



Angiotensinogen T174M and M235T gene polymorphisms are associated with the extent of coronary atherosclerosis

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Abstract

Background: The relations of the angiotensinogen (*AGT*) T174M and M235T gene polymorphisms to the risk of coronary heart disease (CHD) have been investigated in only a few studies with conflicting results. **Results:** Therefore, we analysed the relationship of the *AGT* gene polymorphisms to the presence and extent of CHD in 2250 male Caucasians whose coronary anatomy was defined by means of coronary angiography. The relative frequencies of the T and M alleles of the T174M and of the M235T gene variation did not significantly differ between patients without or with single-, double- or triple-vessel disease and between subjects without or with myocardial infarction (MI). In contrast the mean CHD score—defined by Gensini—was higher within MM homozygotes of the T174M gene variation than within TT genotypes; TM subjects had intermediate values. In M235T genotypes, mean CHD scores were similar in the total sample and in older individuals (≥ 62 years), whereas in younger individuals (< 62 years) a higher CHD score was found within AGT 235 T allele carriers than within MM homozygotes. In younger individuals with high apoAI plasma levels, the mean CHD score was clearly higher within TT homozygotes of the M235T gene variation than within MM genotypes; MT subjects had intermediate values. An interaction between both angiotensinogen gene polymorphisms on the extent of CHD or on the risk of non-fatal MI were not observed when the M allele of AGT T174M was combined either with the T allele or the TT genotype of M235T. **Conclusions:** The present study strengthens the hypothesis of an association of both angiotensinogen gene polymorphisms with the extent of coronary heart disease. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Angiotensinogen gene; Cardiovascular disease; Myocardial infarction; Risk factors; Renin-angiotensin-aldosterone system; Gensini score

1. Introduction

Various studies have presented evidence that the renin angiotensin system (RAS) might play an important role in the development of coronary heart disease (CHD) [1–8]. These findings stimulated investigators to identify genetic variations in main components of RAS which might be associated with CHD [9–17]. Besides the insertion/deletion (I/D) polymorphism of the an-

giotensin 1-converting enzyme (*ACE*) gene [9–14] and the angiotensin II type 1 receptor A1166C gene variation [15], two polymorphisms in the angiotensinogen (*AGT*) gene (T174M, M235T) could be identified [16–21].

With respect to the angiotensinogen gene two scientific groups [17,18]—in contrast to other investigators [19–21]—identified the *AGT* M235T gene variation as a risk factor for CHD. The relation of the *AGT* T174M gene variation to coronary heart disease has only been investigated by Ko et al. [19] and Tiret et al. [21]; the authors did not detect a significant contribution of this gene polymorphism to the risk of MI.

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In a large case-control study the assumption was strengthened that the *ACE* I/D gene polymorphism was associated with the risk of coronary heart disease [14]. It was the aim of the present study to extend these investigations by analysing the relationships of the *AGT* T174M and M235T gene polymorphisms with CHD in the large population of 2250 male Caucasians whose coronary anatomy was defined by means of coronary angiography.

2. Methods

2.1. Study population

The study sample comprised 2250 consecutive male Caucasians who underwent coronary angiography for diagnostic purposes. About 80% of the participants underwent coronary angiography on account of coronary heart disease as verified illness or presumption diagnosis. The remaining part of the group consisted almost completely of patients who underwent coronary angiography for clarification of restricted left ventricular function. All patients who agreed to participate in the study were evaluated with a detailed questionnaire which provided information about coronary risk factors such as smoking, diabetes mellitus and hypertension.

2.2. Detection of coronary artery disease and myocardial infarction

Coronary angiography was performed by the Judkins method. Coronary vessels with at least 50% stenosis were defined as diseased. The severity of coronary heart disease (CHD) was also estimated by calculating the Gensini score [22,23]; this score is designated 'CHD score' in subsequent text. Angina pectoris and acute myocardial infarction were diagnosed according to criteria by the World Health Organisation.

2.3. Definition of variables, measurements of substrates, and detection of the T174M and M235T polymorphisms

Arterial hypertension and diabetes mellitus were defined as binary variables; cigarette consumption was given as pack years. Triglycerides, total cholesterol, apolipoprotein B (apoB), apolipoprotein AI (apoAI), fibrinogen and lipoprotein (a) (Lp(a)) were measured as described in [14]. Genotypes for *AGT* codons 174 [24] and 235 [16] were determined as described.

2.4. Statistical analysis

The relation of the *AGT* gene polymorphisms with continuous variables were tested by Kruskal–Wallis one-way ANOVA. The relation of the gene variations to diabetes and hypertension were checked by χ^2 analysis. Established risk factors for coronary artery disease (CAD) and MI were identified by multiple regression analysis (extent of CAD, CHD score) or multiple logistic regression (absence/presence of CAD or MI). The χ^2 -test was used to test for deviation of genotype distribution from Hardy-Weinberg equilibrium. The relations of the *AGT* gene polymorphisms to the extent of CAD—defined as single-, double-, or triple-vessel disease or as CHD score—were determined by multiple regression with adjustment for other coronary risk factors (including *ACE* I/D gene polymorphism [14]). The relations of the *AGT* gene polymorphisms to myocardial infarction were determined by multiple logistic regression with adjustment for other coronary risk factors (including *ACE* I/D gene polymorphism [14]). Interactions between the two *AGT* gene polymorphisms on the extent of coronary heart disease were tested by a two-factor ANOVA procedure. A two-sided probability value of less than 0.05 was considered to indicate statistical significance. Statistical analyses were performed using the SPSS for Windows 95 software (Version 7.52).

3. Results

3.1. Distribution of angiotensinogen T174M and M235T genotypes within the study population and effect of genotypes on variables of clinical chemistry and clinics

By means of coronary angiography, the study population was divided into subjects without any angiographically detectable CAD or with coronary arterial stenoses less than 50% (no vessel disease; $n = 511$; mean age 58.5 ± 10.6 years) and individuals with single-vessel disease ($n = 453$; mean 61.2 ± 9.7 years), double-vessel disease ($n = 488$; mean age 62.6 ± 9.8 years), and triple-vessel disease ($n = 798$; mean age 63.6 ± 8.6 years). Mean age was 61.3 ± 9.9 years in persons without myocardial infarction ($n = 1192$) and 62.1 ± 9.5 years in subjects with MI ($n = 1058$) (Table 1). In controls, the T174M and M235T genotype distributions were compatible with Hardy-Weinberg expectations. Age, total cholesterol, triglycerides, apoAI, apoB, Lp(a), fibrinogen, prevalence of diabetes and arterial hypertension, body mass index and cigarette consumption were almost identical between the angiotensinogen genotypes of the total study population (data not shown). Similar observations were made in subgroups of patients with-

out or with single- or multi-vessel disease, of subjects without MI, and of MI patients (not shown). Both polymorphisms were in pronounced linkage disequilibrium ($P < 0.0001$), since the M174 allele was preferentially found in a subgroup of individuals with at least one T235 allele (Fig. 1).

3.2. Relation of established coronary risk factors and of AGT T174M and M235T gene variations to coronary heart disease

3.2.1. Coronary risk factors

ApoB ($P < 0.0001$), Lp(a) ($P < 0.005$), diabetes mellitus ($P < 0.02$), hypertension ($P < 0.01$), smoking habit ($P < 0.01$) and age ($P < 0.0001$) could be demonstrated as risk factors for coronary artery disease, and apoAI ($P < 0.0001$) and high apoAI/apoB ratios ($P < 0.0001$) as protective factors against CAD. Age ($P < 0.002$), apoB ($P < 0.0002$), fibrinogen levels ($P < 0.05$) and cigarette consumption ($P < 0.002$) were identified as risk factors for myocardial infarction, and apoAI ($P < 0.0001$) and high apoAI/apoB ratios ($P < 0.0002$) as protective factors against MI (data not shown).

3.2.2. AGT T174M gene polymorphism

The relative frequencies of the T and M alleles of the T174M gene variation did not significantly differ between patients without or with single-, double- or triple-vessel disease (Table 1). In contrast, the mean CHD score was higher within MM homozygotes of the T174M gene variation than within TT genotypes; TM subjects had intermediate values ($P < 0.05$; Fig. 2A). Differences in CHD scores were also observed in

younger individuals (< 62 years, mean age of population) (Fig. 2A; $P < 0.05$) which were no longer significant after further restriction to subjects with high apoAI (> 1.43 g/l; mean value of population) (Fig. 2A). An association of the AGT T174M gene polymorphism with non-fatal MI was not detected; this observation applies to the total study sample (Table 1) and to various high and low risk populations (data not shown).

3.2.3. AGT M235T gene polymorphism

Also the relative frequencies of the M and T alleles of the M235T gene polymorphisms were essentially the same in patients without or with single-, double- or triple-vessel disease (Table 1). In M235T genotypes, mean CHD scores were similar in the total sample (Fig. 2B) and in older individuals (≥ 62 years) (data not shown), whereas in younger individuals (< 62 years) a higher CHD score was found within AGT 235 T allele carriers than within MM homozygotes (Fig. 2B; $P < 0.02$). In younger individuals with high apoAI plasma levels, the mean CHD score was clearly higher within TT homozygotes of the M235T gene variation than within MM genotypes; MT subjects had intermediate values ($P < 0.01$; Fig. 2B). Similar to the AGT T174M gene variation, the AGT T235M gene polymorphism was not linked to the risk of MI either in the total sample (Table 1) or in low or high risk populations (data not shown).

3.2.4. Potential interactions between both AGT gene polymorphisms

In the total sample and in subpopulations, interactions between both AGT gene variations were not ob-

Table 1
Distribution of angiotensinogen T174M and M235T genotypes in subjects without any or single-, double-, or triple-vessel disease, individuals without MI and patients with myocardial infarction^a

Disease	Age (years)	n	Frequencies									
			AGT 174					AGT 235				
			Alleles		Alleles		Alleles		Alleles			
TT	TM	MM	T (95% CI)	M (95% CI)	MM	MT	TT	M (95% CI)	T (95% CI)			
<i>CAD</i>												
NVD	58.5 ± 10.6	511	389	115	7	0.87 (0.85–0.89)	0.13 (0.11–0.15)	168	247	96	0.57 (0.54–0.60)	0.43 (0.40–0.46)
SVD	61.2 ± 9.7	453	356	90	7	0.88 (0.86–0.91)	0.12 (0.09–0.14)	141	244	68	0.58 (0.55–0.61)	0.42 (0.39–0.45)
DVD	62.6 ± 9.8	488	384	99	5	0.89 (0.87–0.91)	0.11 (0.09–0.13)	156	256	76	0.58 (0.55–0.61)	0.42 (0.39–0.45)
TVD	63.6 ± 8.6	798	613	173	12	0.88 (0.86–0.89)	0.12 (0.11–0.14)	239	420	139	0.56 (0.54–0.59)	0.44 (0.42–0.46)
<i>MI</i>												
No MI	61.3 ± 9.9	1192	925	249	18	0.88 (0.87–0.89)	0.12 (0.11–0.13)	385	585	222	0.57 (0.55–0.59)	0.43 (0.41–0.45)
≥1 MI	62.2 ± 9.5	1058	817	228	13	0.88 (0.87–0.89)	0.12 (0.11–0.13)	319	582	157	0.58 (0.56–0.60)	0.42 (0.40–0.44)

^a Coronary angiography was performed by the Judkins method. Vessels were defined as diseased if at least 50% stenosis was demonstrated by means of coronary angiography. Acute myocardial infarction was diagnosed according to criteria by the World Health Organisation. DVD, double vessel disease; NVD, no vessel disease; SVD, single vessel disease; TVD, triple vessel disease. Age is given as mean ± S.D.

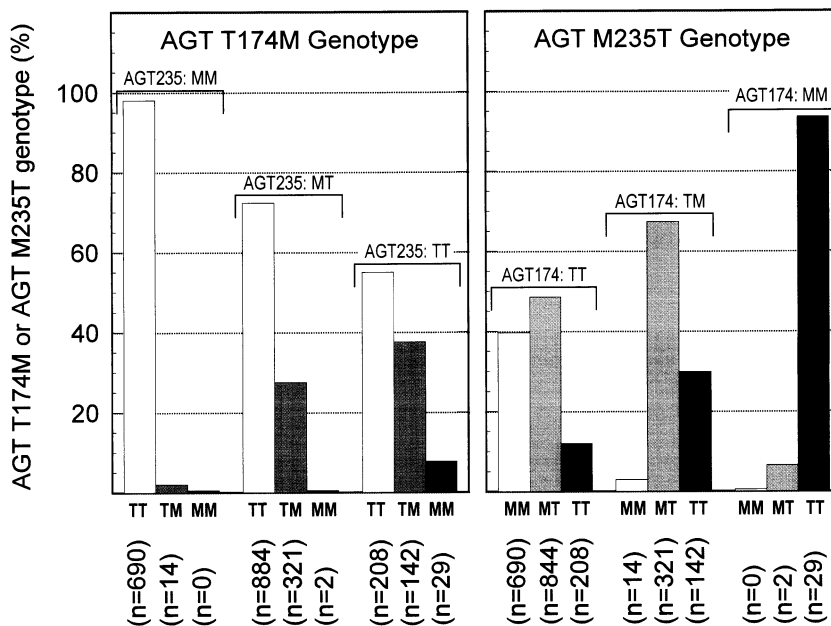


Fig. 1. Distributions of angiotensinogen T174M genotypes according to the *AGT* M235T gene variation and of angiotensinogen M235T genotypes according to the *AGT* T174M gene polymorphism. The relations between both angiotensinogen gene variations were checked by χ^2 analysis. Both polymorphisms were in a pronounced linkage disequilibrium ($P < 0.0001$). Numbers of individuals are given in parentheses.

served. Subjects with at least one M allele of the *AGT* T174M gene polymorphism and the TT genotype of the *AGT* M235T gene variation did not have higher CHD scores than individuals without these genotype combinations (Fig. 2C). Only in subgroups of younger individuals (< 62 years), carriers of 'AGT174 M + AGT235 TT' had higher CHD scores than subjects with the genotype combination 'AGT174 TT + AGT235 MM' ($P < 0.05$). These differences were more pronounced when the younger individuals were restricted to subjects with high apoAI (Fig. 2C). However, these differences did not significantly exceed those between genotypes of the *AGT* M235T gene variation alone (Fig. 2C vs. Fig. 2B). An interaction between both angiotensinogen gene polymorphisms was also not observed when the M allele of *AGT* T174M and the T allele of M235T were combined (data not shown).

4. Discussion

The present investigation was performed to analyse the relationships of the angiotensinogen *AGT* T174M and *AGT* M235T gene polymorphisms with CHD and to search for potential interactions between these gene variations on the risk of coronary heart disease.

4.1. Design of the present study

Although a separate subgroup of control subjects without any symptoms of any type of heart disease

was not established, there is every reason to assume that comparisons within our study group would lead to accurate predictions of new coronary risk factors, since established risk factors of CHD, such as age, hypercholesterolaemia, apoB, Lp(a), fibrinogen levels, diabetes mellitus, hypertension and cigarette consumption, as well as known protective factors against CHD, like apoAI and high apoAI/apoB ratios, could be identified in the present study sample. Furthermore, we would like to place particular emphasis on the fact that we not only divided our study sample into CHD – and CHD + subjects, but also analysed the extent of CHD of all participants of our large case-control study by coronary angiography and subsequent calculation of a CHD score, defined by Gensini [22,23]. This calculation enabled us to determine the precise extent of coronary heart disease.

4.2. Distributions of *AGT* T174M and *AGT* M235T genotypes

The frequencies of the T and M alleles in the present study sample show similarities to those of previous investigations [21,24,26]. In controls, *AGT* T174M M allele frequencies of 0.88 [21], 0.82 [24], 0.88 [26] and 0.87 (present study) were calculated. Similar observations were made with respect to the *AGT* M235T gene variation, since in controls T allele frequencies of 0.38 [17], 0.41 (ARIC white [18]), 0.43 (Framingham [18]), 0.40 [21], 0.44 [25] and 0.43 (present study) were detected.

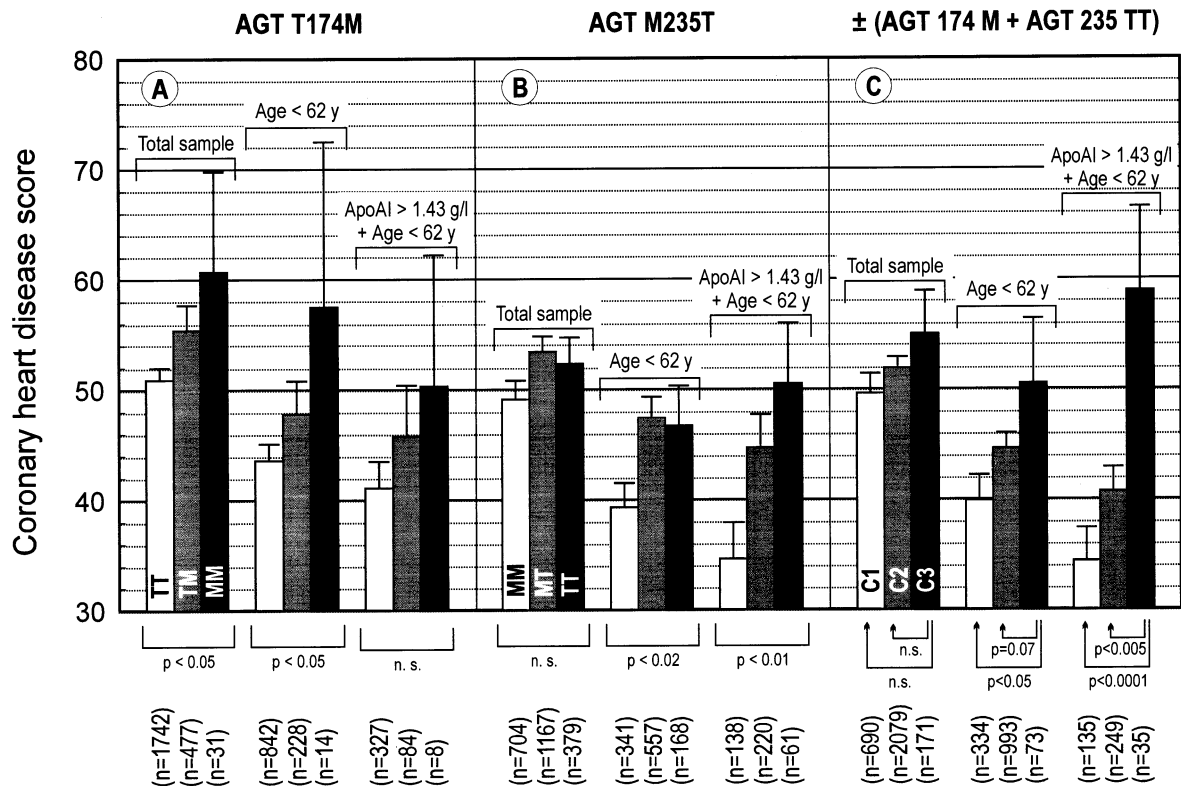


Fig. 2. Comparison of mean CHD scores between subjects with different AGT T174M and AGT M235T genotypes. The mean CHD scores of the different subpopulations were compared by multiple regression analysis after adjustment for coronary risk factors. Values are means \pm S.E.M. Abbreviations: C1, individuals who were homozygous with respect to the AGT T174M T allele and the AGT M235T M allele; C2, individuals whose AGT T174M and M235T genotype combination did not match with the genotype constellation of C3; C3, individuals who simultaneously had at least one AGT T174M M allele and an AGT M235T TT genotype. Numbers of individuals are given in parentheses.

4.3. Association of both angiotensinogen gene variations with CHD

The results of the present study strengthens the hypothesis that both angiotensinogen gene polymorphisms are associated with the extent of CHD. These results cannot be compared with the results of other investigations, since only in the present study was the extent of CHD in all participants analysed by calculating a coronary heart disease score, defined by Gensini [22,23]. With respect to the *AGT* T174M gene variation, none of the three studies ([19,21] and present study) gave any support for an association of this gene polymorphism with an increased risk of non-fatal MI. With respect to the relation of the *AGT* M235T gene variation to MI, the present study was also in line with the results of other investigators [19–21]; the genotype distributions did not differ between survivors of myocardial infarction and controls. However, in Caucasians of the ARIC study group but not of the Framingham study sample, Ludwig et al. [18] observed an association of the *AGT* M235T TT genotype with the risk of MI. In addition, Katsuya et al. [17] also identified the *AGT* M235T TT genotype as an independent risk factor of non-fatal MI. Reasons for these discrepancies are not known.

4.4. Putative mechanism(s) for the increased susceptibility of AGT T174M M and M235T T carriers to cardiovascular disease

At present it is still a matter of debate or completely unknown whether the *AGT* gene polymorphisms may have causative roles in the development of CHD or simply act as neutral markers. The angiotensinogen M235T gene variation has been hypothesised to have a causal role, since the M235T T allele was associated with an increased concentration of circulating angiotensinogen [27]. Further studies are clearly needed to clarify the relation of both angiotensinogen gene polymorphisms to CHD and to explore the putative mechanism(s) in the case of an increased susceptibility of *AGT* T174M M and M235T T carriers to cardiovascular disease.

References

- [1] Powell JS, Clozel JP, Muller RKM, Kuhn H, Hefti F, Hosang M, et al. Inhibitors of angiotensin-converting enzyme prevent myointimal proliferation after vascular injury. *Science* 1989;245:186–8.
- [2] Chobanian AV, Haudenschild CC, Nickerson C, Drago R. Antiatherogenic effect of captopril in the Watanabe heritable hyperlipidemic rabbit. *Hypertension* 1990;15:327–31.

- [3] Aberg G, Ferrer P. Effects of captopril on atherosclerosis in cynomolgus monkeys. *Cardiovasc Pharmacol* 1990;15(Suppl 15):S65–S72.
- [4] Wang DH, Prewitt RL. Captopril reduces aortic and microvascular growth in hypertensive and normotensive rats. *Hypertension* 1990;15:68–77.
- [5] The SOLVD investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *New Engl J Med* 1991;325:293–302.
- [6] Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ, Cuddy TE, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. *New Engl J Med* 1992;327:669–77.
- [7] Ambrosioni E, Bacchelli S, Degli Esposti D, Borghi C. ACE-inhibitors and atherosclerosis. *Eur J Epidemiol* 1992;8(Suppl 2):129–33.
- [8] Dzau VJ, Sasamura H, Hein L. Heterogeneity of angiotensin synthetic pathways and receptor subtypes: physiological and pharmacological implications. *J Hypertens* 1993;11:S13–8.
- [9] Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992;359:641–4.
- [10] Singer DRJ, Missouriis CG, Jeffery S. Angiotensin-converting enzyme gene polymorphism. What to do about all the confusion? *Circulation* 1996;94:236–9.
- [11] Samani NJ, Thompson JR, O'Toole L, Channer K, Woods KL. A meta-analysis of the association of the deletion allele of the angiotensin-converting enzyme with myocardial infarction. *Circulation* 1996;94:708–12.
- [12] Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer M, Grodstein F, La Motte F, et al. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. *N Engl J Med* 1995;332:706–11.
- [13] Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Sorensen TIA, Jensen G, Tybjaerg-Hansen A. ACE gene polymorphism: ischemic heart disease and longevity in 10150 individuals. A case-referent and retrospective cohort study based on the Copenhagen City Heart Study. *Circulation* 1997;95:2358–67.
- [14] Gardemann A, Fink M, Stricker J, Nguyen QD, Humme J, Katz N, Tillmanns H, Hehrlein FW, Rau M, Haberbosch W. ACE I/D gene polymorphism: presence of the ACE D allele increases the risk of coronary artery disease in younger individuals. *Atherosclerosis* 1998;139:153–9.
- [15] Tiret L, Bonnardeaux A, Poirier O, et al. Synergistic effects of angiotensin-converting enzyme and angiotensin-II type 1 receptor gene polymorphisms on risk of myocardial infarction. *Lancet* 1994;344:910–3.
- [16] Russ AP, Maerz W, Ruzicka V, Stein U, Gross W. Rapid detection of the hypertension-associated Met235 → Thr allele of the human angiotensinogen gene. *Hum Mol Genet* 1993;2:609–10.
- [17] Katsuyama T, Koike G, Yee TW, Sharpe N, Jackson R, Norton R. Association of angiotensinogen gene T235 variant with increased risk of coronary artery disease. *Lancet* 1995;345:1600–3.
- [18] Ludwig EH, Borecki EB, Ellison RC, Folsom AR, Heiss G, Hiffins M, et al. Associations between candidate loci angiotensin-converting enzyme and angiotensinogen with coronary heart disease and myocardial infarction: the NHLBI family heart study. *Ann Epidemiol* 1997;7:3–12.
- [19] Ko YL, Ko YS, Wang SM, Chu PH, Teng MS, Cheng NJ, et al. Angiotensinogen and angiotensin I converting enzyme gene polymorphisms and the risk of coronary artery disease in Chinese. *Hum Genet* 1997;100:210–4.
- [20] Yamakawa-Kobayashi K, Arinami T, Hamaguchi H. Absence of association of angiotensinogen gene T235 allele with increased risk of coronary heart disease in Japanese. *Lancet* 1995;346:515.
- [21] Tiret L, Ricard S, Poirier O, Arveiler D, Cambou JP, Luc G, et al. Genetic variation at the angiotensinogen locus in relation to high blood pressure and myocardial infarction. *J Hypertens* 1995;13:311–7.
- [22] Gensini GG. Coronary arteriography. In: Braunwald E, editor. *Heart disease*. Philadelphia: Saunders, 1980:352–3.
- [23] Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol* 1983;51:606.
- [24] Hegele RA, Brunt H, Connelly PW. A polymorphism of the angiotensinogen gene associated with variation in blood pressure in a genetic isolate. *Circulation* 1994;90:2207–12.
- [25] Schmidt S, Giessel R, Bergis KH, Strojek K, Grzeszczak W, Ganten D, et al. Angiotensinogen gene M235T polymorphism is not associated with diabetic nephropathy. *Nephrol Dial Transplant* 1996;11:1755–61.
- [26] Tarnow L, Cambien F, Rossing P, Nielsen FS, Hansen BV, Ricard S, et al. Angiotensinogen gene polymorphisms in IDDM patients with diabetic nephropathy. *Diabetes* 1996;45:367–9.
- [27] Jeunmaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, et al. Molecular basis of human hypertension: role of angiotensinogen. *Cell* 1992;71:169–80.