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# Molecular Variants of the Renin-Angiotensin System Components in the Slovak Population

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## Summary

Molecular variants of individual components of the renin-angiotensin system (RAS) are reported to constitute the inherited predisposition to some cardiovascular diseases in man, e.g. essential hypertension or myocardial infarction. The frequency of these variants depends highly on the race and population. Therefore, we examined the M235T molecular variant of the angiotensinogen gene and the I/D polymorphism of the ACE gene in Slovak healthy population, in patients with diagnosed essential hypertension and in patients who had undergone myocardial infarction. DNA from 241 subjects was tested for the presence of M235T and I/D molecular variants. The frequency of both these polymorphisms in the Slovak population is similar to other Caucasian populations. In the group of hypertensive patients, the frequency of the M235T molecular variant was increased compared to controls, predominantly in males (0.45 vs. 0.28), while in the I/D polymorphism the incidence of the D allele was the same for both controls and hypertensives (0.49 vs. 0.50). A significant increase in the D allele frequency compared to the controls occurred in the group of infarcted patients (0.63). The increased frequency of the M235T allele in hypertensive patients compared to the healthy population confirms that the M235T variants associated with increased blood pressure in the Slovak population. In the Slovak population, I/D polymorphism of the ACE gene is associated with myocardial infarction rather than with hypertension.

**Key words**
M235T polymorphism – Hypertension – I/D polymorphism – Slovak population

## Introduction

Hypertension is a common trait of multifactorial determination imparting an increased risk of myocardial infarction, stroke and terminal-stage renal disease. The fact that a large proportion of interindividual variations in blood pressure and the development of myocardial infarction is genetically determined suggests that the genetic approach to the identification of primary determinants of some cardiovascular diseases should be employed. The premise of such efforts is the assumption that identification of the genetic determinants of human hypertension and myocardial infarction will provide the key to the unravelling the pathogenesis of this trait and will permit identification of individuals with a specific underlying inherited predisposition. This should ultimately permit intervention in either preclinical or clinical stages with therapy tailored to these underlying abnormalities (Lifton 1995). Genes encoding the renin-angiotensin system seem to play a pivotal role among hypertension candidate genes and also in some other cardiovascular diseases. Polymorphism in exon 2 of the angiotensinogen (AGT) gene was shown to be bound to increased blood pressure. However, the degree of this polymorphism varies in different races and populations. In the black population, the frequency of M235T molecular variant was found to be much higher (Rotimi *et al.* 1994, Barley *et al.* 1994) compared to the Caucasian population and polymorphism was not found to be associated with hypertension. On the other hand, increased frequency of the D allele of I/D

polymorphism on the angiotensin converting enzyme (ACE) gene was found to be associated with myocardial infarction rather than with hypertension (Cambien *et al.* 1992, Oike *et al.* 1995).

The aim of our study was to analyse both I/D polymorphism of ACE gene and M235T polymorphism of the angiotensinogen gene in an understudied Eastern European population. Essential hypertension and myocardial infarction are major public health complications which dramatically decrease life expectancy in Slovakia. In this study, we analyzed a molecular variant of the angiotensinogen gene, M235T, and ACE I/D polymorphism and compared the frequency of their occurrence in a group of hypertensive patients and patients who had undergone myocardial infarction with control subjects.

## Materials and Methods

*Subjects*

Blood samples from 241 participants included in this trial were obtained from the Clinic of Internal Medicine, Ruzinov Hospital and the Institute of Cardiovascular Diseases, Bratislava and divided into three groups:

- control individuals (age 58.5 ± 0.6 years);
- individuals with essential hypertension (systolic B.P. higher than 140 mm Hg; age 57.5 ± 7.0 years);
- patients who have had myocardial infarction (age 48.2 ± 2.6 years).

*Preparation of genomic DNA*

Genomic DNA was extracted from 500 $\mu$l of whole blood (in EDTA) by the phenol/chloroform procedure. Briefly, 1 ml of lysing buffer (0.32 mol/l sucrose, 1 % Triton X-100, 5 mmol/l $MgCl_2$ and 12 mmol/l Tris pH 7.5) were added to 500 $\mu$l of peripheral blood (in 0.5 mol/l EDTA pH 8.0). The mixture was vortexed and centrifuged at 10 000 x *g* for 3 min. The pellet was washed with 500 $\mu$l of redistilled water, centrifuged at 10 000 x *g* for 2 min and the sediment was resuspended in 100 $\mu$l of 0.375 mol/l NaCl, 0.12 mol/l EDTA at pH 8.0 with 200 $\mu$l $H_2O$, mixed thoroughly and 10 $\mu$l of 10 % SDS were added. Afterwards, 100 $\mu$l of 5.0 mol/l NaCl and 400 ml phenol chloroform mixture (pH 8.0) were added, the sample was mixed and centrifuged for 3 min at 10 000 x *g*. 96 % ethanol in 2-fold excess was added to the water phase. The precipitate was centrifuged at 10 000 x *g* for 3 min, final DNA sediment was washed with 70 % ethanol and suspended in 200 $\mu$l of redistilled water.

*Polymerase chain reaction (PCR)*

Genotypes for the AGT codon 235 were enzymatically amplified in a thermal cycler (Techne UK) with Taq polymerase (Pharmacia Biotech). The primers were designed according to Russ *et al.* (1993) so that in the presence of exchange of methionine (M) to threonin (T) at the codon 235 a restriction site or Tth 111 I was completed. The upstream primer was: 5'-CCGTTTGTGCAGGGCCTGGCTCTCT-3' and the downstream primer was: 5'-CAGGGTGCTGTCCACA CTGGACCCC-3'. PCR products were digested with Tth 111 I restriction endonuclease (Amersham) (5 U per reaction) for four hours at 65 °C and analyzed in 2 % agarose (Pharmacia Biotech) gel. For I/D polymorphism following primers were used: the upstream primer 5'-CTGGAGACCACTCCCATC CTTTCT-3' and the downstream primer 5'-GATGT GGCCATCACATTCGTCAGA-3' (Rigat *et al.* 1992). These primers allowed detection of a genomic DNA segment of 490 bp corresponding to the I allele as well as a segment of 190 bp corresponding to the D allele.

*Allele frequencies and statistical analysis*

Allele frequencies between patients with diagnosed hypertension or infarction and the controls were compared by chi-square analysis.

**Table 1.** Molecular variants of AGT gene in the Slovak population

| Subjects | NI | −/− | −/+ | +/+ | q |
|---|---|---|---|---|---|
| Controls | 108 | 50 | 47 | 11 | 0.32* |
| Controls HW | | 49.9 | 47.0 | 11.1 | |
| Hypertensive | 89 | 30 | 43 | 16 | 0.42* |
| Hypertensive HW | | 29.9 | 43.4 | 15.7 | |

** $p < 0.05$, NI – number of individuals tested, q – frequency, HW – Hardy-Weinberg calculation*

## Results

Presence of the M235T variant was identified after the restriction with restriction endonuclease Tth 111 I. The heterozygous form was indicated by the appearance of two fragments with the size 165 bp and 141 bp, while the homozygous form revealed only one band with the size 141 bp and individuals lacking this mutation showed only one band sized 165 bp.

Frequency of the molecular variant M235T was significantly higher in hypertensive individuals compared to the controls (0.42 versus 0.32) (Table 1). Hypertensive males exhibited the highest frequency of M235T allele (0.45) in all the groups tested. In the group of females, the frequency of M235T allele was not significantly different from that in the group of control females (0.36 versus 0.37) (Table 2). The frequency of the D allele of I/D polymorphism in the healthy Slovak population was 0.49 (Table 3). Almost the same frequency of D allele was observed in the group of hypertensive patients (0.51), while in the group of patients, who had undergone the myocardial infarction, this frequency was significantly increased (0.63) (Table 2).

**Table 2.** Molecular variants of AGT gene in the Slovak population according to sex

| Subjects | NI | n | q | p | %H |
|---|---|---|---|---|---|
| Hypertensive males | 52 | 104 | 0.45 | | 45 |
| Control males | 50 | 100 | 0.28 | $p<0.001$ | 44 |
| Hypertensive females | 37 | 74 | 0.36 | | 60 |
| Control females | 58 | 116 | 0.37 | ns | 43 |
| Hypertensive subjects | 89 | 178 | 0.42 | | 51 |
| Control subjects | 108 | 216 | 0.32 | $p<0.05$ | 43 |

*NI – number of individuals tested, n – number of chromosomes, q – frequency, %H – percentage of heterozygosity*

**Table 3.** Molecular variants of ACE gene in the Slovak population

| Subjects | NI | II | ID | DD | q |
|---|---|---|---|---|---|
| Controls | 103 | 27 | 52 | 24 | $0.485^{*+}$ |
| Controls HW | | 27.3 | 51.4 | 24.2 | |
| Hypertensive | 89 | 22 | 44 | 23 | $0.505^{+}$ |
| Hypertensive HW | | 22.1 | 44.2 | 22.7 | |
| Infarcted | 49 | 7 | 22 | 20 | 0.632* |
| Infarcted HW | | 6.6 | 22.8 | 19.6 | |

*$^{*}p=0.011$, $^{+}p$ = ns, NI – number of individuals tested, q – frequency, HW – Hardy-Weinberg calculation*

## Discussion

The renin-angiotensin system acts as a powerful pressor system with a major influence on salt and water homeostasis. Genes encoding individual components of this system are under intensive investigation in many laboratories because of their possible association with hypertension and some cardiovascular diseases. The involvement of angiotensinogen in the pathogenesis of essential hypertension has been suggested in several studies. Strong and consistent evidence was found, supporting the linkage to essential hypertension of regions within or close to the angiotensinogen gene (Caulfield *et al.* 1994). This conclusion is based on the observation of genetic linkage (Jeunemaitre *et al.* 1992) in hypertensive siblings, of a higher occurrence of the molecular variant M235T of the angiotensinogen gene in hypertensive patients compared to control subjects, and of increased plasma angiotensinogen concentration

in subjects carrying the angiotensinogen molecular variant M235T. The occurrence of the M235T molecular variant was found to be a predisposing factor for hypertension in Caucasians (Jeunemaitre *et al.* 1992) and in Japanese (Hata *et al.* 1994). Moreover, significant association between coronary atherosclerosis (Ishigami *et al.* 1995b) and/or coronary heart disease (Katsuya *et al.* 1995) and M235T of the angiotensinogen gene has also been observed.

Frequency of the M235T polymorphism on angiotensinogen gene in the Slovak population corresponds to that observed in other Caucasian populations (Jeunemaitre *et al.* 1992) with the exception of that published by Barley *et al.* (1994). However, the Slovak population exhibited a marked difference in the frequency of angiotensinogen molecular variants according to sex. While there was no difference found in the frequency of this polymorphism in women, a considerable increase in this frequency was revealed in male hypertensive patients.

A polymorphism of the ACE gene was found to be associated with several cardiovascular diseases. However, the results are still controversial and such association has not yet been established conclusively. Therefore, further studies are required to elucidate the implications of the ethnic differences throughout the world in order to provide more definite conclusions. In a study of the Japanese population, no association between the ACE gene polymorphism and essential hypertension has been reported (Ishigami *et al.* 1995a). This negative result is consistent with the results previously reported in Dutch, US (Jeunemaitre *et al.* 1992) and some other populations. Controversy in the results also exists about the association of this polymorphism with myocardial infarction. However, a meta-analysis of currently available studies supports an association of the ACE DD genotype with the risk of myocardial infarction (Samani *et al.* 1996). In our study we observed a significant difference in the frequency of ID polymorphism in a group of patients after myocardial infarction.

In conclusion, our results contribute to the evidence that M235T molecular variant is associated with hypertension in the Caucasian population, while I/D polymorphism on the ACE gene is associated with myocardial infarction rather than with hypertension.

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