Environmental Mutagens & Carcinogens 22-4 : 223-228 (2002)

DNA Polymorphisms of the Human CYP11B2 and γ Subunit of ENaC Genes in Korean Hypertensives

Byung Yong Kang[1], Joon Seol Bae[2], Ki Tae Kim[2] and Kang Oh Lee[3*]
[1]*Research Institute for Life Science, Sahmyook University, Seoul 139--742, Korea*
[2]*Seoulin Bioscience Institute, Seoulin Bioscience, Co., Ltd, Seoul 134-030, Korea*
[3]*Department of Life Science, Sahmyook University, Seoul 139-742, Korea*

(Received October 20, 2002 / Accepted November 15, 2002)

ABSTRACT: **Hypertension is characterized by multiple genetic and environmental factors. To establish the genetic basis of hypertension in Koreans, we investigated the genetic variations of two candidate genes (aldosterone synthase (CYP11B2), γ subunit of the amiloride-sensitive epithelial sodium channel (ENaC)) in the Korean patients with hypertension and normotensive controls. There were no significant differences in the genotype and allele frequencies between two groups, respectively. However, there was the significant difference between Korean and Caucasian populations in allele frequency of RFLPs in the two candidate genes. Therefore, these studies also need to be confirmed in other ethnic groups, although our results do not support a possible role of these genes on hypertension in Korean population**

Keywords : **Aldosterone, Genotype, Hypertension and Sodium Channel**

Introduction

Hypertension affects approximately 25% of the adult population and is a major risk factor for heart attack, stroke and kidney failure. Although blood pressure is known to have a strong genetic determination, the genes responsible for susceptibility to hypertension are mostly unknown. From studies in human and animal models it is clear that several genetic loci are involved in regulation of blood pressure and hypertension (Szpirer *et al.*, 1993).

Aldosterone is a mineralocorticoid hormone which, via renal actions, controls sodium balance and intravascular volume and thus helps to regulate blood pressure (White, 1994). In addition, aldosterone may have several direct actions on the heart including the development of cardiac hypertrophy and fibrosis (Brilla *et al.*, 1993; Young *et al.*, 1994). Aldosterone is synthesized in the adrenal cortex from deoxycorticosterone by a mitochondrial cytochrome P450 enzyme, aldosterone synthase (CYP11B2). The activity of the CYP11B2 gene is primarily regulated by the rennin-angiotensin system via the actions of angiotensin II (White, 1994).

Variations in the CYP11B2 gene that may influence its activity have been described (White and Slutsker, 1995). Specifically, a polymorphism in the promoter region of the gene at nucleotide −344 from the translation start site, where the residue could be a cytosine (−344C) or thymidine (−344T), is immediately adjacent to the binding site for the transcription factor SF-1, which is a key regulator of steroidogenic enzyme expression(Lala *et al.*, 1992). Two studies have reported that the plasma aldosterone level or urinary aldosterone excretion vary according to genotypes at the −344 position (Hautanena *et al.*, 1998; Pojoga *et al.*, 1998).

Reabsorption of sodium in the distal nephron, the distal colon and other transporting epithelia is a key component in the maintenance of sodium homeostasis and is thus critical in the regulation of blood pressure. There are several classes of sodium channel. The amiloride-sensitive epithelial sodium channel (ENaC) is a highly elective Na channel located in the apical membrane of epithelial cells in various tissues, including the distal nephron, the colon, exocrine gland ducts, the lung and the skin. Entry of sodium into these polarized epithelial cells from the apical space via EnaC is the rate-limiting step for vectorial electrogenetic movement of sodium. ENaC activity in the distal nephrone is regulated by aldosterone and vasopressin, and plays a role in maintaining sodium balance, extracellular volume and blood pressure (Uehara *et al.*, 1998).

ENaC is composed of three subunits, α, β and γ, all of which are required for the normal function of the

*To whom all correspondence should be addressed

channel (Su and Menon, 2001). It has been demonstrated that gain-of-function mutations at the carboxy terminus of β and γ ENaC cause Liddle's syndrome, a rare form of several human salt-sensitive hypertension (Hansson *et al.*, 1995; Shimkets *et al.*, 1994). On the other hand, inactivating mutations at the N-terminus of β and γ ENaC cause pseudohypoaldosteronism type I which is an inherited form of several hypotension and salt wasting (Chang *et al.*, 1996). This feature points out the capital importance of this channel in the regulation of blood pressure, constituting therefore a strong candidate gene for hypertension. Although most cases of Liddles syndrome are due to mutations in the β subunit of ENaC, there are some recent cases in which the disease is caused by a mutation in the γ subunit (second base G to A transition at codon 574 which changes the encoded tryptophan to a termination codon) (Hansson *et al.*, 1995). Also, C to G transversion at codon 649 of the γ subunit has been described and could be used as genetic marker for clinical association (Viaplana *et al.*, 1998).

The purpose of this study was to investigate the association between the genetic variations in the two candidate genes (CYP11B2 and ã subunit of ENaC genes) and hypertension in Koreans.

Materials and Methods

Study subjects

A total of 177 unrelated individuals were randomly chosen from the Seoul Hygiene Hospital, Seoul, Korea. We studied 88 subjects with hypertension. Patients were classified as having hypertension if they had systolic blood pressures above 140 mmHg and diastolic blood pressure above 90 mmHg on at least three separate occasions, and had no clinical signs, symptoms and laboratory findings suggestive of secondary hypertension. In additon, a randomly selected normal population (89 individuals) was analysed as the control groups (blood pressure value, <140/90 mmHg

Determination of serum lipid levels

Blood samples were obtained in EDTA tubes from individuals who had been fasting for 12-16hr. Concentration of serum total cholesterol (TC) and triglyceride were measured by enzymatic colorimetry methods with commercial kit (Boehringer Mannheim, Germany) and chemistry analyzer. Serum HDL-cholesterol level was determined by measuring cholesterol in the supernatant after precipitation of the serum with $MgCl_2$ and dextran sulfate, with a Gilford Impact 400E automated analyzer with reagents and calibrators from Boehringer Mannheim. Also, serum low density lipoprotein (LDL)-cholesterol level was calculated by Friedewald's equation (Friedwald *et al.*, 1972).

DNA analysis

Genomic DNA was isolated from buffy coat by the method of Sambrook *et al* (1989) with slight modification. Polymerase Chain Reaction (PCR) techniques were used for *Hae* III RFLP of CYP11B2 gene (Patel *et al.*, 2000) and *Sac* I RFLP of γ subunit of ENaC gene (Viaplana *et al.*, 1998). Briefly, total 50 μl of the reaction mixture contained 200-400 *ng* of genomic DNA, 100 *ng* of each primer, 200 μl of each dNTP, and buffers recommended by the manufacturer. The sequences of the primers for two polymorphisms studied were:

(a) *Hae* III RFLP in the CYP11B2 gene; sense, 5'-CAG GAG GAG ACC CCA TGT GAC-3' and anti-sense, 5'-CCT CCA CCC TGT TCA GCC C-3' (Patel *et al.*, 2000); (b) *Sac* I RFLP in the γ subunit of ENaC; sense 5'-GCA GAA AGC CAA GGA GTG GTG-3' and anti-sense, 5'-GAT CTG TCT TCT CAA ACC CTG C-3' (Viaplana *et al.*, 1998). Amplification for the *Hae* III RFLP in the CYP11B2 gene was carried out in a Perkin-Elmer DNA thermocycler, in which, after an initial denaturation step at 94°C for 5 min, there were 35 cycles of 94°C, 67°C, and 72°C for 1 min each. After amplification reaction, 15 *ul* of each PCR product was digested with 10 units of the restriction enzyme *Hae* III at 37°C. The 344T allele lacks a *Hae* III site present in the 344C allele, which gives rise to major fragments of –273 bp and 202 bp, respectively, which were resolved on a 2.5% agarose gel stained with ethidium bromide (Fig. 1).

For the detection of *Sac* I RFLP in the γ subunit of

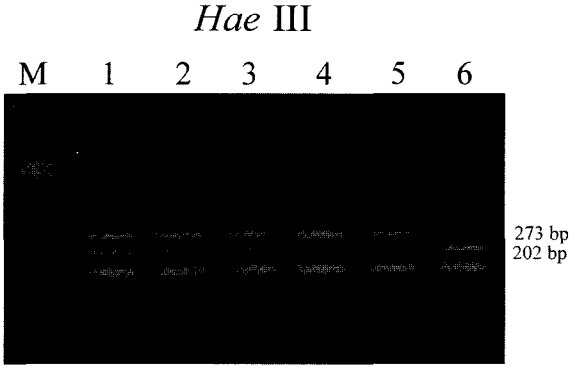

Fig. 1. The *Hae* III RFLP of CYP11B2 gene. Lane 1~3, 5, TC genotypes; lane 4, TT genotype; lane 6, CC genotype.

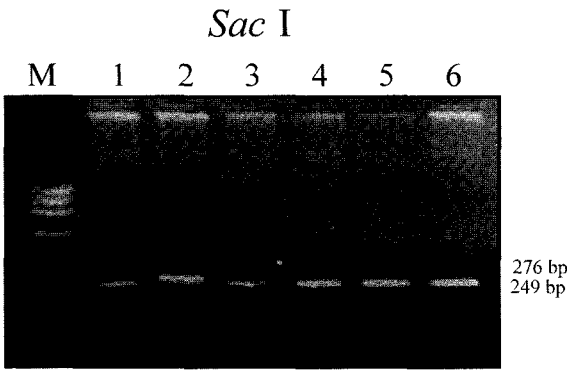

Fig. 2. The *Sac* I RFLP of gamma subunit of epithlial sodium channel gene. Lane 1, 3~6, CC genotypes; lane 2, CG genotype.

ENaC gene, after an initial denaturation step at 96°C for 4 min, samples were ampilified for 35 cycles consisting of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 30 sec, followed by a final extention step at 72°C of 10 min. After amplification reaction, 10 μl of each PCR product was digested with 10 units of restriction enzyme *Sac* I at 37°C. Digested product were size-fractionated on a 2% agarose gel stained with ethidium bromide. The *Sac* I RFLP in the ã subunit of ENaC gene was characterized by two allele, one of 275 bp (designated allele G) and the other of 249 and 27 bp (allele C) (Fig. 2).

Statistical Anaysis

Data are presented as mean ± standard deviation (SD). Allele frequencies were calculated from the genotypes of all subjects. Hardy-Weinberg equilibrium was assessed by χ^2-fitness test with one degree of freedom. The heterozygosity and polymorphism information content (PIC) was estimated by the methods of Bostein *et al.*, (1980). Significant differences between the total chromosomes for the hypertensives and normotensives were assessed by χ^2-independence test with one degree of freedom. The association between genotypes and hypertension was evaluated by χ^2-independence test with two degree of freedom. Differences in the clinical data among genotypes were assessed by Students t-test or one-way analysis of variance (one-way ANOVA) test. A P value of less than 0.05 was considered significant. All statistical analysis was performed by the computer program of SPSSWIN (version 8.0).

Results

Genotype distribution

In the present study, we attempted to clarify the distribution of two polymorphisms in the CYP11B2 and ã subunit of ENaC genes in Koreans. Tables 1 and 2 display each data presenting the gene frequencies and

Table 1. Genotype and allele frequencies of *Hae* III RFLP of the CYP11B2 gene in normotensives and hypertensives

	Genotype No. (%)			Allele No. (%)		H^1	PIC^2
	TT	TC	CC	T	C		
Normotensives	43 (48)	37 (42)	9 (10)	123 (69)	55 (31)	0.4270	0.3359
Hypertensives	37 (41)	46 (51)	7 (8)	120 (67)	60 (33)	0.4444	0.3457
X^2		1.6700		0.2430			
P		0.4340		0.6220			
Odds ratio (CI)[3]			1.10 (0.61-1.99)				

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.

Table 2. Genotype and allele frequencies of *Sac* I RFLP in gamma subunit of the epithelial sodium channel gene in normotensives and hypertensives

	Genotype No. (%)			Allele No. (%)		H^1	PIC^2
	GG	CG	CC	G	C		
Normotensives	0 (0)	9 (11)	72 (89)	9 (6)	153 (94)	0.1049	0.0994
Hypertensives	2 (2)	10 (12)	76 (86)	14 (8)	162 (92)	0.1464	0.1357
X^2		1.8740		0.7660			
P		0.3920		0.3820			
Odds ratio (CI)[3]			1.47 (0.62-3.49)				

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.
Observed genotype distribution was not in Hardy-Weinberg equilibrium in Hypertensives (χ^2 = 0.7660, df = 1, P = 0.0356).

the values of heterozygosity and PIC for *Hae* III RFLP of the CYP11B2 gene and *Sac* I RFLP of the γ subunit of ENaC gene in Korean normotensives and hypertensive groups, respectively. In the case of *Hae* III RFLP, the genotype and allele frequencies were not significantly different between normotensives and hypertensives. The observed genotype distributions of this polymorphism were not significantly different from those expected for Hardy-Weinberg equilibrium. The frequencies of TT, TC and CC genotypes were 48, 42 and 10% in normotensives, and 41, 51 and 8% in hypertensives, respectively. The heterozygosity and PIC values of *Hae* III RFLP of the CYP11B2 gene represented the values of 0.4270 and 0.3359 for normotensives, and 0.4444 and 0.3457 for hypertensives, respectively.

For *Sac* I RFLP of the γ subunit of ENaC gene, there were also no significant differences in allele and genotype frequencies between two groups. The frequencies of GG, CG and CC genotypes were 0, 11 and 89% in normotensives, and 2, 12 and 86% in hypertensives, respectively. The observed genotype distribution was in Hardy-Weinberg equilibrium. The heterozygosity and PIC values of *Sac* I RFLP represented the values of 0.1049 and 0.0994 for normotensives, and 0.1464 and 0.1357 for hypertensives, respectively. According to the heterozygosity and PIC values, *Sac* I RFLP of the γ subunit of ENaC gene showed a relatively low degree of polymorphism in the both groups compared with the *Hae* III RFLP in the CYP11B2 gene.

Association with biochemical parameters

Table 3 presented the comparision of anthropometric data and intermediate phenotypes across *Hae* III RFLP in the CYP11B2 gene. The *Hae* III RFLP in the CYP11B2 gene was not significantly associated with any anthropometrical parameters or serum lipid levels. The comparison of the anthropometric data and serum lipid levels across *Sac* I RFLP in the γ subunit of ENaC gene is shown in Table 4. Likewise CYP11B2 gene, The *Sac* I RFLP in the γ subunit of ENaC gene was not also significantly associated with any anthropometrical parameters or serum lipid levels.

Discussion

Hypertension is thought to be a polygenic disease. Many genetic polymorphisms have been proposed as possible marker for hypertension, including insertion/deletion (I/D) polymorphism of the angiotensin I-converting enzyme (ACE) gene (Rigat *et al.*, 1990), A1166C polymorphism of the angiotensin II type I receptor gene (Bonnardeaux *et al.*, 1994) and M235T angiotensinogen polymorphism (Caulfield *et al.*, 1994; Jeunemaitre *et al.*, 1992). The rennin-angiotensin system is a key mechanism in the

Table 3. Clinical characteristics of subjects according to genotypes of *Hae* III RFLP at the CYP11B2 gene

Variables	Genotypes		
	TT (No.)[6]	TC (No.)	CC (No.)
Age (year)	60.5 ± 9.7 (79)	60.2 ± 12.0 (82)	62.0 ± 10.4 (16)
BMI (kg/m^2)[1]	23.4 ± 2.5 (73)	23.9 ± 2.6 (74)	23.6 ± 2.3 (14)
Tg (mg/dl)[2]	135.0 ± 93.0 (66)	125.5 ± 59.1 (64)	89.9 ± 25.7 (13)
TC (mg/dl)[3]	150.8 ± 34.5 (66)	152.6 ± 35.5 (64)	152.2 ± 41.1 (13)
LDL-chol (mg/dl)[4]	97.2 ± 32.0 (66)	99.9 ± 37.0 (64)	110.0 ± 37.6 (13)
HDL-chol (mg/dl)[5]	27.1 ± 8.9 (66)	26.9 ± 8.9 (64)	30.5 ± 5.3 (13)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol and [6]Number. Values are mean ± SD (Standard Deviation).

Table 4. Clinical characteristics according to genotypes of *Sac* I RFLP in gamma subunit of epithelial sodium channel gene

Variables	Genotypes		
	GG (No.)[6]	CG (No.)	CC (No.)
Age (year)	52.0± 2.8 (2)	59.7± 9.3 (19)	60.44±11.0 (146)
BMI (kg/m^2)[1]	24.7± 1.8 (2)	24.9± 2.5 (19)	23.5± 2.5 (131)
Tg (mg/dl)[2]	207.5±112.4 (2)	159.4±122.4 (16)	121.8±66.0 (118)
TC (mg/dl)[3]	198.0± 41.0 (2)	144.4± 40.2 (16)	151.7±34.6 (118)
LDL-chol (mg/dl)[4]	121.0± 62.8 (2)	90.4± 44.3 (16)	100.0±32.9 (118)
HDL-chol (mg/dl)[5]	35.5± 0.7 (2)	23.9± 15.1 (16)	27.0± 8.6 (118)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol and [6]Number. Values are mean ± SD (Standard Deviation).

Table 5. Comparison of allele frequencies of *Hae* III RFLP in the CYP11B2 gene from various ethnic groups

Populations	Sample number	Allele frequencies		P[1]	Reference
		T	C		
(Normotensives)					
Scotch	129	0.53	0.47	<0.05	Davis *et al.*, 1999
Japanese	227	0.74	0.26	NS[2]	Tamaki *et al.*, 1999
Korean	89	0.69	0.31		Present study
(Hypertensives)					
Scotch	129	0.60	0.40	NS	Davis *et al.*, 1999
Japanese	255	0.68	0.32	NS	Tamaki *et al.*, 1999
Korean	90	0.67	0.33		Present study

[1]Probability, [2]Not significant.

Table 6. Comparison of allele frequencies of *Sac* I RFLP in the in gamma subunit of epithelial sodium channel gene from various populations

Populations	Sample number	Allele frequencies		P[1]	Reference
		G	C		
(Normotensives)					
Spanish	34	0.29	0.71	<0.05[2]	Viaplana *et al.*, 1998
Korean	81	0.06	0.94		Present study
(Hypertensives)					
Spanish	38	0.24	0.76	<0.05	Viaplana *et al.*, 1998
Korean	88	0.08	0.92		Present study

[1]Probability, [2]Significant.

regulation of blood pressure. In addition to the vasoactive action, angiotensin II is a potent stimulus of aldosterone synthesis, which results in sodium and water retention.

The biosynthesis of aldosterone is controlled by P450c11AS, an enzyme encoded by the CYP11B2 gene, and is regulated by concentrations of angiotensin II and potassium (Fardella and Miller, 1996; Zhang and Miller, 1996). Davis *et al.*, (1999) found that the 344T allele in CYP11B2 gene was associated with higher aldosterone excretion in white patients with hypertension.

In the present study, we failed to demonstrate the significant association between the *Hae* III RFLP of the CYP11B2 gene and hypertension or other cardiovascular risk factors in Koreans. Therefore, it is unlikely that this polymorphism may influence the etiology of hypertension or other cardiovascular diseases in our subjects. In normotensives, T allele frequency of Korean (0.69) was higher than that of Scotch (0.53), but similar to that of Japanese (0.74) (Table 5), while in hypertensives, T allele frequency of Korean (0.67) similar to those of Scotch (0.60) and Japanese (0.68). The small difference of allele distribution between this study and others may be due to the difference in control groups. As a possible explanation for this phenomenon, the difference in genetic background could be considered. It seems to be important for carefully designed studies to minimize the ethnic heterogeneity of the case and control populations (Pollak *et al.*, 2000).

In the case of *Sac* I RFLP in the γ subunit of ENaC gene, Viaplana *et al.*, (1998) reported that this RFLP was not associated with hypertension in Spanish population. There was also no significant association with any cardiovascular risk factors as well as hypertension in Korean population. Therefore, this polymorphism may not linked to the pathogenic allele and/or gene of hypertension in Koreans as well as Caucasians.

G allele frequency of Korean (0.06-0.08) was significantly lower than that of Spanish (0.24-0.29) population in the both groups (Table 6). This phenomenon may be explained by the difference in genetic background, because the G allele frequency of γ subunit of ENaC gene in Korean was different from that of Spanish in the both groups. To our knowledge, the association study between *Sac* I RFLP in the γ subunit of ENaC gene and hypertension in Asian population were firstly performed in our study group. Thus, studies in other racial or ethnic groups including black Africans will be of great interest.

Reference

Bonnardeaux, A., Davis, E., Jeunemaitre, X., Féry, I., Charru,

A., Clauser, E., Tiret, L., Cambien, F., Corvol, P. and Soubrier, F. (1994): Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension, *Hypertension,* **24,** 63-69.

Bostein, D., White, R.L., Skolnick, M. and Davis, R.W. (1980): Construction of a genetic linkage map in man using restriction fragment length polymorphisms, *Am. J. Hum. Genet.*, **32,** 314-331.

Brilla, C.G., Matsubara, L.S. and Weber, K.T. (1993): Anti-aldosteronetreatment and the prevention of myocardial fibrosis in primary and secondary prevention, *J. Mol. Cell Cardiol.*, **25,** 563-575.

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

Chang, S.S., Grunder, S., Hanukoglu, A., Rösler, A., Mathew, P.M., Hanukoglu, Y., Shild, L., Lu, Y., Shimkets, R.A., Nelson-Williams, C., Rossier, B.C. and Lifton, R.P. (1996): Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1, *Nat. Genet.*, **12,** 248-253.

Davies, E., Holloway, C.D., Ingram, M.C., Inglis, G.C., Friel, E.C., Morrision, C., Anderson, N.H., Fraser, R. and Connell, J.M.C. (1999): Aldosterone excretion rate and blood pressure in essential hypertension are related to polymorphic differences in the aldosterone synthase gene CYP11B2, *Hypertension,* **33,** 703-707.

Fardella, C.E. and Miller, W.L. (1996): Molecular biology of mineralocorticoid metabolism, *Annu. Rev. Nutr.,* **16,** 443-470.

Friedwald, W.T., Levy, L.I. and Fridrickson, D.S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge, *Clin. Chem.*, **18,** 499-502.

Hansson, J.H., Nelson-Williams, C., Suzuki, H., Schild, L., Shimkets, R., Lu, Y., Canessa, C., Iwasaki, T., Rossier, B.C. and Lifton, R.P. (1995): Hypertension caused by a truncated epithelial sodium channel γ subunit: genetic heterogeneity of Liddle syndrome, *Nat. Genet.*, **11,** 76-82.

Hautanena, A., Lankinen, L., Kupari, M., Janne, O.A., Aldercreutz, H., Nikkila, H. and White, P.C. (1998): Associations between aldosterone synthase gene polymorphism and the adrenocortical function in males, *J. Intern. Med.*, **244,** 11-18.

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

Lala, D.S., Rice, D.A. and Parker, K.L. (1992): Steroidogenic factor I, a key regulator of steroidogenic enzyme expression, is the mouse homolog of fushi tarazu-factor I, *Mol. Endocrinol.*, **6,** 1249-1258.

Patel, S., Steeds, R., Channer, K. and Samani, N.J. (2000): Analysis of promoter region polymorphism in the aldosterone synthase gene (CYP11B2) as a risk factor for myocardial infarction, *Am. J. Hypertens.*, **13,** 134-139.

Pojoga, L., Gautier, S., Blanc, H., Guyenne, T.T., Poirier, O., Cambien, F. and Benetos, A. (1998): Genetic determination of plasma aldosterone levels in essential hypertension, *Am. J. Hypertens.*, **11,** 856-860.

Pollak, R.D., Friedlander, Y., Pollak, A., Idelson, M., Bejarano-achache, I. and Blumenfeld, A. (2000): Ethnic differences in the frequency of the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene in healthy Israel populations, *Genet. Test.*, **4,** 309-311.

Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P. and Soubrier, F. (1990): An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels, *J. Clin. Invest.*, **86,** 1343-1346.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989): Molecular Cloning A Laboratory Manual. 2nd ed. Cold Spring Harbor NY, pp. 9.16 9.23.

Shimkets, R.A., Warnock, D.G., Bositis, C.M., Nelson-Williams, C., Hansson, J.H., Schambelan, M., Gill, J.R. Jr., Ulick, S., Milora, R.V., Findling, J.W., Canessa, C., Iwasaki, T., Rossier, B.C. and Lifton, R.P. (1994): Liddles syndrome: heritable human hypertension caused by mutations in the γ subunit of the epithelial sodium channel, *Cell,* **79,** 407-414.

Su, Y. R. and Menon, A.G. (2001): Epithelial sodium channels and hypertension, *Drug Metab. Dispos.*, **29,** 553-556.

Szpirer, C., Rivière, M., Szpirer, J., Levan, G., Guo, D.F., Iwai, N. and Inagami, T. (1993): Chromosomal assignment of human and rat hypertension candidate genes: type 1 angiotensin II receptor genes and the S_A gene, *J. Hypertens.*, **11,** 919-925.

Tamaki, S., Iwai, N., Tsujita, Y. and Kinoshita, M. (1999): Genetic polymorphism of CYP11B2 gene and hypertension in Japanese, *Hypertension,* **33,** 266-270.

Uehara, Y., Sasaguri, M., Kinoshita, A., Tsuji, E., Kiyose, H., Taniguchi, H., Noda, K., Ideishi, M., Inoue, J., Tomita, K. and Arakawa, K. (1998): Genetic analysis of the epithelial sodium channel in Liddles syndrome, *J. Hypertens.*, **16,** 1131-1135.

Viaplana, R., Poch, E., Lario, S., Oriola, J., González, D., Botey, A. and Rivera, F. (1998): *Sac* I identifies a biallelic polymorphism in the coding sequence of the gamma subunit of the epithelial sodium channel (EnaC): a candidate gene for hypertension, *Clin. Genet.*, **54,** 104-105.

White, P.C. (1994): Disorders of aldosterone biosynthesis and action, *N. Eng l. J. Med.*, **331,** 250-258.

White, P.C. and Slutsker, L (1995): Haplotype analysis of CYP11B2, *Endocrine Res.*, **21,** 437-442.

Young, M., Fullerton, M.J., Dilley, R. and Funder, J.W. (1994): Mineralocorticoids, hypertension and cardiac fibrosis, *J. Clin. Invest.*, **93,** 2579-2583.

Zhang, G. and Miller, W.L. (1996): the human genome contains only two CYP11B2 (P450c11) genes, *J. Clin. Endocrinol. Metab.*, **81,** 3254-3256.