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Association of the -344T/C aldosterone synthase gene variant with essential hypertension

Short title: CYP11B2 gene polymorphism in hypertension

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Summary

Background: The aldosterone synthase gene (CYP11B2) is an important candidate gene region in essential hypertension. We therefore studied the association of -344T/C polymorphism of the CYP11B2 gene with the presence and severity of hypertension in case-control study.

Methods: We studied 369 individuals, of whom 213 were hypertensive patients (139 controlled hypertensive, 74 resistant hypertensive) and 156 were healthy normotensive subjects. The -344T/C polymorphism of the CYP11B2 gene was determined using polymerase chain reaction - restriction fragment length polymorphism analysis. *Results:* The distribution of genotypes in normotensive controls and hypertensive subjects were: TT 25.6% vs.31.9%, TC 51.9% vs. 57.3% and CC 22.4% vs. 10.8%. The - 344T/C variant was associated with hypertension. Subjects carrying the -344T allele had greater risk of hypertension compared to those having C allele ($\chi^2 = 5.89$, p<0.05). The frequency of CC genotype was significantly lower in hypertensive patients than in normotensive controls ($\chi^2 = 9.44$, p<0.01). A stepwise logistic regression analysis confirmed these findings. We did not find an association of -344T/C variant with the resistance of hypertensive patients to combination therapy.

Conclusion: We observed an association of -344T/C polymorphism of aldosterone synthase gene with increased risk of hypertension. These results support a potential role of -344T/C CYP11B2 gene polymorphism in genetic predisposition to develop hypertension.

Key words: aldosterone synthase - hypertension - polymorphism - aldosterone

Introduction

The renin-angiotensin-aldosterone system is an important regulator of blood pressure and molecular variants in genes that encode components of this system have been associated with several cardiovascular diseases, such as essential hypertension, myocardial infarction and hypertrophic cardiomyopathy.

Among them the CYP11B2 gene encodes a key enzyme of the aldosterone biosynthesis aldosterone synthase. Aldosterone is an independent risk factor for cardiovascular diseases and development of cardiac hypertrophy and fibrosis. Aldosterone synthase includes the steroid 11 β-hydroxylase, 18-hydroxylase and 18-oxidase activities that are required for the final steps of aldosterone biosynthesis. A related enzyme, 11β-hydroxylase (CYP11B1), is responsible mainly for cortisol biosynthesis, although changes in its activity can also influence biosynthesis of steroid metabolites with mineralocorticoid actions. The two relevant genes (CYP11B2, CYP11B1) are located in close proximity on chromosome 8q22 and it is now apparent from investigation of rare monogenic forms of hypertension and animal models that changes in the expression and activity of these enzymes can affect sodium homeostasis and thereby blood pressure through defects in mineralocorticoid metabolism. CYP11B2 is thus an obvious gene to test for association with hypertension (Freel and Connell 2004; Brand et al. 1998).

To date, three common genetic variants of the aldosterone synthase gene (CYP11B2) have been identified as possible determinants of high blood pressure in patients with essential hypertension (Mulatero et al. 2000; Zhu et al. 2003; White and Slutsker 1995). One is a single nucleotide polymorphism in the 5' promoter region at -344T/C that alters a putative recognition site for the steroidogenic transcription factor-1 (SF-1). The other polymorphism involves intron 2 of CYP11B2, which is partly replaced by the corresponding intron of CYP11B1 gene. These two polymorphisms are in close linkage disequilibrium. The third polymorphism is a point mutation K173R in exon 3.

Several studies of association between -344T/C polymorphism and essential hypertension have been published, with controversial results. Whereas some have found that the T-allele is more common in hypertension and subjects with increased urinary aldosterone excretion rate (Brand et

al.1998; Davies et al. 1999; Casiglia et al. 2005; Kumar et al. 2003), others have demonstrated no association of either allele with hypertension or other cardiovascular disease (Kato et al. 2000; Schunkert et al. 1999; Tsujita et al. 2001; Brand et al. 1999). It was also suggested that the C allele may be associated with genetic predisposition to hypertension in Hani and Yi minorities in China (Tang et al. 2006). Several factors such as ethnicity, gender and age could be involved in the phenotypic expression of this polymorphism.

Some studies evaluated the impact of -344T/C polymorphism of the CYP11B2 on the severity of hypertension and blood pressure-lowering response to antihypertensive drugs (Tiago et al. 2003; Kurland et al. 2002). Because the severity of hypertension determines cardiovascular risk and aldosterone is an important therapeutic target in hypertension, genotyping for the -344T/C polymorphism of the CYP11B2 gene may have prognostic as well as therapeutic potential in hypertensive patients.

The present study was designed to investigate the association of -344T/C polymorphism of the CYP11B2 gene with genetic predisposition to essential hypertension. Further, we examined whether this polymorphism is associated with resistance of hypertensive patient to antihypertensive drugs combination.

Materials and methods

Study groups and clinical assessment

We examined a total of 369 individuals, of whom 213 were patients with essential hypertension and 156 were healthy normotensive subjects. All subjects were unrelated Caucasians from the Bohemia region of the Czech Republic. Hypertensive patients were sequentially enrolled from the outpatient clinic of Second Medical Department – Clinical Department of Cardiology and Angiology, First Faculty of Medicine and General University Hospital, Prague. Hypertension was defined according to the following criteria: (i) systolic blood pressure (BP) \geq 140mmHg or diastolic BP \geq 90mmHg, or both, measured on two consecutive visits for untreated subjects; (ii) patients receiving long-term antihypertensive treatment; (iii) no clinical or biochemical evidence of secondary hypertension. Normotensive subjects were selected from outpatients who participated in voluntary health checks and

hospital staff undergoing annual medical examinations. The subjects were considered to be normotensive when their systolic blood pressure was lower than 140 mmHg and diastolic blood pressure below 90 mmHg. All subjects had normal renal function and no history of diabetes, heart disease or other serious illness. Informed consent was obtained from each subject and the study was approved by the local ethic committee.

To phenotype hypertensive subjects further they were subdivided according to the severity of hypertension into two groups: patients well-controlled after adjustment of lifestyle and medication [controlled hypertension group (CH), n=139] and subjects resistant to combined antihypertensive therapy [resistant hypertension group (RH), n=74]. Hypertensive patients were considered resistant to therapy if they had systolic BP \geq 140mmHg and/or diastolic BP \geq 90mmHg when on a triple antihypertensive therapy including diuretics.

Genotyping

Genomic DNA was isolated form peripheral leukocytes according to the standard method (Miller et al. 1988). The -344T/C polymorphism of the CYP11B2 gene was determined by the analysis of restriction fragment length polymorphism (RFLP) (Komica et al. 2000) (Figure 1). The DNA fragment containing -344T/C of the CYP11B2 gene was amplified by polymerase chain reaction (PCR). The PCR was performed using Taq polymerase Boehring Ingelheim and oligonucleotide primers CTCACCCAGGAACCTGCTCTGGAAACATA and

CAGGAGGGATGAGCAGGCAGAGCACAG using hot start and touchdown PCR to increase specificity of the reaction. PCR was subjected to 94°C for 30s and from 62° to 58°C, two cycles 1°C lower temperature annealing, 72°C for 30s for amplification to the total amount of 35cycles followed by final extension for 5min, producing a fragment of 639bp. This fragment was subsequently cleaved by HaeIII, creating fragments for allele T 402, 138, 51 and 48bp, and for allele C 334, 138, 68, 51 and 48bp, which where subjected to electrophoresis on a metaphoragarose gel and visualized with ethidium bromide. The samples from hypertensive patients and healthy controls were also analyzed by DNA sequence analysis to confirm results of the PCR-RFLP method.

Statistical analysis

Statistical analysis was performed using SYSTAT 10 statistical software (SPSS Inc., 2000). Data are given as means \pm SD. Differences in clinical variables between case and control groups were tested using one-way analysis of variance (ANOVA). The Hardy-Weinberg equilibrium and allele frequency and genotype distributions were tested by $\chi 2$ statistic (Falconer and Mackay 1996). A stepwise logistic regression analysis was conducted to adjust for covariates including age, gender, body mass index (BMI), blood glucose, serum triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol plasma levels. Association of phenotypic parameters (BP levels, age, sex, BMI) with genotype was assessed by one-way ANOVA for continuous variables and χ^2 test for discrete variables. All statistical tests were two-tailed and values of p<0.05 were considered to represent statistically significant differences.

Results

Clinical characteristics of the study groups

The overall characteristics of the study subjects genotyped for the -344T/C CYP11B2 gene polymorphism are listed in Table 1. Systolic and diastolic blood pressure was markedly higher in patients with essential hypertension (H group) than in normotensive group (N group). In addition, as expected, body mass index (BMI) and fasting blood glucose were significantly higher in H group than in N group. No significant differences were detected between groups with respect to height, total cholesterol and LDL-cholesterol levels. Plasma levels of LDL-cholesterol revealed a non-significant tendency to lower concentrations in hypertensive than in normotensive subjects. This finding corresponds to significant number of hypertensive patients treated by statin therapy. However, serum triglycerides were higher and serum HDL cholesterol was lower in hypertensive than in normotensive group.

Association between aldosterone synthase variants and essential hypertension

Table 2 reports the distribution of genotypes and alleles for the -344T/C CYP11B2 gene polymorphism in hypertensive subjects and normotensive controls

Frequencies of genotypes were in accordance with the Hardy-Weinberg equilibrium in normotensive group. On the contrary the observed genotype frequencies deviated from that expected from the Hardy-Weinberg equilibrium in hypertensive group (p<0.01).

We found a significant association of the -344T/C CYP11B2 gene polymorphism with essential hypertension. Subjects carrying the -344T allele had significantly greater risk of essential hypertension compared with those carrying C allele. The frequency of CC genotype was twice as prevalent in the normotensive as in hypertensive patients (Table 2). In the logistic regression analysis we observed significant association of -344T/C aldosterone synthase gene polymorphism and hypertension (TC vs. CC: odds ratio = 3.4, 95% confidence interval 1.3 - 9.1; TT vs. CC: odds ratio = 5.4, 95% confidence interval 1.8 - 16) after adjustment for the covariates of age, gender, BMI, blood glucose and blood lipid levels. We failed to observe any significant difference in the genotype distribution or allele frequencies between hypertensive subjects well-controlled with antihypertensive therapy (CH group) and resistant hypertensive group (RH group) either by chi-square statistics or logistic regression analysis.

Association between phenotypic characteristics and aldosterone synthase genotype were analysed for the normotensive and hypertensive groups separately, and the combined group of normotensive and hypertensive subjects. One-way ANOVA analysis did not show a significant influence of genotype on systolic or diastolic BP. Finally, no significant differences in terms of gender distribution, age, body mass index, glucose and cholesterol concentrations were detected among the genotypes.

Discussion

In the present study, we investigated the association between the -344T/C polymorphism in the promoter region of the human CYP11B2 gene and essential hypertension. Our results showed that the genotype and allele distribution differed significantly between hypertensive and normotensive

groups. The -344T allele and TT genotype were associated with genetic predisposition to develop hypertension.

A number of studies have suggested the implication of CYP11B2 gene polymorphism in the pathogenesis of cardiovascular disease. However, previous data on the association between the -344T/C polymorphism and hypertension or with hypertensive intermediate phenotypes such as plasma renin activity and plasma aldosterone concentration gave controversial results. Our findings are in agreement with several studies previously conducted in Caucasians, also showing association of -344T allele with hypertension or blood pressure levels (Brand et al. 1998; Davies et al. 1999; Casiglia et al. 2005). At variance with these reports, other studies conducted in white populations did not find any association (Schunkert et al 1999; Brand et al. 1999) and a study by Kumar et al. reported that the C allele was associated with hypertension in Caucasian women (Kumar et al. 2003). No association between the T allele and hypertension has also been reported in several studies in Japanese (Kato el al. 2000; Tsujita et al. 2001; Isaji et al. 2005) and in one of them hypertension was associated with the C allele (Tamaki et al. 1999). Similar inconsistencies have characterised the reports linking this polymorphism to plasma or urinary aldosterone levels (Brand et al. 1998; Davies et al. 1999; Connell et al. 2004). Several reasons could account for these inconsistent results. First, associations could be influenced by the different genetic background and environmental factors in geographically separated populations. Further, differences in study design and selection criteria, such as different age (Casiglia et al.2005), gender (Tsujita et al. 2001) or different proportions of individuals with low renin hypertension (Zhu et al. 2003) might be responsible for these discrepancies. Sookoian et al. in the recent meta-analysis showed that subjects homozygous for the -344T allele of the CYP11B2 gene have, at least, a 17% greater risk of essential hypertension than their -344CC counterparts (Sookoian et al. 2007).

The exact mechanism whereby the -344T/C polymorphism of CYP11B2 gene variant may lead to higher blood pressure remains unknown. Recent studies suggest that the -344T/C variant by itself does not directly influence promoter activity despite its location within a SF-1 binding site. Rather, binding of SF-1 to this site downregulates activity of CYP11B2 promoter by making SF-1 less available to functionally affect other CYP11B2 promoter sites, which could alter expression of the

gene (Clyne et al. 1997). Moreover, in vitro studies showed that C allele binds SF-1 fourfold more strongly than it does the T allele (White and Slutsker 1995). This may allow a change in CYP11B2 promoter activity and expression (Matsubara et al. 2004; Hautanen et al. 1998).

In our previous study conducted in normotensive healthy men we observed higher plasma renin activity (PRA) in TT homozygotes and higher values of left ventricular mass index in individuals with TT genotype and high PRA (Heller et al. 2004). In agreement with these findings Sookian et al. in recent meta-analysis also reported higher PRA and no difference in plasma aldosterone levels in homozygous TT individuals (Sookoian et al. 2007). We may speculate, that higher PRA reported in TT homozygous individuals may be adaptive to a lack of adequate response of the CYP11B2 promoter bearing the T allele to the angiotensin II-mediated stimulus and therefore the elevated plasma angiotensin II may make TT homozygous subjects more prone to hypertension (Sookoian et al. 2007; Heller et al. 2004; Staessen et al. 2007).

Evaluation of hypertensive patients selected according to the severity of hypertension may increase the genetic component of hypertension and thus improve the likelihood of detecting any existing genetic association with hypertension. Studies conducted in hypertensive patients that examined the relationship between candidate gene polymorphisms and the severity of hypertension or presence of resistant hypertension has provided contradictory data. While some studies have reported an association of -344T/C polymorphism of aldosterone synthase gene, I/D polymorphism of ACE gene, A1166C polymorphism of AT1-receptor gene and G894T polymorphism of the endothelial nitric oxide synthase gene with severity of BP changes, resistant hypertension, malignant hypertension or hypertensive crisis, others did not confirm these results (Brand et al. 1998; Tiago et al. 2003; Sunder-Plassmann et al. 2002; O'Donnell et al. 1998; Jáchymová et al. 2001; Stefansson et al. 2000). In our study we evaluated the impact of -344T/C polymorphism of CYP11B2 gene on the severity of hypertension by comparing the group of hypertensive subjects well-controlled by conventional therapy and patients with resistant hypertension. We did not observe association between CYP11B2 genotype groups and presence of resistant hypertension. We found high frequency of resistant hypertension in our study. Although the prevalence of resistant hypertension is unknown data from recent hypertension outcome trials (VALUE, ALLHAT, CONVINCE) indicate that it is greater than

previously thought (Julius et al. 2003; Cushman et al. 2002; Black et al. 2003). On the other hand, patients enrolled in hypertension clinic of our hospital are generally those with moderate to severe or uncontrolled hypertension. Thus, we think the number of patients with resistant hypertension in our study overestimates occurrence of resistant hypertension in hypertensive population.

Several studies investigating the role of -344T/C aldosterone synthase gene polymorphism on blood pressure described an association of BP levels with -344T allele (Brand et al. 1998; Tiago et al. 2003; Barbato et al. 2004 Brand et al. 1998; Tiago et al. 2003; Matsubara et al.2001). However, in study by Brand et al. the relationship was significant only in the subgroup of severely hypertensive patients of African ethnicity with BMI > 27 kg/m² (Brand et al. 1998). In contrast, other cross-sectional and case-control studies and the meta-analysis by Sookoian et al. did not confirm the role of aldosterone synthase gene polymorphism in determining BP levels (Tsujita et al. 2001 Brand et al. 1998; Tiago et al. 2003; Kupari et al. 1998 Brand et al. 1998; Tiago et al. 2003; Sookoian et al. 2007). In our study, we did not demonstrate influence of genotype on systolic and diastolic BP levels. However, since we were unable to discontinue therapy in our out-patients, and assessment of pre-treatment blood pressure levels was not available, such analysis is inevitably biased. The genetic effects on BP levels in our studied group may have been modified by the antihypertensive medication.

Finally, we did not observe any significant differences among the genotypes in terms of gender distribution, age, BMI, glucose and cholesterol concentrations.

In summary, we have observed an association of -344T/C polymorphism of aldosterone synthase gene with hypertension in case-control study in Caucasian subjects of Czech Republic. These results support a potential role of -344T/C CYP11B2 gene polymorphism in mechanisms affecting blood pressure regulation. Further studies of this locus should be performed in large-scale populations to confirm our results and to define the underlying physiological and clinical implications of observed association.

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References

BARBATO A, RUSSO P, SIANI A, FOLKERD EJ, MILLER MA, VENEZIA A, GRIMALDI C, STRAZZULLO P, CAPPUCCIO FP: Aldosterone synthase gene (CYP11B2) C-344T polymorphism, plasma aldosterone, renin activity and blood pressure in a multi-ethnic population. *J Hypertens* **22**: 1895-1901, 2004. BRAND E, CHATELAIN N, MULATERO P, FERY I, CURNOW K, JEUNEMAITRE X, CORVOL P, PASCOE L, SOUBRIER F: Structural analysis and evaluation of the aldosterone synthase gene in hypertension. *Hypertension* 32:198-204, 1998.

BRAND E, SCHODE U, RINGEL J, BRITE J, DISTLER A, SHARMA AM: Aldosterone synthase gene
(CYP11B2) polymorphism in Caucasians from the Berlin Salt-Sensitive Trial (BeSST). *J Hypertens*17: 1563-1567, 1998.

CASIGLIA E, TIKHONOFF V, MAZZA A, RYNKIEWICZ A, LIMON J, CAFFI S, GUGLIELMI F, MARTINI B, BASSO G, WINNICKI M, PESSINA AC, SOMERS VK: C-344T polymorphism of the aldosterone synthase gene and blood pressure in the elderly: a population-based study. *J Hypertens* **23** :1991-1996, 2005. BLACK HR, ELLIOTT WJ, GRANDITS G ET AL, FOR THE CONVINCE RESEARCH GROUP: Principal results of the Controlled Onset Verapamil Investigation of Cardiovascular End Points (CONVINCE) trial. *JAMA* **289**: 2073-2082, 2003.

CLYNE CD, ZHANG Y, SLUTSKER L, MYTHUS JM, WHITE PC, RAINEY WE: Angiotensin II and potassium regulate human CYP11B2 transcription through common cis elements. *Mol Endocrinol* **11**: 856-860, 1997.

CONNELL JM, FRASER R, MACKENZIE SM, FRIEL EC, INGRAM MC, HOLLOWAY CD, DAVIES E: The impact of polymorphisms in the gene encoding aldosterone synthase (CYP11B2) on steroid synthesis and blood pressure regulation. *Moll Cell Endocrinol* **217**: 243-247, 2004.

CUSHMAN WC, FORD CE, CUTLER JA, ET AL, FOR THE ALLHAT COLLABORATIVE RESEARCH GROUP: Success and predictors of blood pressure control in diverse North American settings: the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *J Clin Hypertens* **4**: 393-404, 2002. DAVIES E., HOLLOWAY CD, INGRAM MC, INGLIS GC, FRIEL EC, MORRISON C, ANDERSON NH, FRASER R, CONNELL JM: Aldosterone excretion rate and blood pressure in essential hypertension are related to polymorphic differences in the aldosterone synthase gene CYP11B2. *Hypertension* **33**: 703-707, 1999. FALCONER DS, MACKAY FC: Introduction to quantitative genetics, 4th edn. London: Longman Group Ltd; 1996.

FREEL EM, CONNELL JM: Mechanisms of hypertension: the expanding role of aldosterone. *J Am Soc Nephrol* **15**: 1993-2001, 2004.

HAUTANEN A, LANKIEN L, KAPUCI M, JANNE OA, ADLERCREUTZ H, NIKKILA H, WHITE PC: Associations between aldosterone synthese gene polymorphism and adrenocortical function in males. *J Intern Med* **244**: 11-18, 1998.

HELLER S, LINHART A, JINDRA A, JÁCHYMOVÁ M, HORKÝ K, PELEŠKA J, HLUBOCKÁ Z, UMNEROVÁ V, ASCHERMANN M: Association of -344/T/C aldosterone synthase polymorphism (CYP11B2) with left ventricular structure and humoral parameters in young normotensive men. *Blood Press* **13**: 158-163, 2004.

ISAJI M, MUNE T, TAKADA N, YAMAMOTO Y, SUWA T, MERITA H, TAKEDA J, WHITE PC: Correlation between left ventricular mass and urinary podium excretion in specific genotypes of CYP11B2. *J Hypertens* **23**: 1149-1157, 2005.

JÁCHYMOVÁ M, HORKÝ K, BULTAS J, KOŽICH V, JINDRA A, PELEŠKA J, MARTÁSEK P: Association of the Glu298Asp polymorphism in the endothelial nitric oxide synthase gene with essential hypertension resistant to conventional therapy. *Biochem Biophys Res Commun* **284**: 426-430, 2001.

KATO N, SUGIYAMA T, MERITA H, KURIHARA H, FURUKAWA T, ISSHIKI T, SATO T, YAMORI Y, YAZAKI Y: Comprehensive analysis of the renin-angiotensin gene polymorphisms with relation to hypertension in the Japanese. *J Hypertens* **18**: 1025-1032, 2004.

KOMIKA I, YAMADA T, TAKARA M, ASAWA T, SHIMABUKURO M, NISHIMORI T, TAKASU N: Lys(173)Arg and -344T/C variants of CYP11B2 in Japanese patients with low-renin hypertension. *Hypertension* **35**: 699-703, 2000.

KUMAR NN, BENJAFIELD AV, LIN RC, WANG WY, STOWASSER M, MORFIA BJ: Haplotype analysis of aldosterone synthase gene CYP11B2 polymorphism shows association with essential hypertension. *J Hypertens* **21**: 1331-1337, 2003.

KUPARI M, HAUTANENA A, LANKINEN L, KOSKINEN P, VIROLAINEN J, NIKKILA H, WHITE PC: Associations between human aldosterone synthese (CYP11B2) gene polymorphisms and left ventricular size, mass, and function. *Circulation* **97**: 569-575, 1998.

KURLAND L, MELHUS H, KARLSSON J, KAHAN T, MALMQVIST K, LIND L: Aldosterone synthase (CYP11B2) -344C/T polymorphism is related to antihypertensive response: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial. *Am J Hypertens* **15**: 389-393, 2002.

JULIUS S, KJELDSEN SE, BRUNNER H, FOR THE VALUE TRIAL: VALUE trial: long-term blood pressure trends in 13,449 patients with hypertension and high cardiovascular risk. *Am J Hypertens* **16**: 544-548, 2003.

MATSUBARA M, KIKUYA M, OHKUBO T, METOKI H, OMORI F, FUJIWARA T, SUZUKI M: Aldosterone synthase gene (CYP11B2) C-344T polymorphism, ambulatory blood pressure and nocturnal decline in blood pressure in the general Japanese population: the Ohasama Study. *J Hypertens* **19**: 2179-2184, 2001.

MATSUBARA M, SATO T, NISHIMURA T, SUZUKI M, KIKUYA M, METOKI H, MICHIMATA M, TSUJI I,

OGIHARA T, IMAI Y: CYP11B2 polymorphisms and home blood pressure in a population-based cohort in Japanese: the Ohasama Study. *Hypertens Res* **27**: 1-6, 2004.

MILLER SA, DYKES DD, POLESKY HF: A simple salting out procedure for extraction DNA from human nucleated cells. *Nucleid Acids Res* **16**: 1215, 1988.

MULATERO P, SCHIAVONE D, FALLO F, RABBIA F, VEGLIO F: CYP11B2 gene polymorphisms in idiopathic hyperaldosteronism. *Hypertension* **35**: 694-698, 2000.

O'DONNELL CK, 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 but not women in the Framingham Heart Study. *Circulation* **97**: 1766-1772, 1998.

SCHUNKERT H, HENGSTENBERG C, HOLME SR, BROECKEL U, LUCHNER A, MUSCHOLL MW, KURZINGER S, DORING A, HENSE HW, RIEGGER GA: Lack of association between a polymorphism of the aldosterone synthase gene and left ventricular structure. *Circulation* **99**: 2255-2260, 1999.

SOOKOIAN S, GIANOTTI TF, GONZALEZ CD, PIROLA CJ: Association of the C-344T aldosterone synthese gene variant with essential hypertension: a meta-analysis. *J Hypertens* **25**: 5-13, 2007.

STAESSEN JA, LI Y, THIJS L: Meta-analysis of blood pressure and the CYP11B2 polymorphism

highlights the need for better designed studies. J Hypertens 25: 37-39, 2007.

STEFANSSON B, RICKSTEN A, RYMO L, AURELL M, HERLITZ H: Angiotensin-converting enzyme gene I/D polymorphism in malignant hypertension. *Blood Press* **9**: 104-109, 2000.

SUNDER-PLASSMANNG, KITTLER H, BERLE C, HIRSCHL MM, WOISETSCHAGER CH, DERHASCHNIG U,

LAGGNER AN, HORL WH, FODINGER M: Angiotensin concerting enzyme DD genotype is associated with hypertension crisis. *Crit Care Med* **30**: 2236-2241, 2002.

TAMAKI S, IWAI N, TSUJITA Y, KINOSHITA M: Genetic polymorphism of CYP11B2 gene and hypertension in Japanese. *Hypertension* **33**: 266-270, 1999.

TANG W, WU H, ZHOU X, CHEBY B, DONG Y, HE L, YU H, XU L, LU J, LI K, XIAO CH: Association of the C-

344T polymorphism of CYP11B2 gene with essential hypertension in Hani and Yi minorities of China. *Clinica Acta* **364**: 222-225, 2006.

TIAGO AD, BADENHORST D, NKEH B, CANDY GP, BROOKSBANK R, SARELI P, LIBHABER E, SAMANI NJ, WOODIWISS AJ, NORTON GR: Impact of Renin-Angiotensin-Aldosterone System Gene Variants on the Severity of Hypertension in patients with newly diagnosed hypertension. *Am J Hypertens* **16**: 1006-1010, 2003.

TSUJITA Y, IWAI N, KATSUYA T, GIGAMI J, OGIHARA T, TAMAKI S, KINOSHITA M, MANNAMI T, OGATA J, BABA S: Lack of association between genetic polymorphism of CYP11B2 and hypertension in Japanese: the Suita Study. *Hypertens Res* **24**: 105-109, 2001.

WHITE PC, SLUTSKER L: Haplotype analysis of CYP11B2. Endocr Res 21: 437-42, 1995.

ZHU H, SAGNELLA GA, DONG Y, MILLER MA, ONIPINLA A, MAKANDU ND, MAC GREGOR GA: Contrasting associations between aldosterone synthase gene polymorphisms and essential hypertension in blacks and in whites. *J Hypertens* **21**: 87-95, 2003.

Table 1. Clinical characteristics of the study groups

	Normotensive	Hypertensive subjects n= 213		р,
	subjects	Controlled hypertensive	Resistant hypertensive	ANOVA
n	156	139	74	
Age (years)	51.3±9.7	56.6±10.5*	61.4±8.74*	p<0.001
Systolic BP (mmHg)	122±10.4	144±16.9*	152±16.1*	p<0.001
Diastolic BP (mmHg)	79.6±6.45	90.1±8.61*	95.3±8.27*	p<0.001
BMI (kg/m ²)	26.1±3.41	28.5±4.37*	29.8±3.79*	p<0.001
Fasting glucose (mmol/l)	5.29±0.73	5.98±1.74*	6.36 ±2.27*	p<0.001
S-total cholesterol (mmol/l)	5.43±0.94	5.45±1.16	5.19±0.94	n.s.
Triglycerides (mmol/l)	1.43±0.94	1.87±1.22*	1.87±0.84*	p<0.01
HDL-cholesterol (mmol/l)	1.40±0.30	1.29±0.31*	1.23±0.30*	p<0.01
LDL-cholesterol (mmol/l)	3.39±0.77	3.36±0.91	3.11±0.81	n.s.

Data are expressed as means±SD, *p<0.05 for controlled hypertensives, resistant hypertensives versus normotensives by one-way ANOVA.

BP = blood pressure, BMI = body mass index, HDL = high density lipoprotein, LDL = low density lipoprotein, n.s. = not significant, ANOVA = analysis of variance

Table 2. Association analysis of -344T/C CYP11B2 gene polymorphism in hypertensives versus

 normotensives

	Genotype frequencies (n, %)			
Group(n)	TT (n=108)	TC (n=203)	CC (n=58)	р
normotensive subjects	40 (37,0%)	81 (39,9%)	35 (60,3%)	p<0.01
hypertensive patients	68 (63,0%)	122 (60,1%)	23 (39,7%)	χ ² =9.44

	Allele freque		
Group(n)	T (n=419)	C (n=319)	р
normotensive subjects	161(38,4%)	151 (47,3%)	p<0.05
hypertensive patients	258 (61,6%)	168 (52,7%)	χ ² =5.89

Genotypes and alleles indicated as percentage and numer. Statisitcal analysis by $\chi 2$ test



Figure 1. Genotype determination of the -344T/C CYP11B2 gene polymorphism