Association of A1166C polymorphism in AT_1 receptor gene with baroreflex sensitivity

Miroslav Jíra[1, 3], Eva Závodná[1], Nataša Honzíková[1], Zuzana Nováková[1], Anna Vašků[2], Lydie Izakovičová Hollá[2], Bohumil Fišer[1]

[1]Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

[2]Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

[3]Department of Surgery, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic

Full address of corresponding author:

Prof. MUDr. Nataša Honzíková, CSc.

Fyziologický ústav LF MU v Brně

Komenského nám. 2

CZ-662 43 Brno

Czech Republic

Tel.: +420-549 493 748; Fax: +420-549 493 748; E-mail: ***nhonziko@med.muni.cz***

Short title: Genetics of AT_1 receptor and baroreflex sensitivity

Summary

Objective: The aim of this study was to evaluate association of A1166C polymorphism in angiotensin II type 1 receptor (AT_1R) gene with baroreflex sensitivity (BRS in ms/mmHg; BRSf in mHz/mmHg) in man.

Methods: 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.

Results: The frequency of genotypes of AT_1R 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 SNP were found (Kruskal-Wallis: BRS - AA: 7.9±3.3, AC: 8.6±3.6, CC: 5.9±2.3 ms/mmHg; BRSf - AA: 12.0±4.0, AC: 12.0±5.0, CC: 8.0±3.0 mHz/mmHg). Homozygotes in the less frequent allele (CC) comparing carriers of other genotypes (AA+AC) showed significantly lower BRSf (Mann-Whitney: BRSf - AA+AC: 12.0±4.0, CC: 8.0±3.0 mHz/mmHg; $p<0.01$) and borderline lower BRS (BRS - AA+AC: 8.2±3.5, CC: 5.9±2.5 ms/mmHg; $p=0.07$).

Conclusion: We found a significant association of A1166C polymorphism in AT_1 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 mmHg is understood (BRS in ms/mmHg; BRSf in mHz/mmHg).

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 connected 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 (Honzikova *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, Honzikova *et al.* 2000). Recently we showed 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 values of BRS under 3.9 ms/mmHg (Závodná *et al.* 2006). BRS is also dependent on carotid intima-media thickness (Zancheti *et al.* 1998, Lábrová *et al.* 2005, Honzíková *et al.* 2006a).

There are some studies providing evidence that BRS could be influenced by genetic factors. Normotensive and hypertensive subjects with a family history of hypertension exhibit decreased BRS compared with 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 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 decreased BRS (White *et al.* 1999, Ylitalo *et al.* 2000). Gollash *et al.* (2002) showed that some polymorphisms in the gene coding for β_1 subunit of calcium-activated potassium channel are associated with changes in heart rate variability and altered baroreflex function. In 2005 Ormezzano *et al.* suggested a role of the polymorphism in the endothelin system in determination of BRS and found that the T allele of the EDNRA/C+1222T polymorphism is associated with a reduction in BRS in both healthy and hypertensive subjects. Angiotensin II (AngII) is involved in neurotransmission in the baroreflex pathway through AT_1 receptors (Gaudet *et al.* 2000, Matsumura *et al.* 1998). The gene for AT_1 receptor (AT_1R) is located to 3rd chromosome (Curnow *et al.* 1992) and a polymorphism of AT_1R, 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 (Takami *et al.* 1998, Osterop *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 association of the 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. Recently, association studies of

the AT_1R gene were summarized to a review focusing on clinical end-points and physiological responses (Duncan *et al.* 2001).

Although many studies were engaged in polymorphisms in the AT_1R 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 AT_1R 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. A total of 135 healthy volunteers (39 men and 96 women) aged 19-26 years was examined.

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 minutes' 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. In each subject body mass index (BMI) was also counted. The Ethics Committee approved the study and each subject gave his/her informed consent.

Data processing, spectral analysis, baroreflex sensitivity assessment

Baroreflex sensitivity was determined by a spectral method (Honzikova *et al.* 2003, Zavodna *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]:

$$H[f] = Gxy[f] / Gxx[f],$$

where Gxy[f] corresponds to the cross-spectral density between SBP and IBI (or HR); Gxx[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 AT_1R 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 $MgCl_2$, 0.5 μl *d*NTP, 0.5 μl of each primer, and 7.4 μl H_2O. 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 hours. 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 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.

Results

Characterization of the study population

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

Effects of AT_1R 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; Tab. 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 (Tab. 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; Tab. 2). Taking into account the gender, there was no significant difference in distribution of genotypes between males and females (Tab. 2), neither were there any significant differences in BRS and BRSf among them (Mann-Whitney test with the Bonferroni-Holm correction: BRS - male: 8.7±4.1, female: 7.8±3.1 ms/mmHg; p=0.385; BRSf - male: 11.9±4.6, female: 11.6±4.4 mHz/mmHg; p=0.760).

Discussion

Our study indicates that, besides aldosterone synthase, endothelin receptor and β_1 subunit of calcium-activated potassium channel gene polymorphisms (White *et al.* 1999, Ylitalo *et al.* 2000, Ormezzano *et al.* 2005, Gollash *et al.* 2002), angiotensin II AT_1 receptor gene polymorphism A1166C also modified baroreflex sensitivity. Because angiotensin II (AngII) together with aldosterone is part of the renin-angiotensin-aldosterone system, 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 as one of them. It exerts its influence through two types of receptors: AT_1R and AT_2R. Most of its physiological effects are mediated by the activation of AT_1R. The receptors belong to the superfamily of G-protein-coupled receptors and the coupling occurs via Gq proteins. Stimulation of AT_1R activates phospholipase C, increases the levels of diacylglycerol and inositol triphosphate, 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 other experiments on animals (Gaudet *et al.* 1997, Paton *et al.* 1999, Campagnole-Santos *et al.* 1988, Matsumura *et al.* 1998). 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 occur 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 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 (Balkestein *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 AT_1R, losartan, reduces carotid IMT (Sonoda *et al.* 2008) by a direct antihypertensive effect on local blood pressure and pulse pressure (Miyazaki *et al.* 2002), and by inhibition of angiotensin II-induced vascular smooth muscle cell growth (Ludwig *et al.* 2002). This emphasizes the role of AngII blunting baroreflex sensitivity by its action through AT_1R at the receptive site of the baroreflex arch, the carotid artery.

In 1992 the angiotensin II type 1 receptor gene was cloned and mapped to the long arm of human chromosome 3 (3q21-q25) (Curnow *et al.* 1992). In 1994, Bonnardeaux *et al.* also cloned cDNA of the AT_1R and identified several gene variants and, among them, a polymorphism in the non-translated region 3´ (A1166C) corresponding to an A-C replacement in the position of nucleotide 1166 of the mRNA sequence (Bonnardeaux *et al.* 1994). 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 (Takami *et al.* 1998, Osterop *et al.* 1998), and greater coronary artery vasoconstriction induced by methylergonovine maleate in homozygotes for the C allele (Amant *et al.* 1997). Tiret *et al.* (1994) reported a high prevalence of the C allele of this polymorphism in patients with myocardial infarction. Rats with a disrupted gene for AT_1R displayed a hypotensive phenotype. Very recently it was shown that the frequency of the AT_1R 1166C allele and specifically the CC genotype in patients with heart failure was similar to the general population, but associated with an ischemic and not non-ischemic aetiology (Amir *et al.* 2009). The CC genotype was associated with more advanced disease and more severe abnormalities of renal function. A survival analysis showed that AT_1R CC homozygous patients had a significantly higher mortality in comparison with other genotypes (Amir *et al.* 2009). In addition, AT_1R 1166C/A and alpha-adducin Gly460Trp polymorphisms interactively determine the elastic properties of the femoral artery. In ADD1 Trp allele carriers femoral cross-sectional compliance was significantly higher in carriers of the AT_1R C allele than in AT_1R AA homozygotes, with a similar trend of femoral distensibility, and these associations were independent of potential confounding factors, including age (Seidlerova *et al.* 2009). Miller *et al.* (1999) reported association of the AT_1R 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 efferent 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 subjects with the C allele exhibited RBF and ERP higher than those with the A allele. 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 AT_1R C1166 variant with cardiovascular diseases and different phenotypes is still controversial; many studies found negative or opposite 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 AT_1 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 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 AT_1R gene polymorphism. The A1166C variant occurs in the 3´untranslated region of the AT_1R 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 dilation 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 (Miller *et al.* 1999, Miller *et al.* 2000, Amant *et al.* 1997, 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 AT_1R 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 AT_1R A1166C showed a weak but significant linkage disequilibrium with the 810T/A polymorphism in the promoter region of the AT_1R gene and suggested that the A1166C polymorphism may be slightly associated with expression of the AT_1R 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 a possible marker, in linkage disequilibrium with another functionally relevant genetic variant affecting the structure or expression of the AT_1R. In addition, a potential epistatic interaction possibly exists between AT_1R AC or CC and ACE DD genotypes that produces a synergistic effect in some diseases (Ye S *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 AT_1

receptor down-regulation. Taking into account that down-regulation serves as protection against excessive effects of AngII, its alteration causing increased tissue densities of AT_1 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 debatable 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 a 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 receptors' down-regulation as the biological base is most probable.

It is clear from what was mentioned above that not only central but also peripheral influences of AT_1R gene A1166C polymorphism have to be taken into account. The activation of AT_1 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 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) conducted only in hypertensive but not normotensive subjects, the AT_1R genotypes were involved in the regulation of aortic rigidity.

Nevertheless, the peripheral part of the baroreflex outcome would be useful to evaluate, which is planned as a next step of our investigation after this pivotal study, for example by measurement of pulse wave velocity.

It is known that the value of BRS is associated with BMI (Honzikova *et al.* 2006b), age (Schmidt *et al.* 2006, Sleight 1997), 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 co-factors with A1166C polymorphism. It means that significant differences of BRS distribution among the genotypes of this polymorphism are not influenced by these co-factors in our tested group of young healthy subjects. This shows that BRS is connected with A1166C polymorphism independently of them. It is in line, for example, with the study by Neale *et al.* (1992), who showed that the heritability of BRS changes minimally after correction for BMI and blood pressure. Our sample consisted of medical students with a sedentary style of life, none of them featured by special regular physical activity, which excludes this aspect from possible influences. As baroreflex sensitivity depends also 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 a number of limitations to 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 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 our better understanding of the interaction effects of the above-mentioned genetic factors in determination of baroreflex sensitivity.

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Table 1. Characterization of the study population

Parameter	Units	Value
Age	[years]	19-26
SBP	[mmHg]	112.0±10.4
DBP	[mmHg]	64.0±7.7
IBI	[ms]	836.7±130.2
HR	[Hz]	1.2±0.2
BMI	[kg/m^2]	21.7±2.4
BRS	[ms/mmHg]	8.1±3.4
BRSf	[mHz/mmHg]	12.0±4.0

Means ± standard deviation: SBP, systolic blood pressure; DBP, diastolic blood pressure; IBI, inter-beat interval in ms; HR, heart rate in Hz; BMI, body mass index in kg/m^2; BRS, baroreflex sensitivity in ms/mmHg; BRSf, baroreflex sensitivity in mHz/mmHg

Table 2. Differences in age, gender composition, SBP, DBP, IBI, HR, BMI, BRS, and BRSf among genotypes of A1166C SNP (non-parametric Kruskal-Wallis test for multiple comparisons of independent samples)

Parameter	Units	AA	AC	CC	p
n (%)		62 (45.9)	62 (45.9)	11 (8.2)	
Age	[years]	20.8±1.4	20.9±1.4	21.0±1.4	0.445
Gender	[M/F]	17/45	18/44	4/7	0.834
SBP	[mmHg]	112.0±11.3	112.0±9.6	108.0±9.7	0.447
DBP	[mmHg]	64.0±7.9	64.0±7.7	61.0±7.2	0.397
IBI	[ms]	815.8±117.7	855.4±144.4	848.2±101.4	0.398
HR	[Hz]	1.2±0.2	1.2±0.2	1.2±0.2	0.436
BMI	[kg/m^2]	21.9±2.8	21.4±2.1	22.4±2.2	0.379
BRS	[ms/mmHg]	7.9±3.3	8.6±3.6	5.9±2.3	0.037
BRSf	[mHz/mmHg]	12.0±4.0	12.0±5.0	8.0±3.0	0.009

Means ± standard deviation: SBP, systolic blood pressure; DBP, diastolic blood pressure; BRS, baroreflex sensitivity in ms/mmHg; BRSf, baroreflex sensitivity in mHz/mmHg; IBI, inter-beat interval in ms; HR, heart rate in mHz; BMI, body mass index in kg/m^2; Gender, Male/Female; p, p - level

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)

Parameter	**Units**	**AA+CA**	**CC**	**p**
n (%)		124 (91.9)	11 (8.1)	
BRS	[ms/mmHg]	8.2±3.5	5.9±2.5	0.070
BRSf	[mHz/mmHg]	12.0±4.0	8.0±3.0	0.009

Means ± standard deviation: BRS, baroreflex sensitivity in ms/mmHg; BRSf, baroreflex sensitivity in mHz/mmHg; p, p - level

Figure 1. Differences in BRS (left) and BRSf (right) among genotypes of AT_1R A1166C SNP (non-parametric Kruskal-Wallis test for multiple comparisons of independent samples). BRS, baroreflex sensitivity in ms/mmHg; BRSf, baroreflex sensitivity in mHz/mmHg; *p<0.05; **p<0.01

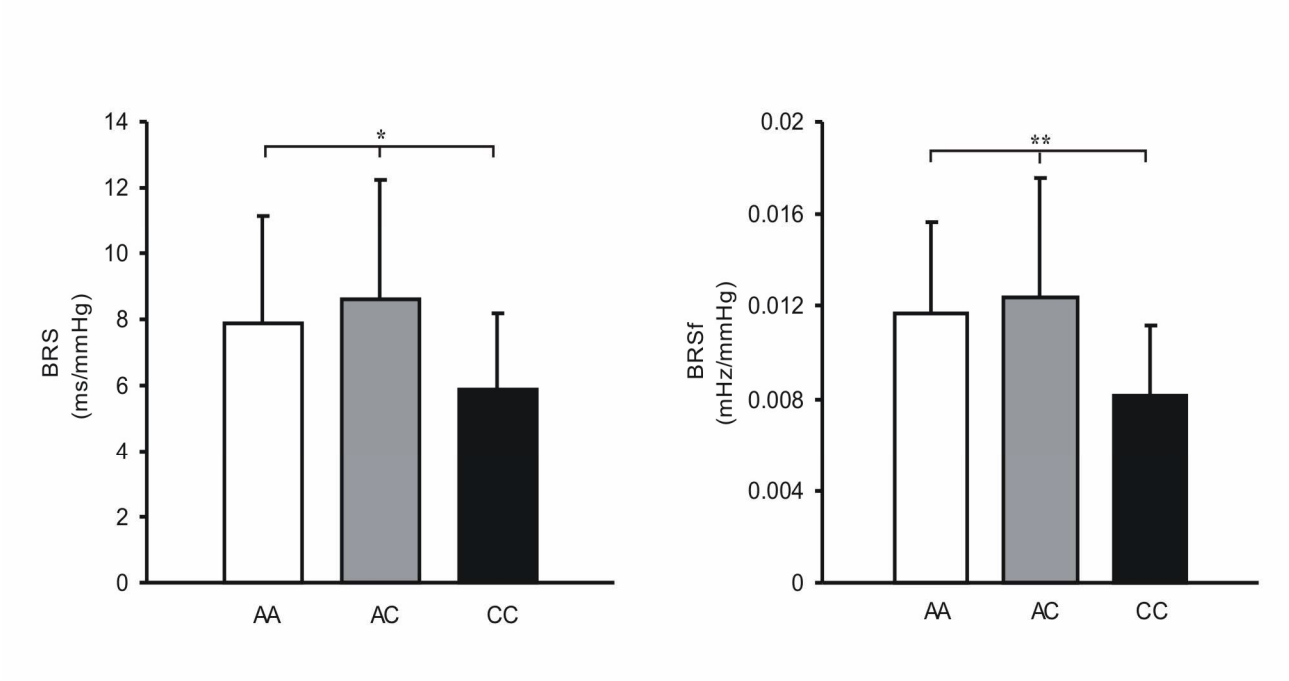

Figure 2. Differences in BRS (left) and BRSf (right) between all carriers of more frequent allele (AA homozygotes and AC heterozygotes) and less frequent allele homozygotes (CC). BRS, baroreflex sensitivity in ms/mmHg; BRSf, baroreflex sensitivity in mHz/mmHg; p, p-level; **p<0.01 (Mann-Whitney test with Bonferroni-Holm correction)