediated by baroreceptors includes the influence on the heart and regulation of the tone of resistance and capacitance vessels, the sensitivity of the baroreceptor-heart rate reflex is studied most frequently (Persson et al. 2001). Under baroreflex sensitivity, usually the change of inter-beat interval in ms or heart rate in mHz due to systolic blood pressure (SBP) change by 1 mm Hg is understood (BRS in ms/mm Hg; BRSf in mHz/mm Hg).

Diminished BRS has been shown to contribute to the pathophysiology of several cardiovascular dysfunctions such as hypertension (Sleight 1997) or cardiac failure (Osterziel et al. 1995). It is associated not only with fully developed hypertension, but it can be seen even in the early stage of blood pressure elevation in adolescents and young adults (Honzíková et al. 2006b, Krontorádová et al. 2008). It is also thought to be a risk factor for sudden cardiac death in patients after myocardial infarction (La Rovere et al. 1988, Honzíková et al. 2000). Recently, we have demonstrated that BRS is an individually characteristic feature and that it characterizes a young healthy individual (Jíra et al. 2006) similarly as the power spectra of blood pressure and heart rate (Honzíková et al. 1990). In young people, in whom
hypertension is not fully developed yet, we can find BRS values under 3.9 ms/mm Hg (Závodná et al. 2006). BRS is also dependent on carotid intima-media thickness (Zancheti et al. 1998, Lábrová et al. 2005, Honziková et al. 2006a).

There are some studies providing the evidence that BRS could be influenced by genetic factors. Normotensive and hypertensive subjects with a family history of hypertension exhibit decreased BRS compared to subjects without a family history of hypertension (Parmer et al. 1992). There is a correlation in BRS among monozygotic twin pairs but not among dizygotic ones (Tank et al. 2001). Neale et al. (1992) showed by statistical methods that the heritability of BRS changes minimally after the correction for body mass index (BMI) and blood pressure (BP), which means that BRS could be influenced by other genes than BP and BMI. It is clear that there must be factors such as neurotransmitters, receptors, and ion channels involved in baroreflex function, and the genetic determination of BRS may be given by variants of genes of any factor involved. Some studies have brought promising results. The polymorphism –344C/T in the promoter region of the aldosterone synthase (CYP11B2) gene is associated with a decreased BRS (White et al. 1999, Ylitalo et al. 2000). Gollash et al. (2002) showed that some polymorphisms in the gene coding for β1 subunit of calcium-activated potassium channel are associated with changes in heart rate variability and altered baroreflex function. Ormezzano et al. (2005) suggested the importance of the polymorphism in the endothelin system for determination of BRS and found that the T allele of the EDNRA/C+1222T polymorphism was associated with a reduction in BRS in both healthy and hypertensive subjects. Angiotensin II (AngII) is involved in neurotransmission in the baroreflex pathway through AT1 receptors (Matsumura et al. 1998, Gaudet et al. 2000). The gene for AT1 receptor (AT1R) is located on the third chromosome (Curnow et al. 1992) and a polymorphism of AT1R, A1166C, belongs to the most studied variants. Angiotensin II (AngII) is involved in neurotransmission in the baroreflex pathway through AT1 receptors (Matsumura et al. 1998, Gaudet et al. 2000). The gene for AT1 receptor (AT1R) is located on the third chromosome (Curnow et al. 1992) and a polymorphism of AT1R, A1166C, belongs to the most studied variants. This polymorphism has been associated with hypertension (Bonnardeaux et al. 1994, Wang et al. 1997, Dzida et al. 2001), aortic stiffness (Benetos et al. 1996), left ventricular mass (Osterop et al. 1998, Takami et al. 1998), and greater coronary artery vasoconstriction induced by methylergonovine maleate in homozygotes for the C allele (Amant et al. 1997). Van Geel et al. (2000) demonstrated the association of C allele with an increased response to angiotensin II in isolated human arteries, and Tiret et al. (1994) reported a high prevalence of the C allele of this polymorphism in patients with myocardial infarction. The association studies of the AT1R gene were summarized to a review focusing on clinical end-points and physiological responses (Duncan et al. 2001).

Although many studies were devoted to the role of polymorphisms in the AT1R gene, there is still a lack of information about the association of its variants with baroreflex sensitivity. Therefore, the aim of this study was to evaluate association of A1166C polymorphism in the AT1R gene with baroreflex sensitivity in man.

Methods

Subjects and protocol

Young healthy individuals (university students) were recruited for this study. Only apparently healthy individuals were included in the study; all of the subjects were without positive personal history of hypertension, myocardial infarction, stroke, or diabetes mellitus. In each subject body mass index (BMI) was calculated. A total of 135 healthy volunteers (39 men and 96 women) aged 19-26 years was examined. The Ethics Committee approved the study and each subject gave his/her informed consent.

Systolic (SBP), diastolic (DBP) blood pressures and inter-beat interval (IBI), and instantaneous values of heart rate (HR) respectively, were recorded beat-to-beat by a non-invasive, continuous method from finger arteries (Finapres, Ohmeda 2300) in subjects sitting at rest for 5 min. The finger cuff was placed on the second phalanx of the middle or ring finger of the subject’s dominant hand. The hand was fixed at the level of the participant’s heart. The examinations were performed in a quiet room (temperature 22 °C), after 15 min sitting at rest. During the recording, regulated breathing (20 breaths per min) was used with respect to the importance of high-frequency paced breathing in a spectral assessment of baroreflex sensitivity (Frederiks et al. 2000). The subjects were allowed to adjust the tidal volume according to their own comfort and had neither objective nor subjective problems to follow the metronome. The examinations were done three times in periods of one week, in the same daytime.

Data processing, spectral analysis, baroreflex sensitivity assessment

Baroreflex sensitivity was determined by a
spectral method (Honzíková et al. 2003, Závodná et al. 2006). The primary signal used was the SBP waveform signal from the output of the Finapres, sampled at 250 Hz sampling frequency and stored on a PC hard disk. From the stored signal, the sequence of SBP, DBP and IBI of consecutive heartbeats was extracted. IBI values were also converted into beat-to-beat values of instantaneous HR. The sequences of instantaneous values of HR instead of IBI were used for calculation of the BRSf index.

For spectral analysis, values of SBP, DBP and IBI (instantaneous values of HR) were linearly interpolated and equidistantly sampled at 2 Hz. The linear trend was removed. The autocorrelation and cross-correlation functions and power spectra as well as cross-spectra and coherence were calculated. The value $H[f]$ of the transfer function between variations in SBP and IBI (or HR) was taken as an index of BRS (or BRSf) at the frequency of 0.1 Hz [$f$]:

$$H[f] = \frac{G_{xy}[f]}{G_{xx}[f]},$$

where $G_{xy}[f]$ corresponds to the cross-spectral density between SBP and IBI (or HR); $G_{xx}[f]$ corresponds to the power spectral density of SBP. The values of the transfer function at 0.1 Hz with a coherence higher than 0.5 were taken into account only.

**Genotyping of AT1 R A1166C polymorphism**

The blood was collected in ethylenediaminetetraacetic acid. DNA was isolated from leukocytes according to standard procedures using proteinase K.

DNA segments were amplified by polymerase chain reaction (PCR) in a total volume of 15 µl containing 0.1 µl Taq, 1.5 µl buffer, 2.5 µl MgCl₂, 0.5 µl dNTP, 0.5 µl of each primer, and 7.4 µl H₂O. The primers used were: 5’-AGAAGCCTGCACCATGTTTT-3’ (sense) and 5’-TGTGGCTTTGCTTTGTCTTG-3’ (antisense). The reaction conditions were as follows: initial denaturation 95 °C 5 min, then 33 cycles of denaturation 94 °C for 30 s, annealing 53 °C for 25 s, and elongation 72 °C for 25 s, final elongation 72 °C for 10 min, and cooling 10 °C for 10 min. The PCR product (233 bp) was digested by 5 U of Dde I for 12 h. The fragments were separated by electrophoresis using 3% agarose gel at 85 V and visualized by ethidium bromide staining under UV light. The AA variant was detected as one fragment (233 bp), AC as three fragments (233, 118, 115 bp), and CC as two fragments (118 and 115 bp).

**Statistical analysis**

Statistical analysis was performed using Statistica version 6.0. A non-parametric Kruskal-Wallis test (ANNOVA) for multiple comparisons of independent samples (groups) was used to reveal differences in BRS (BRSf) among genotypes of the A1166C single nucleotide polymorphism (SNP). A Mann-Whitney test with Bonferroni-Holm correction for multiple comparisons was used to reveal differences in BRS (BRSf) between less frequent allele homozygotes (CC) and all carriers of the more frequent allele (AA homozygotes and AC heterozygotes). The odds ratio (OR) and the 95% confidence interval were calculated to evaluate the risks related to the genotypes of the polymorphism studied. A Fisher exact test was used to calculate the significance of OR.

**Table 1.** Characterization of the study population.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>[years]</td>
<td>19-26</td>
</tr>
<tr>
<td>SBP</td>
<td>[mm Hg]</td>
<td>112.0±10.4</td>
</tr>
<tr>
<td>DBP</td>
<td>[mm Hg]</td>
<td>64.0±7.7</td>
</tr>
<tr>
<td>IBI</td>
<td>[ms]</td>
<td>836.7±130.2</td>
</tr>
<tr>
<td>HR</td>
<td>[Hz]</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>BMI</td>
<td>[kg/m²]</td>
<td>21.7±2.4</td>
</tr>
<tr>
<td>BRS</td>
<td>[ms/mm Hg]</td>
<td>8.1±3.4</td>
</tr>
<tr>
<td>BRSf</td>
<td>[mHz/mm Hg]</td>
<td>12.0±4.0</td>
</tr>
</tbody>
</table>

Means ± S.D., SBP – systolic blood pressure; DBP – diastolic blood pressure; IBI – inter-beat interval; HR – heart rate; BMI – body mass index; BRS – baroreflex sensitivity; BRSf – baroreflex sensitivity.

**Results**

**Characterization of the study population**

Data characterizing all the 135 participants are summarized in Table 1. The values of blood pressure of our study group (aged 19-26 years) evaluated from 5 min recordings were in a physiological range. None of the subjects was hypertensive.

**Effects of AT1 R A1166C polymorphism on BRS and BRSf**

The frequency of genotypes of A1166C polymorphism was: 45.9 % (AA, n=62), 45.9 % (AC, n=62), 8.2 % (CC, n=11), similar to other samples of Caucasian population (Bonnardeaux et al. 1994, Filippi-Codaccioni et al. 2005). Significant differences in BRS
(p<0.05) and BRSf (p<0.01) among genotypes of this SNP were found (non-parametric Kruskal-Wallis test for multiple comparisons of independent samples; Table 2, Fig. 1). The homozygotes for the less frequent allele (CC) showed lower BRS and BRSf comparing carriers of other genotypes (AA homozygotes and AC heterozygotes).

When the Mann-Whitney test with the Bonferroni-Holm correction was used, a significant difference (p<0.01) in BRSf was found between CC homozygotes and carriers of other genotypes (AA homozygotes and AC heterozygotes together). In the BRS index this difference was borderline (p=0.07); the homozygotes for the less frequent allele (CC) also tended to lower values compared with the group of AA homozygotes and AC heterozygotes together (Table 3, Fig. 2).

The CC genotype was more frequent in the group with BRSf lower than the median of the whole study group compared to the group with BRSf above this median (6.7 % vs. 1.5 %; median=0.011067). The difference was statistically significant (p=0.0258, OR: 5.12, 95 % confidence interval: 1.06-24.67).

The frequency of CC genotype was also higher in the group with BRS lower than the median of the whole study group compared to the group with BRS above the median, but the difference was not significant (5.9 % vs. 2.3 %; p=0.108; median=7.5176).

No significant differences in SBP, DBP, IBI, HR, or BMI among genotypes of this SNP were found (non-parametric Kruskal-Wallis test for multiple comparisons of independent samples) (Table 2). Taking into account the gender, there was no significant difference in distribution of genotypes between males and females (Table 2), neither were there any significant differences in BRS and BRSf among them (Mann-Whitney test with the Bonferroni-Holm correction: BRS – males: 8.7±4.1, females: 7.8±3.1 ms/mm Hg; p=0.385; BRSf – males: 11.9±4.6, females: 11.6±4.4 mHz/mm Hg; p=0.760).

**Table 2. Differences in age, gender composition, SBP, DBP, IBI, HR, BMI, BRS, and BRSf among genotypes of A1166C SNP.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>AA</th>
<th>AC</th>
<th>CC</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>n (%)</td>
<td></td>
<td>62 (45.9)</td>
<td>62 (45.9)</td>
<td>11 (8.2)</td>
<td></td>
</tr>
<tr>
<td>Age [years]</td>
<td></td>
<td>20.8±1.4</td>
<td>20.9±1.4</td>
<td>21.0±1.4</td>
<td>0.445</td>
</tr>
<tr>
<td>Gender [Males/Females]</td>
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<td>17/45</td>
<td>18/44</td>
<td>4/7</td>
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</tr>
<tr>
<td>SBP [mm Hg]</td>
<td></td>
<td>112.0±11.3</td>
<td>112.0±9.6</td>
<td>108.0±9.7</td>
<td>0.447</td>
</tr>
<tr>
<td>DBP [mm Hg]</td>
<td></td>
<td>64.0±7.9</td>
<td>64.0±7.7</td>
<td>61.0±7.2</td>
<td>0.397</td>
</tr>
<tr>
<td>IBI [ms]</td>
<td></td>
<td>815.8±117.7</td>
<td>855.4±144.4</td>
<td>848.2±101.4</td>
<td>0.398</td>
</tr>
<tr>
<td>HR [Hz]</td>
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<td>1.2±0.2</td>
<td>1.2±0.2</td>
<td>1.2±0.2</td>
<td>0.436</td>
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<tr>
<td>BMI [kg/m²]</td>
<td></td>
<td>21.9±2.8</td>
<td>21.4±2.1</td>
<td>22.4±2.2</td>
<td>0.379</td>
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<tr>
<td>BRS [ms/mm Hg]</td>
<td></td>
<td>7.9±3.3</td>
<td>8.6±3.6</td>
<td>5.9±2.3</td>
<td>0.037</td>
</tr>
<tr>
<td>BRSf [mHz/mm Hg]</td>
<td></td>
<td>12.0±4.0</td>
<td>12.0±5.0</td>
<td>8.0±3.0</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Means ± S.D., SBP – systolic blood pressure; DBP – diastolic blood pressure; BRS – baroreflex sensitivity; BRSf – baroreflex sensitivity; IBI – inter-beat interval; HR – heart rate; BMI – body mass index. Non-parametric Kruskal-Wallis test was used for multiple comparisons of independent samples.

**Discussion**

Our study indicates that, besides aldosterone synthase, endothelin receptor and β₁ subunit of calcium-activated potassium channel gene polymorphisms (White et al. 1999, Ylitalo et al. 2000, Gollash et al. 2002, Ormezzano et al. 2005), angiotensin II AT₁ receptor gene polymorphism A1166C is also associated with modified baroreflex sensitivity. Because angiotensin II together with aldosterone is part of the renin-angiotensin-aldosterone system (RAAS), the effect on baroreflex sensitivity mediated by modulation of the aldosterone or AngII production offers a simple explanation. However, other alternatives are also plausible.

There are many factors and mediators involved in cardiac autonomic pathways and AngII has been suggested to be one of them. It exerts its influence through two types of receptors: AT₁R and AT₂R. Most of its physiological effects are mediated by the activation of AT₁R. The receptors belong to the superfamily of G-protein-coupled receptors and the coupling occurs via Gq proteins. Stimulation of AT₁R activates phospholipase
C, increases the levels of diacylglycerol and inositol trisphosphate, elevates intracellular Ca\(^{2+}\) concentration, and activates several kinases modulating cell functions.

Besides circulating AngII there is a remarkable production of this peptide in the brain, as the brain possesses its own RAAS system (Danser 1996), and AngII produced locally in the brain contributes to cardiovascular regulation independently of the circulating AngII (Richards et al. 1989). It was demonstrated that intracerebroventricular infusion of AngII decreased baroreflex gain, and blockade of AT\(_1\) receptors with losartan increased baroreflex gain in conscious rabbits (Gaudet et al. 2000). Thus AngII influences baroreflex sensitivity through central mechanisms, which was shown many times also in animal experiments (Campagnole-Santos et al. 1988, Gaudet et al. 1997, Matsumura et al. 1998, Paton et al. 1999). The central mechanism by which AngII modulates the baroreflex depends on the location of AngII action. For example, in the nucleus tractus solitarii it intensifies inhibitory postsynaptic potentials, thus inhibiting neuronal transmission. It has been suggested that the increase of synaptic inhibition occurred at the level of the terminals of inhibitory interneurons (Kasparov et al. 1999). In the caudal ventrolateral medulla, AngII evokes a depressor and sympathoinhibitory response (Sasaki et al. 1990), which can be explained as a consequence of activation of inhibitory interneurons that project directly to sympathoexcitatory neurons in the rostral ventrolateral medulla (Dampney 1994). AngII receptor binding sites also occur in the nodose ganglion and are transported centrally in the vagus to be located on presynaptic terminals in the nucleus tractus solitarii, and also peripherally, where they may be present on terminals of the vagus (Allen et al. 1988).

Besides regulation of the central part of the baroreflex arch in the brain, AngII together with the whole RAAS influences baroreflex heart rate gain also by peripheral mechanisms. Increased carotid intima-media

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>AA+CA</th>
<th>CC</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRS</td>
<td>[ms/mm Hg]</td>
<td>8.2±3.5</td>
<td>5.9±2.5</td>
<td>0.070</td>
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<tr>
<td>BRSf</td>
<td>[mHz/mm Hg]</td>
<td>12.0±4.0</td>
<td>8.0±3.0</td>
<td>0.009</td>
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</table>


**Table 3.** Differences in BRS and BRSf between CC homozygotes and carriers of other genotypes (AA homozygotes and AC heterozygotes together) of A1166C SNP (Mann-Whitney test with the Bonferroni-Holm correction).
thickness (IMT) is connected with decreased baroreflex sensitivity (Zancheti et al. 1998, Lábrová et al. 2005, Honzíková et al. 2006a). The thickening of the vessel wall can be caused by atherosclerotic processes combined with hypertrophy of smooth muscles. AngII plays a pivotal role in the progression of atherosclerosis by promoting fibroblast proliferation and excessive deposition of extracellular matrix (Schmidt-Ott et al. 2000). A polymorphism in the ACE gene (I/D) is connected with increased level of ACE (Rigat et al. 1990) and increased carotid IMT (Balkenstein et al. 2001), which can be explained by increased production of AngII by ACE with enhanced atherosclerotic effect as the result. From the clinical point of view it is important that the inhibitor of AT1R, losartan, reduces carotid IMT (Sonoda et al. 2009). Miller et al. (1999) reported the association of the AT1R C allele with lower glomerular filtration rate (GFR), renal blood flow (RBF) and effective renal plasma flow (ERP), and with greater increase in GFR by losartan. During AngII infusion, AC/CC subjects maintained GFR despite equivalent declines in RBF, suggesting an enhanced effenter arteriolar constrictive response and enhanced intrarenal and peripheral AngII activity in C allele carriers. Other studies (Doria et al. 1997) support the previous suggestion and report a relationship between the C allele and diabetic nephropathy, and are amended by others (Miller et al. 2000), where diabetic patients with the C allele despite they exhibited higher RBF and ERP than those with the A allele at a physiological level of glycemia, they also had a higher renal vascular resistance and exhibited an enhanced pressor response to high glucose. This high-pressure state may contribute to glomerular injury and explain the relationship between the C allele and diabetic nephropathy in patients with poor glucose control. However, the result of association of the AT1R C1166 variant with cardiovascular diseases and different phenotypes is still controversial; many studies yielded negative or contradictory results. For example, Schmidt et al. (1997) found no association between the A1166C polymorphism and hypertension in the German population, and Tiret et al. (1994) demonstrated an association between this polymorphism and hypertension in women only. Although Takami et al. (1998) found an association between the A1166C polymorphism and left ventricular mass as mentioned above, they detected no association with hypertension. Yet hypertensives with a CC genotype had a higher systolic blood pressure than those with AC or AA. In addition, the subjects were older and had higher blood pressures, and the frequency of the C allele was low in the Japanese population (Duncan et al. 2001).

Our study suggests the effect of angiotensin II AT1 receptor gene polymorphism A1166C on baroreflex sensitivity. We showed that the homozygotes for the less frequent allele (CC) had a significantly lower baroreflex
sensitivity compared to carriers of other genotypes (AA homozygotes and AC heterozygotes). We also demonstrated that the CC genotype is significantly more frequent in individuals with low baroreflex sensitivity. Since a lower baroreflex sensitivity is connected with cardiovascular diseases, it seems likely that the presence of CC genotype brings a higher risk for the development of these diseases. This result is in line with others mentioned above. Another important issue is the biological relevance of this AT1R gene polymorphism. The A1166C variant occurs in the 3’ untranslated region of the AT1R gene and is not characterized by any functional diversity. However, recently it was shown that patients with the C allele showed smaller changes in brachial artery flow-mediated dilatation in comparison with patients with the AA genotype, and it was associated with a significantly lower endothelial response to statin treatment (Kiliszek et al. 2007). In explaining biological and physiological impacts of the A1166C polymorphism the studies on different response of blood vessels on AngII in carriers of different genotypes are of great help. Some of them were mentioned above (Amant et al. 1997, Miller et al. 1999, 2000, Kiliszek et al. 2007). Moreover, Spiering et al. (2000) found that the C allele of the polymorphism is associated with increased sensitivity to AngII. Similar results were obtained by van Geel et al. (2000) who demonstrated that the AT1R A1166C polymorphism (namely the C allele) is associated with an increased response to angiotensin II in isolated human arteries. Erdmann et al. (1999) reported that AT1R A1166C showed a weak but significant linkage disequilibrium with the 810T/A polymorphism in the promoter region of the AT1R gene and suggested that the A1166C polymorphism may be slightly associated with expression of the AT1R gene. Plumb et al. (1989) showed that a mutation at position 810T/A destroys the transcriptional factor-binding site for GATA-binding factors. Thus, A1166C polymorphism can be considered as a possible marker in linkage disequilibrium with another functionally relevant genetic variant affecting the structure or expression of the AT1R. In addition, a potential epistatic interaction possibly exists between AT1R AC or CC and ACE DD genotypes that produces a synergistic effect in some diseases (Ye et al. 2003, Bleumink et al. 2004) and may explain some inconsistent results from different populations due to analysis of only one gene polymorphism. Another possibility is that homozygosity for the C allele could influence proteosynthesis on the level of mRNA and cause alteration of AT1 receptor down-regulation. Taking into account that down-regulation serves as protection against excessive effects of AngII, its alteration causing increased tissue densities of AT1 receptors could lead to an enhanced effect of AngII, which diminishes baroreflex. So far, all the explanations about the functional significance of this polymorphism were questionable or based on indirect evidence. Now it has been proved by Martin et al. (2007) that the A1166C polymorphism occurred in a cis-regulatory site, which is recognized by specific microRNA (miRNA) – miR-155. miRNAs are non-coding RNAs that silence gene expression by base-pairing with complementary sequences in the 3’ untranslated region of target RNAs. When the C allele is present, base-pairing complementarity is interrupted, and the ability of miR-155 to interact with the cis-regulatory site is decreased. As a result, miR-155 no longer attenuates translation efficiently enough. Thus, the possibility of failure in the receptor down-regulation as the biological base is most probable.

It is clear that not only central but also peripheral influences of AT1R gene A1166C polymorphism have to be taken into account. The activation of AT1 receptors present in the blood vessel wall by AngII leads to vasoconstriction. Since the C allele was associated with aortic stiffness (Benetos et al. 1996) and smaller changes in brachial artery flow-mediated dilation in comparison with patients with the AA genotype (Kiliszek et al. 2007), one can assume that the A1166C polymorphism affects not only the baroreflex heart rate gain but also the peripheral part of the baroreflex arch. However, in this study we focused on baroreflex heart rate gain because only young healthy individuals were engaged in the study. In these subjects the affection of peripheral blood vessels was not expected on the basis of the recent finding that the age-dependent decrease of baroreflex sensitivity corresponds to structural changes of the carotid wall depending on age (Lábrová et al. 2005). In addition, in the study of Benetos et al. (1996), which was conducted only in hypertensive but not in normotensive subjects, the AT1R genotypes were involved in the regulation of aortic rigidity. Nevertheless, it would be useful to evaluate the peripheral part of the baroreflex outcome, e.g. pulse wave velocity. This is planned as a next step of our further investigations.

It is known that the value of BRS is associated with BMI (Honzíková et al. 2006b), age (Sleight 1997, Schmidt et al. 2006), heart rate (Honzíková et al. 2003),
blood pressure (Hesse et al. 2007), and regular physical training (Gademan et al. 2007), respectively. In our study we found no significant association of these possible cofactors with A1166C polymorphism. It means that significant differences in BRS distribution among the genotypes of this polymorphism are not influenced by these cofactors in our tested group of young healthy subjects. It indicates that BRS is connected with A1166C polymorphism independently of them. This is in line, for example, with the study by Neale et al. (1992), who showed that the heritability of BRS changes minimally after the correction for BMI and blood pressure. Our sample consisted of medical students with a sedentary style of life, none of them performed regular physical activity, which excludes this aspect from possible influences. As baroreflex sensitivity also depends on the average duration of the heart cycle period upon which the baroreflex operates (inter-beat interval or heart rate), we calculated heart-rate baroreflex sensitivity not only as a BRS index but also as a BRSf index, which is less sensitive to differences in heart rate and better reflects sensitivity of baroreceptors than vagal activity (Honzíková et al. 2009). The finding of a higher significance in the differences of the BRSf index among the alleles of A1166C polymorphism underlines that standardization of heart-rate baroreflex sensitivity on the mean heart rate increases the power of association of A1166C polymorphism with baroreflex sensitivity.

There are numerous limitations of this study. The present study is limited by the fact that the heritability of baroreflex sensitivity is a complex phenomenon where a combination of several genes plays a role. Importantly, this limitation could lead to a reduced ability to identify genetic risk factors but would not account for significant positive associations. Second, the case-control approach used is generally quite vulnerable to the population stratification, e.g. due to the different ethnic origin. The present sample, however, is exclusively of Czech subjects of Caucasian origin, restricted to the limited geographical area populated by quite homogenous population.

It is difficult to determine which of the genes (previously described genes, or the gene described in the present study) are more important for baroreflex sensitivity. Complex interactions of genetic factors and demographic and environmental factors could explain heterogeneity in previous studies. Additional functional and prospective studies will be necessary for better understanding of the interaction effects of the above-mentioned genetic factors in determination of baroreflex sensitivity.

Conflict of Interest
There is no conflict of interest.

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