

Angiotensin-Converting Enzyme D/I Gene Polymorphism and Age-Related Changes in Pulse Pressure in Subjects with Hypertension

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Background—Few studies have examined the possible influence of gene polymorphisms on the increase of systolic blood pressure (SBP) and pulse pressure (PP) with age, although in older populations, SBP > 160 mm Hg or PP > 60 mm Hg are strong mechanical factors predicting cardiovascular mortality.

Methods and Results—This cross-sectional study involved 315 men and 154 women with either systolic–diastolic or isolated systolic hypertension. Using polymerase chain reaction, the angiotensin-converting enzyme (ACE) D/I gene polymorphism was investigated separately in men and women, enabling us to determine the relationships between age and PP, SBP, and diastolic blood pressure (DBP) for each genotype in each population. In men, most of which were under 50 years of age, the slope of the age–PP and age–SBP (but not age–DBP) relationships differed significantly between genotypes ($P=0.0096$ and 0.0175). The interslope difference was unmodified after adjustments involving all of the following factors together: plasma glucose, cholesterol, creatinine, potassium, body weight, tobacco consumption, mean blood pressure, and previous antihypertensive therapy. Adjustment of the two latter parameters alone significantly attenuated the interslope difference. Based on logistic regressions, the DD genotype was shown to independently predict a PP > 60 mm Hg but not a SBP > 160 mm Hg.

Conclusions—In men, the ACE D/I gene polymorphism independently modulates age-related increase of PP, and potentially modulates the resulting cardiovascular risk. This finding requires the development of long-term follow-up. (*Arterioscler Thromb Vasc Biol.* 2004;24:782-786.)

Key Words: hypertension ■ gene polymorphism ■ angiotensin-converting enzyme ■ pulse pressure

The predictive value of blood pressure (BP) as a cardiovascular (CV) risk factor is largely influenced by age. According to the Framingham Heart Study,^{1,2} diastolic blood pressure (DBP) is the stronger CV risk factor for subjects under 50 years of age, whereas systolic blood pressure (SBP) and, more significantly, pulse pressure (PP=SBP–DBP) contribute significantly to CV risk for subjects over 50 years of age. This contribution to CV risk is mainly due to myocardial infarction. These observations are influenced by the particularities of the age–SBP, –DBP, and –PP relationships. In subjects under 50 years of age, SBP, DBP, and PP increase parallel to age increase. For subjects over 50 years of age, SBP and PP increase exponentially with age, whereas DBP remains stable or even tends to be slightly reduced, independent of any antihypertensive drug therapy but parallel to an increase of arterial stiffness.³ Cross-sectional and longitudinal studies^{4,5} have confirmed that CV mortality is not only positively correlated to the level of SBP, but also that, at any given value of SBP, CV mortality is higher when DBP is lower, particularly for subjects over 60 years of age. Thus, it is important to evaluate the changes

together of SBP and PP with age, particularly in older populations in which an SBP > 160 mm Hg and/or a PP > 60 mm Hg are independently and significantly associated to CV risk and mortality.²

Long-term follow-up in large populations has emphasized that the levels of SBP and PP, in addition to their rates of change with age, contribute substantially to CV risk.^{3–6} Epidemiology indicates that there are wide intersubject variations in the slope of the curves relating SBP and PP to age.³ Numerous environmental or genetic factors (or a combination of these factors) might substantially influence the steepness of the slope of such relationships. Nevertheless, few studies have examined the role of genetic factors in the mechanism of the changes of SBP and PP with age, even though, in animals and men, a hereditary predisposition has been previously postulated to explain such alterations.⁷ Thus, in subjects both under and over 50 years of age, it is relevant to investigate the influence of gene polymorphisms on the age-related changes of SBP and PP.

Recent investigations in humans have shown that the angiotensin II type I (AT₁) receptor gene polymorphism and

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the Glu298Asp gene polymorphism, or even their combination, contribute to the mechanism(s) of the increase of PP with age, and therefore of an increase in CV risk.^{8–10} However, in such studies, several questions remained to be answered. First, the results were exclusively limited to two gene polymorphisms. It is possible that other gene polymorphisms, particularly those related to the renin-angiotensin system, might be implicated. Second, because of the relatively small number of subjects tested, it was not possible to dissociate the specific influence of gene polymorphisms according to both age and gender. Finally, taken together, these observations suggest the need to investigate specifically the role of the angiotensin-converting enzyme (ACE) D/I gene polymorphism on the age–PP and –SBP relationships. Indeed, this polymorphism is the most commonly studied in subjects with hypertension, but it has never been investigated within the framework of the age–PP or –SBP relationships.^{7,11} On the other hand, pharmacological studies in humans have shown the influence of the ACE D/I gene polymorphism on the contractile properties of conduit arteries,¹² thus suggesting a potential effect on arterial stiffness and PP.

The first objective of this study was to evaluate, in men and women with hypertension, the contribution of the ACE D/I gene polymorphism to the slope of the curve relating SBP and PP to age. The second objective was to investigate whether alterations of environmental and genetic factors provide some insight on the CV risk of hypertensive subjects over 50 years of age. This latter objective takes into account that, for subjects over 50 years of age, isolated systolic hypertension is a characteristic hemodynamic feature, which involves increased PP and is classically observed in women.^{1,13}

Methods

Study Cohort

The study was performed with hypertensive patients who participated in a previously published therapeutic trial conducted in 13 countries.¹⁴ Written informed consent was obtained from each patient, and the protocol was approved by the Ethics Committees, in accordance with local regulations. After a 1-month washout placebo period, the patients entered the therapeutic trial. The present study was performed only in the population of subjects investigated at baseline, ie, at the end of the placebo period.

Hypertensive subjects with either systolic–diastolic hypertension or isolated systolic hypertension were selected on the basis of BP measurements obtained by mercury sphygmomanometer. SBP was constantly above 160 mm Hg whether DBP was < or \geq 90 mm Hg. Subjects with SBP >210 mm Hg and DBP >110 mm Hg were excluded from the cohort. Patients had no clinical or biological signs of secondary hypertension, as classically defined.¹⁴ No subjects had past symptoms of coronary artery disease, heart failure, stroke, or peripheral arterial disease. Finally, 315 men and 154 women, from 19 to 85 years of age (mean=55 years), were investigated in this study. Among these patients, 73% had been previously treated for hypertension and the remaining did not receive any previous antihypertensive therapy (PAT).

All participants were examined in the morning after fasting for at least 12 hours, and all patients underwent the same procedure. After 20 minutes of rest in the supine position, BP was measured on the basis of phases I and V of Korotkoff sounds, using a mercury sphygmomanometer (mean of 3 measurements). Mean BP (MBP) was calculated as $DBP + 1/3 PP$. PP was expressed in absolute value (mm Hg). Based on epidemiological studies, it is well accepted that SBP and PP above the critical levels of 160 and 60 mm Hg, respectively, cause particular risk in patients.^{1–3} At the end of the procedure, blood was drawn for determination of standard biochemical measurements and DNA extraction.

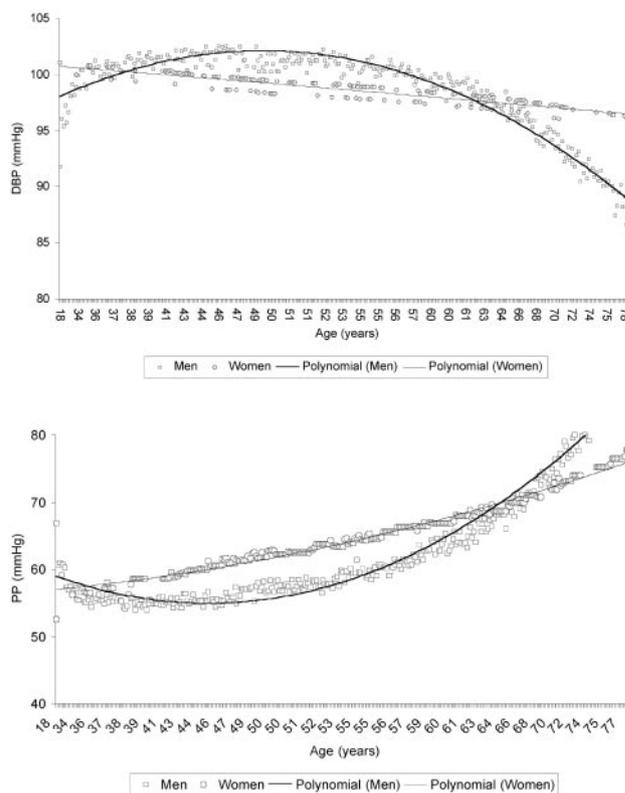


Figure 1. Overall population: relationship between age and DBP (predictive values) and age and PP (predictive values) in males and females. Note that the best model (age–DBP relationship in men) is quadratic and that the nadir of the age–DBP relationship is at 50 years.

Figure 1 shows the age–DBP and age–PP relationships in the totality of the population. As shown by most classical epidemiological studies,^{1,2,13,15} DBP increases with age until around 50 years of age and thereafter tends to decrease markedly, whereas PP and SBP increase progressively with age. The mathematical evaluation of the curvilinear age–DBP relationship (Figure 1) clearly shows that DBP at 50 years of age approximated 90 to 95 mm Hg and was the target value, which enabled us to divide the total population into younger (\leq 50 years of age) and older ($>$ 50 years of age) subjects. The clinical and biological characteristics of the subjects are presented according to gender in Table I (available online at <http://atvb.ahajournals.org>). Note that when women are compared with men, particularly over 50 years of age, women's plasma glucose and creatinine were lower and their heart rate was higher.

Genotype Investigation and Classification of Patients

In this genetic study, standard salting-out techniques and polymerase chain reaction amplification were used. The set of primers for amplification of relevant DNA regions, the probes, and the methods used to detect the ACE D/I gene polymorphisms have been described previously in detail.^{8–10} The washing temperature in saturated sodium citrate was adjusted according to the melting temperature of the probes. Table II (available online at <http://atvb.ahajournals.org>) summarizes the repartition of subjects according to the different genotypes, classically called II, ID, and DD.

Statistical Analyses

Quantitative data were expressed as mean \pm SD (1 standard deviation) or mean \pm SE (1 standard error) when adjusted on one or several of the different studied parameters. Qualitative data were expressed as percent of subjects in each modality. Allele and genotype

frequencies were analyzed by using the gene-counting method and the Hardy–Weinberg equilibrium, checked by a χ^2 test, and were considered to be maintained in all polymorphisms as described.^{8–11}

First, we used Levene's test of equality of variance to verify the well-established necessary conditions that enable the comparison of the different subgroups composed of gender and ACE D/I genotypes.

Second, we studied the age–PP, age–SBP, and age–DBP relationships in men and women according to a linear regression, which gave the regression coefficient of age and its standard error in each group. Thus, we described for each gender the age–PP, age–SBP, and age–DBP slopes according to the three different ACE D/I genotypes given by linear regression. We tested the slope difference between men and women as well as between each ACE D/I genotype in men. For this we used a test of heterogeneity of slopes (which represents a natural extension of covariance analysis) according to the genotype classification. Using Bonferroni's test, we considered $P < 0.025$ significant.

Last, we reclassified the PP, SBP, and DBP parameters into two classes each, considering that the cutting point for each parameter for the prediction of CV risk is 60 mm Hg, 160 mm Hg, and 95 mm Hg, respectively. Such cutting points, which are well known for SBP and DBP,¹⁵ have been established for PP on the basis of epidemiological studies^{16–18} indicating the lower level of PP at which renal, cerebral, and most ischemic cardiopathies (myocardial infarctions) occur. Note that the cutting point for PP (60 mm Hg) also represents the reference value for clinical PP in a nonselected population¹⁹ and that, in contrast with SBP and DBP, PP is poorly sensitive to placebo.²⁰ Using this procedure, a stepwise logistic regression was performed involving age in classes (≤ 50 years of age/ > 50 years of age), gender, ACE genotypes (II, ID, DD), tobacco consumption (yes or no), total plasma cholesterol in class (≤ 6.21 / > 6.21 mmol/L), plasma glucose in class (≤ 6.88 / > 6.88 mmol/L), plasma potassium in class (≤ 3.1 / > 3.1 mg/L), plasma creatinine in class (≤ 130 / > 130 μ mol/L), PAT (yes or no), and MBP (expressed in cm Hg for simplicity of calculations).

Results

Studies of Mean Values in Each Genotype Studied for Each Gender

Table III (available online at <http://atvb.ahajournals.org>) shows the mean values of MBP, PP, SBP, and DBP in the II, ID, and DD genotypes for each gender. No significant difference was observed between groups in the men or in the women. Similar findings were observed regarding PAT and CV and renal risk factors (data not shown).

Statistical Differences in the Slopes of the Pulse Pressure and Systolic Blood Pressure Versus Age Relationships

Table IV (available online at <http://atvb.ahajournals.org>) shows the regression coefficients of the age–PP relationship in the overall population and in the II, ID, and DD genotype groups in both men and women. These slope values did not change with any adjustment for PAT and MBP. The slopes were significantly different between gender ($P = 0.0082$) when adjusted for age classes (≤ 50 years and > 50 years) and between age classes ($P < 0.001$) when adjusted for gender.

In men, the difference between the slopes of the age–PP relationship according to genotypes (II, ID, and DD) was significant ($P = 0.0096$). Similar results were observed for SBP ($P = 0.0175$), but not for DBP or MBP (data not shown). All of these findings were observed in men but not in women.

As indicated in Table IV, the slope of the age–PP relationship in men was slightly steeper in the II genotype than in the DD or ID genotypes. We identified that this difference was mainly

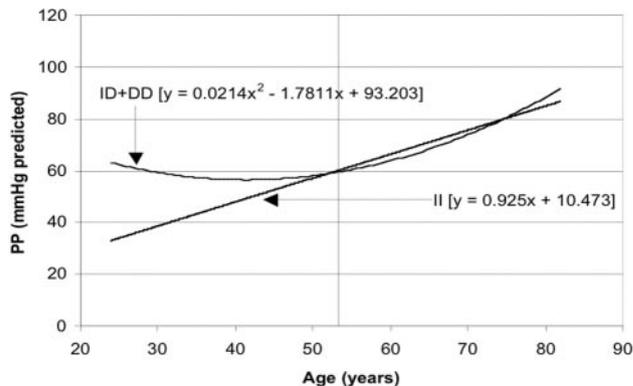


Figure 2. ACE D/I genotypes: curves relating age to PP in men. A notable difference was observed between the two curves: the ID+DD curve corresponds to a curvilinear model, and the II curve corresponds to a linear model. The difference was observed even after adjustment for tobacco consumption, total plasma cholesterol, plasma glucose, plasma potassium, plasma creatinine, PAT, and MBP.

observed in younger (≤ 50 years of age) subjects and not in older subjects. Figure 2 shows a clear model of the age–PP relationship in the II genotype as compared with the model of the ID+DD genotype. These models indicate the difference in the time course of the age–PP relationships by the curvilinearity of the ID+DD curve. Similar results are observed when the II, ID, and DD curves are presented separately.

Factors Influencing the Interslope Comparison in Men

In Table V (available online at <http://atvb.ahajournals.org>), the probability value of the interslope difference for men was studied before and after several adjustments, including CV risk factors, renal risk factors, and MBP and PAT combined. As we noted previously, the probability value before adjustment is $P = 0.0096$. The probability value was poorly modified when the adjusted factors were studied together. However, some factors studied alone or in combination, such as plasma potassium, MBP, and PAT, markedly attenuated the probability value of the interslope difference. Note that plasma glucose did not interfere substantially in the overall results.

Factors Influencing the SBP and PP Levels in the Overall Population

In Table VI (available online at <http://atvb.ahajournals.org>), the stepwise logistic regression indicates that the ACE D/I genotype is significantly linked to PP, even when age and MBP are entered in the model. The odds ratio (OR) for ACE D/I was 0.73 with 95% confidence intervals from 0.54 to 0.98 ($P = 0.0232$). Thus, a homozygote for the ACE I allele results in a lower OR (0.73) compared with D allele carriers, indicating a lower PP and hence a lower CV risk of myocardial infarction.

When SBP and DBP were analyzed in the same way, we observed no significant role of the ACE D/I genotype (data not shown). Age was the only factor influencing the level of SBP or DBP. Interestingly, the OR of age was > 1 for SBP and < 1 for DBP.

Discussion

This study examined the age-PP and age-SBP relationships in a population of subjects with either systolic-diastolic or isolated systolic hypertension. The patients were divided according to gender. In the case of the ACE gene polymorphism, the slopes of the age-SBP and age-PP relationships significantly differed between the II, ID, and DD genotypes in men but not in women. In men, the time course of the age-PP curves differed between the II genotype versus the ID+DD genotypes (Figure 2). However, the interslope difference was slightly attenuated after adjustment of CV and renal risk factors and, to a greater extent, after adjustment of one of the following 3 parameters: MBP, plasma potassium, and PAT. However, when all adjustments were made at the same time, the probability value did not differ from the data without further adjustment. These results were obtained using a cross-sectional analysis. We and others have shown that an adequate concordance may be observed between cross-sectional and longitudinal investigations relating SBP and PP to age.^{2,21}

In most previous reports on the genetics of hypertension, the hypothesis that genetic variability could lead to hypertension had been tested on the basis of comparison of mean DBP values in patients with different genotypes (see, for example, the review by Luft¹¹). The classification of hypertensive subjects was based on the measurement of DBP, a single point on the BP curve, whereas SBP and PP, the two mechanical factors which have the higher predictive value in term of CV risk, were often neglected in studies.¹⁻³ Using this well-established procedure, negative results were observed in the present investigation, as previously observed in many other reports.¹¹ Our principal goal was to test the hypothesis that the ACE D/I gene polymorphism might modulate the influence of age on SBP and PP, and therefore play a role in CV risk through an alteration of the age-related change of these mechanical factors. Because PP is no more than the difference between SBP and DBP, it is important to show that the present results were not influenced by a statistical artifact reflecting the well-established correlations between MBP and PP, and especially between SBP and PP.³ In the present study, this issue raised minor concern because the modulating influence of the studied gene polymorphism was evaluated in parallel for SBP, DBP, and PP, with PP being evaluated both before and after adjustment to MBP. Clearly, in several issues of this investigation, PP differed from MBP (Table V) and SBP from PP (Table VI).

The slope of the curve relating PP to age constitutes a very interesting phenotype. First, the slope was highly influenced by gender, being steeper in men than in women. The observed differences might suggest the role of sex hormones and/or their genotype in PP and arterial stiffness, with different consequences on CV risk according to gender. Second, the genotype itself influenced the slope and, to a greater extent, the time course of age-PP relationship in men but not in women. The relevant finding was that the time course of the age-PP relationships differed markedly between the II versus the ID+DD genotypes (Figure 2), but also that this time course may be modulated by many other factors, mainly of environmental origin (Table V).

The ACE gene locus has been linked to BP in spontaneously hypertensive rats.²²⁻²⁴ Although the ACE gene ID allelic variant in humans has been implicated in arteriosclerotic cardiovascular disease, cardiac hypertrophy, restenosis, and progression of diabetic renal disease, its role in the mechanism of high BP in essential hypertension has remained difficult to evaluate. O'Donnell et al found evidence for association and genetic linkage of the ACE gene with hypertension and blood pressure in men, but not in women, when they analyzed over 3000 participants from the Framingham Heart Study.²⁵ Fornage et al studied 583 three-generation pedigrees from Minnesota, United States, and were able to show that variations in a microsatellite marker within the growth hormone gene, which is close to the ACE gene locus, influenced interindividual blood pressure differences in young white men but not in women.²⁶ Our study confirms the gender influence observed for the ACE gene polymorphism and shows that, in the presence of the D allele, there is a steeper increase of PP with age in older subjects than in younger subjects (Figure 2). To our knowledge, few data have addressed the relationships between PP and gene polymorphism related to the renin-angiotensin system in rats.²⁷ In hypertensive subjects, the present results suggest that the D variant of the ACE gene polymorphism contributes to modulation of the BP pulsatility according to age.

The finding that the DD genotype might influence arterial pulsatility is difficult to interpret. From association and linkage studies, there is strong evidence that the ACE D allele accounts for almost half of the variance in ACE plasma levels.²⁸⁻³⁰ Our results provide an interesting contribution to this problem because the D allele might contribute to the increase of pulsatility in older subjects but not in younger subjects. Pharmacological studies indicate that the ACE D/I gene polymorphism influences not only angiotensin II generation but also the cross-talk of this hormone with bradykinin and even nitric oxide.¹² It seems likely that the combination of all these vasoactive compounds changes with age and contributes in turn to the age-related changes in arterial stiffness and, thus, in PP in subjects with DD genotype. Furthermore, the present findings agree with reports suggesting the influence of the ACE gene polymorphism on the mechanisms of systolic hypertension, particularly in older subjects in which structural changes of the arterial wall are associated.³¹

In the present study, the interslope difference between genotypes in men was largely influenced by many associated genetic and environmental factors. As shown in Table V, all of these factors taken together provide some compensation between them because the probability value of the interslope difference did not differ without or with the totality of studied adjustments. However, adjustment of three of these parameters might reduce the interslope difference considerably. First, the role of MBP is not surprising, since it is well known that an increase in MBP may mechanically generate an increase in PP. Second, the role of plasma potassium may indirectly reflect the well-established influence of cations in the mechanisms of systolic hypertension in the older subjects. In this variety of hypertension, the ACE D/I gene polymorphism has been shown to play a role in association with the gene polymorphism of alpha adducin, a compound that contributes greatly to sodium sensitivity.³² Finally, the interslope difference tends to

be considerably attenuated after adjustment for PAT. This previously observed finding²⁵ suggests that longitudinal genetic studies are required, in addition to the present cross-sectional study, in order to understand the subtle links between PP and the ACE D/I gene polymorphism. In particular, it has been shown that, after one year of treatment in hypertensive subjects, ACE inhibition in association with diuretic reduces SBP and PP to a greater extent than the beta-blocking agent atenolol for the same DBP reduction.¹⁴

Finally, the present investigation has shown that the ACE D/I gene polymorphism may modulate the relationship between age and SBP or PP. This finding, which is age- and gender-dependent, principally affects the slope of the age-PP curve in older subjects and, as shown in Table VI, might play a role in the mechanism of CV risk. Clearly these results require further investigation involving long-term follow-up.

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References

- Kannel WB, Gordon T, Schwartz MJ. Systolic versus diastolic blood pressure and risk of coronary heart disease: the Framingham Study. *Am J Cardiol.* 1971;27:335–346.
- Franklin SS, Gustin W IV, Wong ND, Larson MG, Weber MA, Kannel WB, Levy D. Hemodynamic patterns of age-related changes in blood pressure: the Framingham Heart Study. *Circulation.* 1997;96:308–315.
- Safar ME. Pulse pressure, arterial stiffness, and cardiovascular risk. *Curr Opin Cardiol.* 2000;15:258–263.
- Benetos A, Zureik M, Morcet J, Thomas F, Bean K, Safar M, Ducimetiere P, Guize L. A decrease in diastolic blood pressure combined with an increase in systolic blood pressure is associated with high cardiovascular mortality. *J Am Coll Cardiol.* 2000;35:673–680.
- Blacher J, Gasowski J, Staessen JA, Gierd X, This L, Liu L, Wang JG, Fagard R, Safar ME. Pulse pressure not mean pressure determines cardiovascular risk in older hypertensive patients. *Arch Intern Med.* 2000;160:1085–1089.
- Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Prospective Studies Collaboration. *Lancet.* 2002;360:1903–1913.
- Avolio A. Genetic and environmental factors in the function and structure of the arterial wall. *Hypertension.* 1995;26:34–37.
- Benetos A, Gautier S, Ricard S, Topouchian J, Asmar R, Poirier O, Larosa E, Guize L, Safar M, Soubrier F, Cambien F. Influence of angiotensin-converting enzyme and angiotensin II type I receptor gene polymorphisms on aortic stiffness in normotensive and hypertensive patients. *Circulation.* 1996;94:698–703.
- Lajemi M, Labat C, Gautier S, Lacolley P, Safar M, Asmar R, Cambien F, Benetos A. Angiotensin II type I receptor¹⁵³ A/G and ¹¹⁶⁶ A/C gene polymorphisms and increase in aortic stiffness with age in hypertensive subjects. *J Hypertens.* 2001;19:407–413.
- Mourad JJ, Ducailar G, Rudnicki A, Lajemi M, Mimram A, Safar ME. Age-related increase of pulse pressure and gene polymorphisms in essential hypertension: a preliminary study. *J Renin Angiotensin Aldosterone Syst.* 2002;3:109–115.
- Luft FC. Molecular genetics of human hypertension. *J Hypertens.* 1998;16:1871–1878.
- Buikema H, Pinto YM, Rooks G, Grandjean JG, Schunkert H, van Gilst WH. The deletion polymorphism of the angiotensin-converting enzyme gene is related to phenotypic differences in human arteries. *Eur Heart J.* 1996;17:787–794.
- Kannel WB, Dawber TR, McGee DL. Perspectives on systolic hypertension: the Framingham Study. *Circulation.* 1980;61:1179–1182.
- Asmar RG, London GM, O'Rourke ME, Safar ME, REASON Project Coordinators and Investigators. Improvement in blood pressure, arterial stiffness and wave reflections with a very-low-dose perindopril/indapamide combination in hypertensive patients: a comparison with atenolol. *Hypertension.* 2001;38:922–926.
- Framingham Heart Study. Kannel WB, Wolf P, Garrison R, eds. Framingham study: an epidemiological investigation of cardiovascular disease. Section 35: survival following initial cardiovascular events: 30 year follow-up. Washington, D.C.: Government Printing Office, 1988. NIH Publication No. 88–2969.
- Madhavan S, Ooi WL, Cohen H, Alderman MH. Relation of pulse pressure and blood pressure reduction to the incidence of myocardial infarction. *Hypertension.* 1994;23:395–401.
- Benetos A, Safar M, Rudnicki A, Smulyan H, Richard JL, Ducimetiere P, Guize L. Pulse pressure: a predictor of long-term cardiovascular mortality in a French male population. *Hypertension.* 1997;30:1410–1415.
- Benetos A, Rudnicki A, Safar M, Guize L. Pulse pressure and cardiovascular mortality in normotensive and hypertensive subjects. *Hypertension.* 1998;32:560–564.
- Asmar R, Vol S, Brisac AM, Tichet J, Topouchian J. Reference values for clinic pulse pressure in a nonselected population. *Am J Hypertens.* 2001;14:415–418.
- Asmar R, Safar M, Queneau P. Evaluation of the placebo effect and reproducibility of blood pressure measurement in hypertension. *Am J Hypertens.* 2001;14:546–552.
- Benetos A, Adamopoulos C, Bureau JM, Temmar M, Labat C, Bean K, Thomas F, Pannier B, Asmar R, Zureik M, Safar M, Guize L. Determinants of accelerated progression of arterial stiffness in normotensive subjects and in treated hypertensive subjects over a 6-year period. *Circulation.* 2002;105:1202–1207.
- Jacob HJ, Lindpaintner K, Lincoln SE, Kusumi K, Bunker RK, Mao YP, Ganten D, Dzau VJ, Lander ES. Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. *Cell.* 1991;67:213–224.
- Deng Y, Rapp JP. Cosegregation of blood pressure with angiotensin-converting enzyme and atrial natriuretic peptide receptor genes using Dahl salt-sensitive rats. *Nat Genet.* 1992;1:267–272.
- Hilbert P, Lindpaintner K, Beckmann JS, Serikawa T, Soubrier F, Dubay C, Cartwright P, De Gouyon B, Julier C, Takahashi S, Vincent M, Ganten D, George M, Lathrop GM. Chromosomal mapping of two genetic loci associated with blood pressure regulation in hereditary hypertensive rats. *Nature.* 1991;353:521–529.
- O'Donnell CJ, Lindpaintner K, Larson MG, Rao VS, Ordovas JM, Schaefer EJ, Myers RH, Levy D. Evidence for association and genetic linkage of the angiotensin-converting enzyme locus with hypertension and blood pressure in men but not women in the Framingham study. *Circulation.* 1998;97:1766–1772.
- Fornage M, Amos CI, Kardia S, Sing CF, Turner ST, Boerwinkle E. Variation in the region of the angiotensin-converting enzyme gene influences interindividual differences in blood pressure levels in young white males. *Circulation.* 1998;97:1773–1779.
- Dubay C, Vincent M, Samani N, Hilbert P, Kaiser MA, Beressi JP, Kotelevtsev Y, Beckmann JS, Soubrier F, Sassard J, Lathrop GM. Genetic determinants of diastolic and pulse pressure map to different loci variation in Lyon hypertensive rats. *Nat Genet.* 1993;3:354–357.
- Tiret L, Rigat B, Visvikis S, Breda C, Corvol P, Cambien F, Soubrier F. Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. *Am J Hum Genet.* 1992;51:197–205.
- Williams RR, Hunt SC, Hopkins PN, Stults BM, Wu LL, Hasstedt SJ, Barlow GK, Stephenson SH, Lalouel JM, Kuida H. Familial dyslipidemic hypertension: evidence from 58 Utah families for a syndrome present in approximately 12% of patients with essential hypertension. *JAMA.* 1988;259:3579–3586.
- Busjahn A, Knoblauch H, Knoblauch M, Bohlender J, Menz M, Faulhaber HD, Becker A, Schuster H, Luft FC. Angiotensin-converting enzyme and angiotensinogen gene polymorphisms, plasma levels, cardiac dimensions: a twin study. *Hypertension.* 1997;29:165–170.
- Balkenstein EJ, Staessen JA, Wang JG, van der Heijden-Spek JJ, Van Bortel LM, Barlassina C, Bianchi G, Brand E, Herrmann SM, Struijker-Boudier HA. Carotid and femoral artery stiffness in relation to three candidate genes in a white population. *Hypertension.* 2001;38:1190–1197.
- Staessen JA, Wang JG, Brand E, Barlassina C, Birkenhäger H, Herrmann SM, Fagard R, Tizzoni L, Bianchi G. Effects of three candidate genes on prevalence and incidence of hypertension in a Caucasian population. *J Hypertens.* 2001;19:1349–1358.