M235T Polymorphism of the Angiotensinogen Gene and Insertion/Deletion Polymorphism of the Angiotensin-1 Converting Enzyme Gene in Essential Arterial Hypertension in Caucasians

(M235T polymorphism of the angiotensinogen gene / ID polymorphism of the *ACE* gene / essential arterial hypertension / association study)

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Abstract. In order to investigate the contribution of candidate genes in the RAAS in pathogenesis of EAH, we analysed the M235T polymorphism of the angiotensinogen gene, and the I/D polymorphism of *ACE* gene in a group of adult Caucasians (Slovene population) with EAH. Four-hundred and thirteen unrelated subjects with the diagnosis of EAH were included in the association study and they were compared to 414 subjects with normal blood pressure (the control group). The M235T angiotensinogen genotype distribution in patients with EAH (TT = 23.2%, MT = 48.7%, MM = 28.1%) did not differ from genotype distribution in controls (TT = 21.1%, MT = 49.0%, MM = 29.9%), and the TT genotype was not associated with EAH (OR 1.1; 95% CI 0.7–1.7; P = 0.6). Moreover, The I/D ACE genotype distribution in patients with EAH (DD = 32.0%, ID = 48.2%, II = 19.8%) did not differ from genotype distribution in controls (DD = 32.2%, ID = 49.0%, II = 18.8%), and the DD genotype was not associated with EAH (OR 1.0; 95% CI 0.7–1.3; P = 0.9). In conclusion, we failed to demonstrate that the M235T angiotensinogen polymorphism and the ACE I/D polymorphism were genetic markers for EAH in adult Caucasians.

Introduction

Essential arterial hypertension (EAH) is a complex disease influenced by various genetic and environmental factors (Jeunemaitre et al., 1992). Several complex physiological systems affect blood pressure, including the renin-angiotensin-aldosterone system (RAAS) (Jeunemaitre et al., 1992). In this case-control association study we analysed the contribution of genetic variability of the RAAS to the predisposition to EAH. Moreover, Caulfield et al. (1994) reported a linkage of the angiotensinogen gene locus (1q42-43) to EAH. For this purpose we analysed the M235T polymorphism (a missense mutation in exon 2) of the angiotensinogen gene and the insertion/deletion (I/D) polymorphism of the angiotensin-1 converting enzyme (*ACE*) gene in a group of adult Caucasians (Slovene population) with EAH.

Material and Methods

The study population consisted of 413 hypertensive and 404 normotensive unrelated adult Caucasians (Slovene population). EAH was diagnosed according to WHO guidelines.

M235T angiotensinogen gene polymorphism and I/D polymorphism of the *ACE* gene were evaluated as described previously (Rigat et al., 1992; Katsuya et al., 1995). Chi-square (χ^2) test was used to compare discrete variables. Genotypic odds ratios (OR) for EAH with 95% confidence intervals (CI) with two-tailed P values were calculated. Statistical analysis was performed using the SPSS program for Windows 2000 version 13 (SPSS Inc., Chicago, IL).

Results and Discussion

The genotype distribution of the M235T polymorphism of the angiotensinogen gene and the I/D polymorphism of the *ACE* gene in patients with EAH and controls were compatible with Hardy-Weinberg expectations (Table 1; angiotensinogen: patients $\chi^2 = 0.25$, P = 0.6; controls $\chi^2 = 0.06$, P = 0.8; ACE: patients $\chi^2 = 0.2$, P = 0.65; controls $\chi^2 = 0.002$, P = 0.97). No significant differences were found in lipid parameters, age (58.0 ± 9.3 years vs. 57.8 ± 8.4 years) and incidence of smoking (39% vs. 38.1%) between the patients and control subjects, whereas the patients had higher body mass index (28.1 ± 4.2 vs. 26.9 ± 4.0 kg/m^2; P < 0.001) in comparison to the control group.

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Abbreviations: ACE – angiotensin-1 converting enzyme, CI – confidence interval, I/D – insertion/deletion, EAH – essential arterial hypertension, OR – odds ratio, RAAS – renin-angiotension aldosterone system.

In cross-sectional study we failed to demonstrate an association between the TT genotype of the angiotensinogen M235T polymorphism and EAH (Table 1). Contrary to our cross-sectional study, few studies reported an association between the TT genotype of the M235T angiotensinogen polymorphism and adult EAH (Jeunemaitre et al., 1992; Johnson et al., 1996). Johnson et al. (1996) studied elderly hypertensive subjects (aged ≥60 years), and the female : male ratio was 0.85, whereas hypertensive subjects in our study were younger (mean age 58 years), and the female : male ratio was 0.38 in the EAH group and 0.44 in the control group. Moreover, the contribution of the M235T angiotensinogen polymorphism may also be dependent on interacting factors such as sex, age and body mass index (Johnson et al., 1996; Ogihara et al., 2000). In our study, however, neither in men (26.8% vs. 20.8%; OR 1.4, 95% CI 0.6–1.4; P = 0.2) nor in women (19.2% vs. 21.1%; OR 0.9, 95% CI 0.5–1.2; P = 0.7) the TT genotype of the M235T polymorphism of the angiotensinogen gene was associated with EAH.

In cross-sectional study we also failed to demonstrate an association between the DD genotype of the I/D polymorphism of the *ACE* gene and EAH (Table 1). Additionally, neither in men (30% vs. 31.7%; OR 0.9, 95% CI 0.6–1.4; P = 0.7) nor in women (33.7% vs. 32.8%; OR 1.0, 95% CI 0.7–1.6; P = 0.9) the DD genotype of the I/D polymorphism of the *ACE* gene was associated with EAH. Our findings are in accordance with the PROGRESS study that failed to demonstrate an association between the DD genotype and EAH (Harrap et al., 2003). Contrary to our cross-sectional study, Ogihara and co-workers (2000) reported that the I/D *ACE* gene polymorphism was a male-specific genetic risk factor for EAH in Japanese population. We speculate that the difference is population-specific (Japanese vs Slovene population). Different populations represent different gene pools, suggesting that gene-disease associations can be expected to vary between populations due to the differences in the complex genetic background.

In conclusion, neither the TT genotype of the angiotensinogen M235T polymorphism nor the DD genotype of the *ACE* I/D polymorphism were associated with EAH in Slovene population, and therefore they may not be used as genetic markers for EAH in Caucasians.

References

Caulfield, M., Lavender, P., Farrall, M., Munroe, P., Lawson, M., Turner, P., Clark, A. J. (1994) Linkage of the angiotensinogen gene to essential hypertension. *N. Engl. J. Med.* **330**, 1629-1633.

Harrap, S. B., Tzourio, C., Cambien, F., Poirier, O., Raoux, S., Chalmers, J., Chapman, N., Colman, S., Leguennec, S., MacMahon, S., Neal, B., Ohkubo, T., Woodward, M.; PROGRESS Collaborative Group. (2003) The ACE gene I/D polymorphism is not associated with the blood pressure and cardiovascular benefits of ACE inhibition. *Hypertension* **42**, 297-303.

Katsuya, T., Koike, G., Yee, T. W., Sharpe, N., Jackson, R., Norton, R., Horiuchi, M., Pratt, R. E., Dzau, V. J., MacMahon, S. (1995) Association of angiotensinogen T 235 variant with increased risk of coronary artery disease. *Lancet* **345**, 1600-1603.

Jeunemaitre, X., Soubrier, F., Kotelevtsev, Y. V., Lifton, R. P., Williams, C. S., Charru P., Hunt, S. C., Hopkins, P. N., Williams, R. P., Lalouel, J. M., Corvol, P. (1992) Molecular basis of human hypertension: role of angiotensinogen. *Cell* **71**, 169-180.

Johnson, A. G., Simons, L. A., Friedlander, Y., Simons, J., Davis, D. R., MaCallum, J. (1996) M235—>T polymorphism of the angiotensinogen gene predicts hypertension in the elderly. *J. Hypertens*. **14**, 1061-1065.

Ogihara, T., Katsuya, T., Higaki, J. (2000) Genetic analysis of essential hypertension in Japanese populations. *Ann. N. Y. Acad. Sci.* **902**, 8-16.

Rigat, B., Hubert, C., Corvol, P., Soubrier, F. (1992) PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene. *Nucleic Acids Res.* **20**, 1433.

Table 1. Genotype distribution of the M235T polymorphism of the angiotensinogen gene and the I/D polymorphism of the ACE gene in patients with EAH and control group

	AH	Control group	OR (95% CI)[1]	P value
M235T polymorphism				
Genotype TT	96 (23.2)	85 (21.1)	1.1 (0.7-1.7)[3]	0.6[3]
Genotype MT	201 (48.7)	198 (49.0)		
Genotype MM	116 (28.1)	121 (29.9)		
ACE I/D polymorphism				
Genotype DD	132 (32.0)	130 (32.2)	1.0 (0.7-1.3)[2]	0.9[2]
Genotype ID	199 (48.2)	198 (49.0)		
Genotype II	82 (19.8)	76 (18.8)		

[1]Odds ratio (95% confidence interval), [2]Odds ratio (95%confidence interval) and P value for the recesive model (DD vs. II plus ID), [3]Odds ratio (95% confidence interval) and P value for the recesive model (TT vs. MM plus MT)