発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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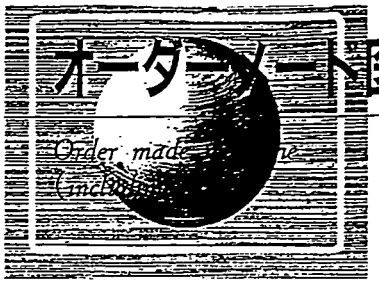
Ⅳ. 研究成果の刊行物・別刷り



オーダーメード医療(SNPを含む)

神出　計/河野雄平

高血圧診療において患者個人のもつ背景を考慮して治療方針を選択する必要があり，とくに遺伝素因から個人に合ったベストの治療をするオーダーメード医療の確立が望まれている．

オーダーメード医療とは

ポストゲノム時代の今，高血圧診療においても一塩基多型SNP(single nucleotide polymorphism；SNP)を解析することによって高血圧の発症や合併症を予測し，治療薬の選択を行うといった個人の遺伝子情報に基づいた診療，オーダーメード医療(テーラーメード医療，個別化医療)の確立に期待がかけられている．すでに癌やリウマチの治療においてはSNPを調べることで薬剤の副作用や効果を予測し，処方の際の情報とするオーダーメード医療が行われているが，高血圧診療においてはまだ研究段階で実現されていない．国立循環器病センターでは，オーダーメード医療の確立とゲノム創薬を目標に掲げた遺伝子解析計画であるミレニアム・ゲノム・プロジェクト(MGP)において高血圧を担当し，5年間で数多くの高血圧関連遺伝子を同定してきた[1]．MGPにより高血圧関連のゲノム情報の基盤は整備され，得られた膨大なゲノム情報はここ数年のうちに臨床の現場に応用されていくことは間違いないと考えられる．

オーダーメード医療への応用

1．高血圧原因遺伝子

高血圧への遺伝素因の関与は多岐にわたる．本態性高血圧(EHT)の病態の根幹をなすレニン-アンジオテンシン(RA)系や交感神経(SN)系の活性化，食塩感受性やインスリン抵抗性の形成など，すべての機序に遺伝因子は関与すると考えられる．また数多くの薬剤がこれらの病態をターゲットにして降圧作用を発揮する(図)．これまで数多く行われてきた候補遺伝子アプローチによる高血圧原因遺伝子同定の試みは，アンジオテンシン変換酵素(ACE)I/D多型に代表されるように，RA系やSN系の受容体や酵素の遺伝子をターゲットにして，ケース・コントロールを用いた解析が主流となってきた．さらに近年ではゲノム網羅的な方法で50万SNPをDNAマイクロアレイで調べる方法を用いて検討されているが[2]，糖尿病などと比較して非常に強い関連性をもつ高血圧原因遺伝子多型がいまだに同定できていないのが現状である．倫理問題を含め，高血圧原因遺伝子多型を調べることで将来の高血圧発症を予測することは当面難しいと思われる．

2．高血圧性臓器障害関連遺伝子

高血圧患者の予後を左右するのは心・血管・腎の合併症であり，遺伝子情報を用いたオーダーメード医療により遺伝子多型から合併症の進展が予測できれば，より厳格な降圧を試みたり，それぞれの臓器障害に有効とされる薬剤を早期から服薬させることにより，発症を予防することが可能となると考えられる．

3．降圧薬感受性遺伝子

降圧薬服用者は高血圧治療患者の半数以上を占める．したがって遺伝的に規定されている降圧薬に対する感受性を薬剤選択の際に考慮できれば，効率のよい降圧薬治療を実現することが可能となる．

用語解説———DNAマイクロアレイ(チップ)
種々のDNAをスライドグラスもしくはシリコンの基盤に非常に繊細に並べたもので，ハイブリダイゼーション法によりDNAの多型を一度に大量に解析できるもの．最近では50〜100万個のSNPを解析するチップが出ている．

Recommended Readings
❶ Turner ST et al : J Hypertens 19 : 1-11, 2001
❷ Arnett DK et al : Circulation 115 : 2878-2901, 2007

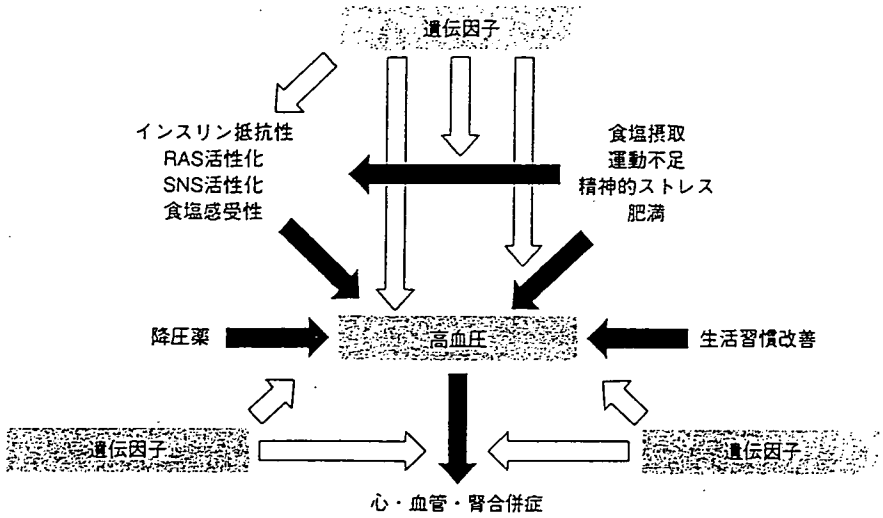


図　高血圧における遺伝因子の影響
(Kamide K et al : Jpn Heart J 45 : S69, 2004 より引用)

こういった観点から降圧薬感受性遺伝子多型を明らかにするために，近年 pharmacogenomics なアプローチがなされてきた．しかしながら，多くの研究が RA 系や SN 系の酵素や受容体の一つから数個の多型の関与を調べた検討のみで，結果は非常にコントロバーシャルであった[3]．より関連性の強い薬剤応答性・感受性遺伝子の同定のためには，多数例の無治療高血圧患者に前向きに降圧薬を投与し，正確に降圧の程度を把握し，数多くの薬物代謝酵素や薬理作用機序関連の遺伝子多型との相関を検討する必要がある．これまでわが国にこのような研究はなかったが，現在，国立循環器病センターでは，全国の大学・医療センターなどとともに降圧薬感受性遺伝子多型同定のための多施設共同研究(GEANE 研究)を施行している．GEANE 研究では，無投薬の軽・中等症 EHT 患者にサイアザイド系利尿薬，ARB，Ca 拮抗薬を 3 カ月ごとに内服してもらい，観察期も含め合計 10 カ月間で投薬を終了するデザインで施行中である．降圧効果のみならず，副作用や代謝性の異常も解析予定でゲノム網羅的に複数の SNP を検討し，これら 3 種類の薬剤の感受性遺伝子多型ならびに副作用関連遺伝子多型を検索している．これにより同定された遺伝子多型を実際の臨床に応用し，オーダーメード医療を確立することを構想している．

オーダーメード医療の実現に向けて

高血圧のオーダーメード医療実現には，適確な研究成果の集積と，出てきた遺伝子多型を用いた迅速遺伝子診断システムの開発，このような遺伝子診断システムを導入した場合の有用性を確かめる前向き試験，遺伝子診断を考慮した新しい高血圧診療ガイドラインの制定などが必要と考えられ，道程は長い．しかしながら確実な研究成果の集積により必ずや実現できるであろう．無駄が少なく，より安全で，合併症を減少させることができるような高血圧診療を患者に提供することを最終目標に研究を進めることが重要である．

References
1) 神出　計 ほか : 血管 28 : 79-85, 2005
2) The Wellcome Trust Case Control Consortium : Nature 447 : 661-678, 2007
3) Arnett DK et al : Circulation 111 : 3374-3383, 2005

関連事項
吹田研究 ▶▶ 50 頁
ACE 多型 ▶▶ 62 頁
ミレニアム・プロジェクト ▶▶ 72 頁
高血圧の遺伝因子 ▶▶ 150 頁

Protein tyrosine kinase 2β as a candidate gene for hypertension

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Protein tyrosine kinase 2β (PTK2B) is a member of the focal adhesion kinase family and is activated by angiotensin II through Ca^{2+}-dependent pathways. An evidence exists that PTK2B is involved in cell growth, vascular contraction, inflammatory responses, and salt and water retention through activation of the angiotensin II type 1 receptor. To examine the contribution of PTK2B, we sequenced the *PTK2B* gene using 48 patients with hypertension, identified 62 genetic polymorphisms, and genotyped six representative single nucleotide polymorphisms in population-based case–control samples from 3655 Japanese individuals (1520 patients with hypertension and 2135 controls). Multivariate logistic regression analysis after adjustments for age, body mass index, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking) showed −22A>G to have an association with hypertension in men (AA vs. AG + GG: odds ratio = 1.27; 95% confidence interval: 1.02–1.57; *P* = 0.030). Another polymorphism, 53484A>C (K838T), in linkage disequilibrium with −22A>G showed a marginal association with hypertension in men (AA vs. AC + CC: odds ratio = 1.25; 95% confidence interval: 0.99–1.57; *P* = 0.059). Diastolic blood pressure was 1.6 mmHg higher in men with the AC + CC genotype of 53484A>C than those with the AA genotype (*P* = 0.003), after adjustments for the same factors. These polymorphisms are in linkage disequilibrium with others in a range of 113 kb in *PTK2B*. The intracellular distribution of the recombinant PTK2B protein and that of the mutant protein with T838 were indistinguishable even after angiotensin II stimulation, both proteins localizing at a focal point in the peripheral area in the cells. Thus, a haplotype in *PTK2B* may play a role in essential hypertension in Japanese. *Pharmacogenetics and Genomics* 17:931–939

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Keywords: focal adhesion kinase 2, genetic variation, hypertension, protein tyrosine kinase 2β

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Introduction

Angiotensin II (Ang II) is a multifunctional hormone that regulates the functions of cardiovascular cells through intracellular signaling events initiated via interaction with cell surface Ang II type 1 (AT1) and Ang II type 2 (AT2) receptors [1,2]. Ang II was initially described as a vasoconstrictor. Recent studies, however, demonstrate that Ang II has growth factor and cytokine-like properties. The multiple actions of Ang II are mediated by specific intracellular signaling pathways that are stimulated following initial binding of the peptide to its specific receptors. In the vasculature, AT1 receptors are mainly present in vascular smooth muscle cells (VSMCs) [3]. In the heart, AT1 receptors are expressed in cardiomyocytes and fibroblasts. AT1 receptors mediate most of the physiological actions of Ang II.

Protein-tyrosine kinase 2β (PTK2B), also known as proline-rich tyrosine kinase-2, focal adhesion kinase 2 (FAK2), cell-associated kinase β, related focal tyrosine kinase or calcium-dependent tyrosine kinase, exhibits a considerable level of structural homology to FAK, a nonreceptor tyrosine kinase which targets sites of integrin clustering [4]. Unlike FAK, PTK2B is expressed in a highly cell type and tissue-specific manner. PTK2B is activated by phosphorylation on tyrosine residues in response to various stimuli, depending on the cell type, including G protein-coupled receptor agonists (such as Ang II, thrombin, and lysophosphatidylcholine) and cellular stress (from ultraviolet irradiation, tumor necrosis factor-α, hyperosmotic shock, etc.) [5]. Activation of PTK2B requires intracellular calcium release [6]. In contrast to FAK, which is localized to adhesion plaques at the basal side of the cell, PTK2B is localized in the cytosol but can be recruited to plasma membrane, the perinuclear region, or the nucleus in response to different stimuli [7].

PTK2B is very similar to FAK, containing a kinase domain and two proline-rich domains, as well as several phosphorylated residues including an autophosphorylation site (T402), sites involved in kinase activation (T579, T580),

and a site (T881) homologous to the Grb2-binding site in FAK [6,8].

AT1 receptors activate PTK2B in a calcium-dependent manner. As PTK2B may act to regulate c-Src and to link G protein-coupled vasoconstrictor receptors with protein kinase-mediated contractile, migratory, and growth responses, it may be a potential point of convergence between Ca^{2+}-dependent signaling pathways and protein kinase pathways in VSMCs. Thus, PTK2B may play a role in hypertension through AT1 receptors.

In this study, we attempted to evaluate the *PTK2B* gene in relation to hypertension using population-based case–control samples from 3655 Japanese individuals (1520 patients with hypertension and 2135 controls). First we identified genetic variations, mainly single nucleotide polymorphisms (SNPs), in all exons of *PTK2B*. Next, we examined the association of SNPs with the presence of hypertension in the Japanese general population. Finally, we examined the intracellular localization of a mutant PTK2B with the missense mutation K838T.

Methods

Participants of the study population

The selection criteria and design of the Suita study were described previously [9,10]. Briefly, the participants had been selected randomly from the municipal population registry and stratified based on sex and age (stratified in 10-year intervals). They were all invited, by letter, to receive medical and behavioral examinations every 2 years at the Division of Preventive Cardiology, National Cardiovascular Center, Japan. DNA from the leukocytes was collected from participants who visited the National Cardiovascular Center. In this study, 3655 individuals including 1520 patients with hypertension (779 men, 741 women) and 2135 controls (930 men, 1205 women) were genotyped. All of the participants were Japanese. For DNA sequencing, 48 Japanese patients with essential hypertension at the Division of Hypertension and Nephrology, National Cardiovascular Center, Japan, were recruited. Only those who gave their written informed consent for genetic analyses were included in this study. The study protocol was approved by the Ethical Review Committee of the National Cardiovascular Center.

Measurements

Blood pressure was measured after at least 10 min of rest in a sitting position. Systolic and diastolic blood pressures (SBP and DBP) were the means of two measurements (recorded > 3 min apart). In this study, two criteria were used to define hypertension. SBP of ≥ 140 mmHg and/or DBP of ≥ 90 mmHg, or the current use of antihypertensive medication. To exclude marginal hypertension, hypertension was defined as SBP of ≥ 160 mmHg and/or DBP of ≥ 95 mmHg, or the current use of antihypertensive medication. Diabetes mellitus was defined as a fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl) or nonfasting plasma glucose level ≥ 11.1 mmol/l (200 mg/dl) or the taking of antidiabetic medication or HbA1c ≥ 6.5%. Hyperlipidemia was defined as a total cholesterol concentration ≥ 5.68 mmol/l (220 mg/dl) or the taking of antihyperlipidemia medication. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared.

Blood samples drawn from the participants after 12 h of fasting were collected in ethylenediaminetetraacetate-containing tubes. Total cholesterol and high-density lipoprotein cholesterol levels were measured with an autoanalyzer (Toshiba TBA-80, Tokyo, Japan) in accordance with the Lipid Standardization Program of the US Centers for Disease Control and Prevention through the Osaka Medical Center for Health Science and Promotion, Japan.

Direct sequencing for discovering polymorphisms and genotyping of single nucleotide polymorphisms

We sequenced the entire coding region of *PTK2B* in 48 hypertensive samples in which hypertension susceptive polymorphisms would be much concentrated. The methods of direct sequencing were described previously [11,12]. SNPs having a minor allele frequency of greater than 5% were candidates for genotyping using the TaqMan-polymerase chain reaction (PCR) system (Applied Biosystems, Foster City, California, USA) [13,14]. As a consequence, we genotyped six SNPs in the population-based samples.

Linkage disequilibrium and single nucleotide polymorphism blocks in the *PTK2B* gene

SNP genotype data for the Japanese population were downloaded from the International HapMap Consortium (*www.hapmap.org*) [15]. Positions of SNP sites were renumbered by NCBI human chromosome sequences (build 35). Pair-wise *D'* and LOD values were calculated and SNP blocks were inferred by Haploview [16] using its default setting parameters.

Expression of wild-type and mutant rat PTK2B

PTK2B is a rat ortholog of human PTK2B. PTK2B cDNA was inserted into an EGFP-tagging mammalian expression vector, pEGFP-C1 (BD Biosciences, San Jose, California, USA). A missense mutation, K838T, was introduced into PTK2BcDNA by site-directed mutagenesis using PCR. The mutation was confirmed by sequencing. Human umbilical vascular endothelial cells (HUVECs) were cultured in HuMedia-2 (Kurabo, Osaka, Japan) supplemented with a growth additive set and used for experiments before passage 7. HUVECs cultured on glass-bottom dishes transfected with either pEGFP-wild-type PTK2B or pEGFP-mutant PTK2B using FuGene6 (Roche Diagnostics, Basel, Switzerland) were imaged under a fluorescence microscope (IX-81, Olympus,

Tokyo, Japan). Furthermore, both wild-type and mutant cells were incubated with vehicle or 1 μmol/l Ang II (Sigma, St Louis, Missouri, USA) for 5 min to investigate the difference of cell maturity.

Statistical analysis

Student's *t*-test was used to compare mean values between groups. Frequencies were compared by χ^2 analysis. The relationships in men and women between genotypes and the presence of hypertensives were expressed in terms of odds ratios (ORs) adjusted for possible confounding effects including age, BMI, antihypertensive drug use, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking) by logistic regression analysis. For multivariate risk predictors, adjusted ORs were given with 95% confidence intervals (CIs).

Association-based analyses in each sex of genotypes with blood pressures were investigated through analysis of covariance considering potential confounding risk variables, including age, BMI, present illness (hyperlipidemia and diabetes mellitus), lifestyle (smoking and drinking), and antihypertensive medication.

Statistical analyses were performed with SAS statistical software (release 6.12, SAS Institute Inc., Cary, North Carolina, USA). The linkage disequilibrium (LD) of genotyped SNPs was calculated by using SNPAlyze version 2.1 (DYNACOM Co., Ltd, Mohara, Japan).

Results

Basic characteristics of participants of the study population

The characteristics of the 3655 participants (1709 men, 1946 women) are summarized in Table 1a. Age, SBP, DBP, BMI, percentage that are current smokers, percentage that are current drinkers, prevalence of hypertension, and prevalence of diabetes mellitus were significantly higher in men than in women. Total cholesterol, high-density lipoprotein cholesterol, and percentage that have hyperlipidemia were significantly higher in women than in men. Table 1b shows patients characteristics divided by two criteria of hypertension. Age, BMI, and percentage that are current drinkers, have diabetes and have hyperlipidemia were higher in hypertensive patients than normotensives for both criteria.

Polymorphisms in *PTK2B* and genotyping of single nucleotide polymorphisms

We sequenced 96 alleles from 48 Japanese patients with hypertension, and identified 62 polymorphisms, including four nonsynonymous and 11 synonymous SNPs (Table 2). The four nonsynonymous SNPs, 45344G > A, 48255A > G, 48273G > A, and 53484A > C, encode for the missense mutation R698H with a minor allele frequency of 0.010,

Table 1a Basic characteristics of the participants

	Women (n=1946)	Men (n=1709)
Age (year)	63.5±11.1	66.1±11.3*
Systolic blood pressure (mmHg)	128.3±19.8	130.8±19.1*
Diastolic blood pressure (mmHg)	76.5±9.7	79.2±10.3*
Body mass index (kg/m^2)	22.4±3.2	23.3±3.0*
Total cholesterol (mg/dl)	216.1±31.3*	198.7±31.4
HDL cholesterol (mg/dl)	64.9±15.2*	54.9±14.3
Current smokers (%)	6.0	30.0†
Current drinkers (%)	27.3	66.9†
Present illness (%)		
Hypertension	38.1	45.6†
Hyperlipidemia	54.9†	31.5
Diabetes mellitus	6.1	13.0†

Values are the mean±SD or a percentage. Hypertension indicates a systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or antihypertensive medication; hyperlipidemia, total cholesterol ≥ 5.68 mmol/l (220 mg/dl) or antihyperlipidemia medication; diabetes, fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl) or nonfasting plasma glucose ≥ 11.1 mmol/l (200 mg/dl) or antidiabetic medication. HDL, high-density lipoprotein.
*P<0.05 between women and men by the Student's *t*-test.
†P<0.05 between women and men by the χ^2 test.

Table 1b Characteristics of the patients divided by two definitions of hypertension

	NT1 (n=2135)	HT1 (n=1520)	NT2 (n=2557)	HT2 (n=1098)
Age (year)	61.8±11.6	68.8±9.4*	62.7±11.4	69.5±9.4*
Sex (F/M)	1205/930	741/779+	1426/1131	520/578+
Body mass index (kg/m^2)	22.3±3.0	23.6±3.2*	22.4±3.0	23.8±3.2*
Systolic blood pressure (mmHg)	118.0±12.0	145.6±16.5*	122.6±15.3	145.5±18.9*
Diastolic blood pressure (mmHg)	73.9±8.3	83.2±9.8*	75.6±9.0	82.9±10.5*
Total cholesterol (mg/dl)	208.3±32.8	207.5±32.2	208.7±32.5*	206.2±32.5
HDL cholesterol (mg/dl)	61.3±15.8*	58.7±15.2	61.1±15.8*	58.1±14.9
Current smokers (%)	19.3+	14.2	18.9+	13.4
Current drinkers (%)	43.6	49.0+	44.3	49.3+
Present illness (%)				
Diabetes mellitus	6.9	12.8+	7.4	13.8+
Hyperlipidemia	40.6	48.6+	41.8	49.1+

Values are the mean±SD or a percentage. HDL, high-density lipoprotein; HT, hypertension; HT1, hypertension indicates a systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or antihypertensive medication; HT2, hypertension indicates a systolic blood pressure >160 mmHg and/or diastolic blood pressure >95 mmHg or antihypertensive medication; hyperlipidemia, total cholesterol ≥ 5.68 mmol/l (220 mg/dl) or antihyperlipidemia medication; diabetes mellitus, fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl) or nonfasting plasma glucose ≥ 11.1 mmol/l (200 mg/dl) or antidiabetic medication; NT, normotension.
*P<0.05 between cases and controls by the Student's *t*-test.
+P<0.05 between cases and controls by the χ^2 test.

Table 2 List of polymorphisms and their allele frequency in *PTK2B* identified by direct sequencing in 48 hypertensive patients

SNP	LD	Amino acid substitution	Region	Allele 1 frequency	Allele 2 frequency	Flanking sequence	Typing	dbSNP ID
-86282C>A			Promoter	0.870	0.130	cccggctgccaa[c/a]gcccgcgacccg	Taqman	rs7006183
-86255C>T	a		Promoter	0.924	0.076	tgctgggaatcg[c/t]ccagtcccttcc		rs12679503
-86253C>T			Promoter	0.989	0.011	ctgggaatcgcc[c/t]agtcccttcccc		
-86200G>A	b		Promoter	0.967	0.033	caatcgtgcgggg[g/a]gggatggcgagg		
-86188G>A	b		Promoter	0.967	0.033	ggggatggcgag[g/a]gggagg-gagggg		
-86141G>A	c		Promoter	0.761	0.239	cttccggtgtgc[g/a]cgggaaatcttg	Taqman	rs7005244
-85972A>T	c		5′-UTR	0.771	0.229	AAAGGAGCCTCT[A/T] CCTTAACCAATC		rs6988218
-85868C>T	a		Intron 1	0.927	0.073	GCACCgtgagtg[c/t]aatcaccactta		rs12679570
-75144G>A	d		5′-UTR	0.979	0.021	TTGTGAAGACAA[G/A] CTAGACGGCAGA		
-74037T>C	c		Intron 5	0.719	0.281	cattattgcaac[t/c]tatgccatatgg		
-99G>A	e,g,i,k,m		Intron 6	0.589	0.411	gctgtccctggg[g/a]ccatgaggtatg		
-22A>G	e,g,i,k,m		5′-UTR	0.589	0.411	TGCAATGTGCCG[A/G] TCTTAGCTGCTG	Taqman	rs2241649
27T>C	e,g,i,k,m	S9S	Exon 7	0.589	0.411	CGAGCCCCTGAG[T/C] CGAGTAAAGTTG		rs1045510
45G>A	e,g,i,k,m	T15T	Exon 7	0.589	0.411	AAAGTTGGGCAC[G/A] TTACGCCGGCCT		rs1045511
162A>G	e,g,i,k,m	K54K	Exon 7	0.589	0.411	CAATCCTGGGAA[A/G] AACTTCAAACTG		rs1045512
224C>T	e,g,i,k,m		Intron 7	0.589	0.411	tgaagtgtctgc[c/t]ctgtccatctgt		rs2241650
22313G>A		E63E	Exon 8	0.990	0.010	tcctctgcagGA[g/a] ATCATCACCTCC		
22436G>A	f	T110T	Exon 8	0.875	0.125	CCCACAGATGAC[G/A] GTGGGTGAGGTG	Taqman	rs1030526
24604G>A	e,g,h,k,l		Intron 9	0.427	0.573	ttgtttgtggtg[g/a]ggtgggtggctg		rs2241652
32896T>A			Intron 12	0.865	0.135	gagtgtaaggga[t/a]gaggctggggct	Taqman	rs2241653
32932T>C	f		Intron 12	0.885	0.115	gagaagccaggg[t/c]atctgcgcggcc		rs7827965
33213T>C			Intron 12	0.927	0.073	agcagtgggcag[t/c]ctctcagcgaga		
33938C>T			Intron 14	0.957	0.043	gggaggtcgtcc[c/t]ctctctgctgcc		
34834T>C			Intron 15	0.979	0.021	tataatggcaga[t/c]tgggagctcttg		rs2303881
34862T>C			Intron 15	0.979	0.021	agaccaaaagtc[t/c]gtgacacacagg		rs2303882
36097G>A			Intron 16	0.979	0.021	ccacagcccagc[g/a]ggaagcttccag		
36456T>C	g,h,k,l		Intron 16	0.417	0.583	gtcagtcaccca[t/c]ccaggtccctgt		rs919493
36567A>G			Intron 17	0.979	0.021	acaatggggtgc[a/g]gaggacagggcc		
36648C>T	b		Intron 17	0.948	0.052	catagtttctgg[c/t]tttcaggcccag		rs12056620
38234G>T	e,i,j,k,m		Intron19	0.615	0.385	ccccgccacagc[g/t]accgtagtcaag		rs11774417
38312C>T			Intron 19	0.989	0.011	ttcctcctttat[c/t]ctcccttcgtgc		
38764C>T	b	H447H	Exon 20	0.958	0.042	CTACACAAATCA[C/T] gtgagttctagg		rs7005936
38881C>T			Intron 20	0.990	0.010	gggccccttgtc[c/t]ctaagggcctct		
38888G>A	b		Intron 20	0.958	0.042	ttgtccctaagg[g/a]cctcttgtccac		rs2241654
39431C>G	g,h,k,l		Intron 20	0.426	0.574	taggagaaaggg[c/g]cctttcctggca		rs2163176
39505G>C	b		Intron 20	0.957	0.043	agcactgggctg[g/c]accaaggggtcc		rs7005954
39722T>C	g,h,k,l		Intron 21	0.426	0.574	tggaggaggggt[t/c]cccgtcctccca		rs6996922
41359-41360delTC			Intron 23	0.990	0.010	agattcttggtc[tc/-]ttttccatctg		
42101T>C	b		Intron 25	0.958	0.042	aagacgaaactc[t/c]gtgacttattct		rs11995441
42595T>C	g,h,k,l		Intron 25	0.448	0.552	ggcgatggtgct[t/c]ctgggtgggagg		rs2241657
42977A>G	g,h,k,l		Intron 26	0.448	0.552	agggtcaaggac[a/g]ggaggctgaagc		rs2241658
45344G>A		R698H	Exon 27	0.990	0.010	AGAGGAATGCTC[G/A] CTACCGAACCCC		
46624C>G	i,j	P717P	Exon 28	0.755	0.245	ctctctccagCC[C/G] AGCCGACCTAAG		
48255A>G		M754V	Exon 29	0.989	0.011	CTCACCAGCCCT[A/G] TGGAGTATCCAT		
48273G>A	d	V760F	Exon 29	0.979	0.021	TATCCATCTCCC[G/A] TTAACTCACTGC		
48640T>A			Intron 29	0.989	0.011	ggggtaggggga[t/a]ctgtggcagctt		
53437G>A	b		Intron 30	0.957	0.043	tcttagtccttc[g/a]ctcttgtttctt		rs751018
53443G>A			Intron 30	0.989	0.011	tccttcgctctt[g/a]tttcttcctctg		
53484A>C	e,g,h,i,k,l,m	K838T	Exon 31	0.489	0.511	ATATGAATGATA[A/C] GTCCCCATTGgt	Taqman	rs751019
53748A>G	g,h,k,l,m		Intron 31	0.448	0.552	cagaaaggtcac[a/g]ttgggtcacgag		rs2251430
53860C>T	e,i,k,l,m		Intron 31	0.615	0.385	tgtctccacagc[c/t]gcatgagtgacg		rs2278319
55445A>G	g,h,k,l		Intron 32	0.448	0.552	tggtagagggga[a/g]ggggctcatttg		rs3735758
56602T>C	g,h,k,l	T876T	Exon 34	0.448	0.552	CCTGGACCGGAC[T/C] GATGACCTGGTG		rs1879184
56804-56805delCT			Intron 34	0.990	0.010	ccagcagatcct[ct/-]tagagcaagctg		
56939C>T	n		Intron 34	0.956	0.044	ctgccccttct[c/t]cccccagAATGT		rs10093964
57034G>A	n		Intron 35	0.956	0.044	ACAGAGgtgagc[g/a]tcccattccaga		rs7007145

Table 2 (*continued*)

SNP	LD	Amino acid substitution	Region	Allele 1 frequency	Allele 2 frequency	Flanking sequence	Typing	dbSNP ID
60775A>G	g,h,k,l	A960A	Exon 36	0.435	0.565	GATGCGGCTGGC[A/G] CAGCAGAACGCC		rs1879182
60799A>G	g,h,k,l	L968L	Exon 36	0.435	0.565	CGTGACCTCCCT[A/G] AGTGAGGAGTGC		rs1879181
60835A>G		S980S	Exon 36	0.967	0.033	GCTGACGGCTTC[A/G] CACACCCTGGCT		
60926C>T			3′UTR	0.989	0.011	CCTGCAGAGTGA[C/T] GGAGGGTGGGGG		
61000T>C			3′UTR	0.989	0.011	TGCTGTTGGTCA[T/C] GTGGGTCTTCCA		
61016G>A	b		3′UTR	0.957	0.043	GGTCTTCCAGGG[G/A] GAAGGCCAAGGG		rs2271920

The A of the ATG of the initiator Met codon is denoted nucleotide + 1, as recommended by the Nomenclature Working Group (*Hum Mut* 1998; 11:1–3). The uppercase and lowercase letters are the sequence in the exon and intron region, respectively. The nucleotide sequence (GenBank Accession ID: NT_023666.16) was used as a reference sequence. The apparent linkage disequilibrium (LD), defined by an r^2 of more than 0.5, was indicated by a–m, which shows different LD group.
SNP, single nucleotide polymorphism; UTR, untranslated region; Taqman, the SNP was successfully genotyped by the Taqman method.

for M754V with a minor allele frequency of 0.011, for V760F with a minor allele frequency of 0.021, and for K838T with a minor allele frequency of 0.489, respectively. 53484A > C has been deposited in the public database with the dbSNP number, rs751019. Considering the allele frequency and the LD, we selected six SNPs for genotyping in large-scale population-based samples.

Association of single nucleotide polymorphisms with hypertension

The multivariate logistic regression analysis after adjustments for age, BMI, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking) revealed that −22A > G showed an association with the presence of hypertension in men (AA vs. AG + GG: OR = 1.27; 95% CI: 1.02–1.57; $P = 0.030$). Another polymorphism, 53484A > C, accompanied by the missense mutation K838T in LD with −22A > G, showed a marginal association with the presence of hypertension in men (AA vs. AC + CC: OR = 1.25; 95% CI: 0.99–1.57; $P = 0.059$) (Table 3a). Power analyses using the SNPs with hypertension were performed. These two significant SNPs, −22A > G and 53484A > C (K838T), showed higher power, 68 and 53%, respectively. Furthermore, DBP was 1.6 mmHg higher in men with the AC + CC genotype of 53484A > C than those with the AA genotype ($P = 0.003$), after adjustments for the same factors described above (Table 4).

When hypertension was defined as SBP of > 160 mmHg and/or DBP of > 95 mmHg, or the current use of antihypertensive medication, −22A > G showed an association with the presence of hypertension in men (AA vs. AG + GG: OR = 1.38; 95% CI: 1.10–1.73; $P = 0.006$). Another polymorphism, 53484A > C (K838T), showed a significant association with the presence of hypertension in men (AA vs. AC + CC: OR = 1.31; 95% CI: 1.03–1.68; $P = 0.031$) (Table 3b). These two significant SNPs, −22A > G and 53484A > C (K838T), showed power, 90 and 68%. Taken together, *PTK2B* was associated with the presence of hypertension in men.

The pair-wise LD parameters, r^2 and D', calculated from the genotype data for these SNPs, are shown in Table 5. Two SNPs, −22A > G (rs1045510) and 53484A > C (K838T: rs751019), were in LD with an r^2 of more than 0.5. These polymorphisms showed LD extensively to make a haplotype block with more than 20 SNPs, as shown in Table 2.

To understand more about the LD in *PTK2B*, we retrieved genotype data on *PTK2B* from the public database, HapMap Project. The pair-wise D' value is shown in supplement Fig. 1. A hypertension-associated polymorphism, 53484A > C (K838T: rs751019), was in LD with rs1879181, rs1583092, rs1019832, rs4733058, rs725787, rs2322718, rs1045510, rs919495, rs11776858, rs11785606, rs10109834, and rs3735759, which are present in a stretch of 113 kb in *PTK2B*. Among the polymorphisms in LD with 53484A > C (K838T: rs751019), rs1045510 (27T > C) is a synonymous SNP encoding S9S, and the others are present in the 5′-untranslational region, in an intron, or in the 3′-untranslational region.

Expression of mutant PTK2B

As described, a haplotype of *PTK2B* including the missense mutation K838T was associated with the presence of hypertension. Figure 1 shows an amino acid sequence alignment of human, ape, dog, and mouse, in the area surrounding K838. The amino acid sequence around K838 was highly conserved among mammals, suggesting a functional role. To understand the functional roles of the K838T mutation, the rat ortholog, PTK2B, was expressed in HUVECs to see the effects on the intracellular localization of the recombinant protein. Both EGFP-tagged wild-type PTK2B and mutant PTK2B were

Table 3a Allele frequency and odds ratio of the presence of hypertension by genotypes of *PTK2B* polymorphisms by sex

		Men		Women	
SNP (allele frequency)	Genotype group	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
-86282C>A	CC	1	0.427	1	0.074
(0.890/0.110)	CA+AA	0.90 (0.70–1.16)		1.26 (0.98–1.62)	
	CC+CA	1	0.914	1	0.181
	AA	1.05 (0.45–2.45)		1.78 (0.76–4.16)	
-86141G>A	GG	1	0.588	1	0.428
(0.671/0.329)	GA+AA	1.06 (0.86–1.30)		1.09 (0.89–1.33)	
	GG+GA	1	0.571	1	0.722
	AA	1.10 (0.79–1.55)		0.94 (0.67–1.32)	
-22A>G	AA	1	0.030	1	0.521
(0.597/0.403)	AG+GG	1.27 (1.02–1.57)		1.07 (0.87–1.32)	
	AA+AG	1	0.375	1	0.617
	GG	1.14 (0.86–1.50)		0.93 (0.70–1.24)	
22436G>A	GG	1	0.456	1	0.452
(0.820/0.180)	GA+AA	0.92 (0.74–1.14)		0.92 (0.74–1.14)	
	GG+GA	1	0.483	1	0.764
	AA	1.27 (0.65–2.45)		1.10 (0.58–2.09)	
32896T>A	TT	1	0.935	1	0.254
(0.889/0.111)	TA+AA	1.01 (0.79–1.29)		1.15 (0.90–1.48)	
	TT+TA	1	0.276	1	0.926
	AA	1.59 (0.69–3.67)		0.96 (0.37–2.50)	
53484A>C	AA	1	0.059	1	0.874
K838T	AC+CC	1.25 (0.99–1.57)		0.98 (0.79–1.22)	
(0.527/0.473)	AA+AC	1	0.600	1	0.633
	CC	1.07 (0.83–1.37)		0.94 (0.73–1.21)	

All adjusted for age, body mass index, antihypertensive drug use, present illness (hyperlipidemia, diabetes mellitus), and lifestyle (smoking and drinking). Hypertension was defined as a systolic blood pressure of >140 mmHg and/or diastolic blood pressure of >90 mmHg, or the current use of antihypertensive medication.
CI, confidence interval; SNP, single nucleotide polymorphism.

Table 3b Allele frequency and odds ratio of the presence of hypertension by genotypes of *PTK2B* polymorphisms by sex

		Men		Women	
SNP (allele frequency)	Genotype group	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
-86282C>A	CC	1	0.426	1	0.288
(0.890/0.110)	CA+AA	1.11 (0.86–1.44)		1.16 (0.88–1.52)	
	CC+CA	1	0.881	1	0.657
	AA	1.07 (0.45–2.51)		1.24 (0.48–3.15)	
-86141G>A	GG	1	0.940	1	0.100
(0.671/0.329)	GA+AA	1.01 (0.81–1.25)		1.20 (0.96–1.50)	
	GG+GA	1	0.470	1	0.305
	AA	1.14 (0.80–1.60)		0.82 (0.57–1.19)	
-22A>G	AA	1	0.006	1	0.819
(0.597/0.403)	AG+GG	1.38 (1.10–1.73)		1.03 (0.82–1.29)	
	AA+AG	1	0.067	1	0.985
	GG	1.31 (0.98–1.74)		1.00 (0.74–1.37)	
22436G>A	GG	1	0.060	1	0.490
(0.820/0.180)	GA+AA	0.80 (0.64–1.01)		0.92 (0.73–1.16)	
	GG+GA	1	0.735	1	0.360
	AA	1.13 (0.56–2.26)		1.36 (0.70–2.63)	
32896T>A	TT	1	0.420	1	0.475
(0.889/0.111)	TA+AA	1.11 (0.86–1.43)		1.10 (0.84–1.44)	
	TT+TA	1	0.691	1	0.487
	AA	1.18 (0.52–2.68)		1.42 (0.53–3.84)	
53484A>C	AA	1	0.031	1	0.554
K838T	AC+CC	1.31 (1.03–1.68)		1.07 (0.85–1.36)	
(0.527/0.473)	AA+AC	1	0.986	1	0.386
	CC	1.00 (0.77–1.30)		0.89 (0.67–1.16)	

CI, confidence interval; SNP, single nucleotide polymorphism.
All adjusted for age, body mass index, antihypertensive drug use, present illness (hyperlipidemia, diabetes mellitus), and lifestyle (smoking and drinking). Hypertension was defined as a blood pressure of >160 mmHg and/or diastolic blood pressure of >95 mmHg, or the current use of antihypertensive medication.

observed at the focal adhesions. Figure 2a and b indicated that the missense mutation, K838T, of PTK2B does not extensively alter the intracellular localization of PTK2B. As shown in Fig. 2c and d, both EGFP-tagged wild-type PTK2B and mutant PTK2B were observed at the cytosol and the immature focal adhesions without the stimulation. After Ang II stimulation, EGFP-tagged wild-type PTK2B and mutant PTK2B were partially located at the mature focal adhesions as reported previously [17] and had similar localization manner. These results indicated that the missense mutation, K838T, of PTK2B does not extensively alter the intracellular localization of PTK2B.

Table 4 Blood pressure levels by genotypes of a *PTK2B* polymorphism, 53484A>C (K838T), in men

	AA	AC	CC	*P*	AA+AC	CC	*P*	AA	AC+CC	*P*
n	459	876	366		1335	366		459	1242	
DBP	78.0±0.5	79.9±0.3	78.9±0.5	0.123	79.2±0.3	78.9±0.5	0.544	78.0±0.5	79.6±0.3	0.003
SBP	130.8±0.8	131.0±0.6	130.5±0.9	0.882	130.9±0.5	130.5±0.9	0.720	130.8±0.8	130.8±0.5	0.921

Values are mean±SD. All adjusted for age, body mass index, antihypertensive drug use, present illness (hyperlipidemia, diabetes mellitus), and lifestyle (smoking and drinking).
DBP, diastolic blood pressure; SBP, systolic blood pressure.
DBP and SBP are expressed in mmHg.

Table 5 Linkage disequilibrium of six genotyped *PTK2B* polymorphisms expressed by r^2 and absolute D'

	−86282 C>A	−86141 G>A	−22 A>G	22436 G>A	32896 T>A	53484 A>C
−86282C>A	–	0.24	0.17	0.02	0.41	0.09
−86141G>A	1.00	–	0.14	0.07	0.07	0.04
−22A>G	0.94	0.42	–	0.13	0.09	0.53
22436G>A	0.79	0.40	0.97	–	0.02	0.02
32896T>A	0.66	0.50	0.68	0.89	–	0.13
53484A>C	0.82	0.27	0.85	0.36	0.94	–

Upper right represents r^2 and lower left shows absolute D'.

Fig. 1

		*	
Human	830	DPMVYMNDKSPLTPEKEV	847
Ape	923	DPMVYMNDKSPLTPEKEV	940
Dog	830	DPMLYMNDKSPLTPEKEA	847
Mouse	830	DPMVYMNDKSPLTPEKEA	847
Rat	788	DPMVYMNDKSPLTPEKEA	805

Amino acid sequences of human, ape, dog, mouse, and rat PTKB2 are aligned. Numbers on left and right side indicate positions of amino acid residues. *K838.

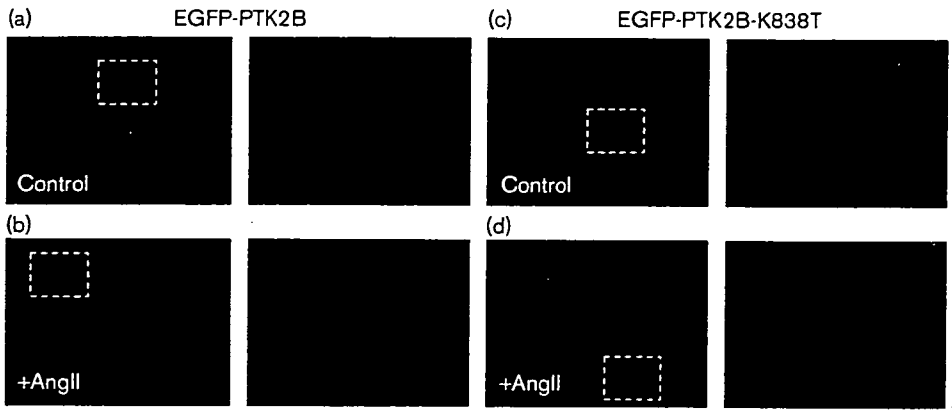
Discussion

In this study, we evaluated *PTK2B* as a candidate for a susceptibility gene for hypertension using population-based case–control samples including 3655 Japanese individuals (1520 patients with hypertension and 2135 controls). The multivariate logistic regression analysis after adjustments for confounding factors showed that −22A > G and 53484A > C (K838T) in *PTK2B* showed an association with the presence of hypertension in men. This association was stronger when hypertension was defined as SBP of ≥ 160 mmHg and/or DBP of ≥ 95 mmHg, or the current use of antihypertensive medication. Both SNPs were in LD with other polymorphisms in *PTK2B*, thus comprising an extensive haplotype block 113 kb in length. Therefore, this extensive haplotype block in *PTK2B* may be an important determinant for hypertension.

PTK2B is involved in the signaling pathways of Ang II and endothelin-1 (ET-1), two important vasoconstrictors, in cardiovascular cells [5,18,19], and nitric oxide, an important vasodilator, inhibited Ang II-induced activation of PTK2B [20]. In addition, PTK2B-mediated Ang II or ET-1-augmented migration and protein synthesis in VSMCs [17,21,22]. The augmented migration and protein synthesis by VSMCs could lead to medial thickening and progressive luminal narrowing of resistant blood vessels and result in hypertension [23,24]. Moreover, VSMCs from spontaneously hypertensive rats exhibited increased cell growth compared with those from normotensive Wistar–Kyoto rats [25], and increased PTK2B activity was involved in this effect [26]. All these results suggest that genetic variations of *PTK2B* influence the net-effects of vasoactive factors on VSMC phenotype and contribute to hypertension. Furthermore, *PTK2B* was originally identified in the human hippocampus and its mRNA was detected mainly in human brain and kidney [27]. An evidence to suggest that Ang II is a neurotransmitter and upregulation of the renin-angiotensin system in brain contributes to hypertension exists [28]. Therefore, an effect of genetic variations of *PTK2B* on the regulation of the cardiovascular system by the central nervous system is expected. Transgenic and knockout techniques for the *PTK2B* gene *in vivo* are necessary to clarify this point.

In this study, we genotyped six SNPs. Therefore, after applying the Bonferroni correction for multiple testing, the level of significance is $P < 0.0083$ (0.05/6 for 6 loci). −22A > G showed a significant association with hypertension in men ($P = 0.006$) even with use of a strict Bonferroni correction, when hypertension is defined as SBP of ≥ 160 mmHg and/or DBP of ≥ 95 mmHg, or the current use of antihypertensive medication. In addition, 53484A > C still showed a significant association with blood pressure levels in men ($P = 0.003$) after the Bonferroni correction. Power analysis also showed that these two SNPs, −22A > G and 53484A > C, had higher power more than 50%, and rest of SNPs did not have

Fig. 2



Fluorescent imaging of wild-type and mutant PTK2B molecules. HUVECs transfected with the plasmids encoding EGFP-PTK2B (a, b) and EGFP-PTK2B K838T (c, d) were starved for 4 h, and stimulated with vehicle (a, c) or 1 μmol/l Ang II for 5 min (b, d). Right side images of each panel show magnified view of the area in squares. HUVECs, human umbilical vascular endothelial cells.

power above 50%. Thus, *PTK2B* is speculated to be a susceptibility gene for hypertension.

The mechanisms by which the two SNPs (−22A > G and 53484A > C) might be associated with hypertension in men only are unknown. No association in women was observed. This inconsistency might be derived from sex differences. Regarding sex differences, the incidence and rate of progression of hypertension was markedly higher in men than in age-matched premenopausal women and, after menopause, this relationship no longer existed [29]. In various hypertensive animal models, males showed higher blood pressure levels than females owing to greater levels of Ang II-NADPH oxidase-mediated upregulation of the production of reactive oxygen species [30,31], Ang II-induced enhancement of sympathetic nerve activity [32], decreased nitric oxide production [33], and a high ratio of AT1/AT2 receptors of Ang II [34]. In addition, the elevation in blood pressure after the administration of ET-1 was much higher in male rats than in female rats [35], because estrogen might reduce ET-1-induced vasoconstriction [36]. As PTK2B is involved in the signaling pathways of Ang II and ET-1 and nitric oxide inhibits Ang II-induced activation of PTK2B [20], sex differences in the relationship between *PTK2B* polymorphisms and hypertension may be ascribed to the influences of these vasoactive factors.

The missense mutation K838T seemed to be important to the function of PTK2B. We expressed the mutant protein in mammalian cells and examined its intracellular localization by fluorescence imaging. It was clear that the mutant did not show extensive changes in terms of localization even after Ang II stimulation. This imaging, however, only looked at the localization. We have to examine the kinase activity of the mutant protein in a future study. In addition, −22A > G and SNPs in LD with 53484A > C (−99G > A, rs919495, rs11776858, rs11785606, rs10109834, and rs3735759) are present in the 5′-untranslational region. Whether they could influence *PTK2B* gene expression needs to be clarified.

In summary, we have found an association between hypertension and SNPs of *PTK2B*. Association-based studies are not consistently reproducible due to false positive results, false negative results, or true variability in associations among different populations [37]. Therefore, confirmation of the result in additional cohorts may be required.

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ORIGINAL ARTICLE

Association of single nucleotide polymorphisms in endothelin family genes with the progression of atherosclerosis in patients with essential hypertension

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Endothelin-1 (ET-1) is a potent vasoconstrictive peptide and its activity is mediated by the receptors ET type A (EDNRA) and ET type B (EDNRB). Although ET-1 is thought to play an important role in the development of atherosclerosis, it remains unclear whether polymorphisms of ET-1 family genes, including the ET-1 gene (*EDN1*), *EDNRA*, *EDNRB* and the genes for endothelin converting enzymes 1 and 2 (*ECE1* and *ECE2*), are associated with the progression of atherosclerosis. We investigated the relationship between 11 single nucleotide polymorphisms (SNPs) of ET-1 family genes (including three in *EDN1*, one in *EDNRA*, two in *EDNRB*, four in *ECE1* and one in *ECE2*) and atherosclerotic changes assessed using pulse wave velocity (PWV) and carotid ultrasonography in 630 patients with essential hypertension (EHT). In male subjects, we found significant differences in brachial-ankle PWV (baPWV) in additive and recessive models in *EDNRB*-rs5351 after Bonferroni correction. Also in male subjects, there were significant differences in mean intima-media thickness (IMT) in additive and recessive models in *EDNRA*-rs5333 after Bonferroni correction. We found no significant correlation between any SNPs in the ET family genes and baPWV, IMT and Plaque score (PS) in female subjects. Furthermore, after multiple logistic regression analysis, only *EDNRB*-rs5351 indicated as an independent risk of atherosclerosis in male hypertensive subjects. Of the endothelin-related genes, *EDNRB*-rs5351 was the most susceptible SNP associated with atherosclerosis in male hypertensives, and the genetic background may be involved in the progression of atherosclerosis in EHT patients.
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Keywords: endothelin 1 (ET-1) family genes; single nucleotide polymorphisms (SNPs); atherosclerosis

Introduction

Endothelin-1 (ET-1) is a potent vasoconstrictive peptide produced primarily by vascular endothelial cells and appearing in many other organs.[1] ET-1 is thought to play an important role in the development of atherosclerosis through endothelial dysfunction and the proliferation of vascular smooth muscle cells (VSMCs). ET-1 may be a marker for arterial vascular disease; Lerman *et al.*[2] showed a significant correlation between plasma endothelin levels and the number of vascular disease sites. Some reports have linked plasma levels of ET-1 to hypertension, while others have argued against this relationship. Hirai *et al.*[3] suggested that high ET-1 levels are not related to hypertension, but rather to subclinical renal dysfunction and smoking. The expression of ET-1 is mediated by the activation of specific receptors: ET type A (EDNRA) and ET type B (EDNRB). The former is the predominant ET receptor on VSMCs, and signalling via EDNRA causes long-lasting vasoconstriction.[4,5] EDNRB is located primarily on endothelial cells and its signalling promotes the formation of nitric oxide, as well as the clearance and reuptake of ET-1.[6–9] Endogenous ET-1, which acts via EDNRA, increases resistance-vessel tone in subjects with hypertension to a level greater than that in smokers and in subjects with hypercholesterolemia.[10] Plasma ET-1

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Table 3b Comparisons between ET-1 gene SNPs and baPWV in female subjects

Genes	*SNPs*	*Allele1/Allele2*		n	*baPWV (cm/s)*	P-*dominant*	P-*additive*	P-*recessive*
EDN1	A201- (4A/3A)	3A/4A	3A3A	198	1831.0±329.4	0.4510	0.7278	0.9152
			3A4A	84	1798.0±305.9			
			4A4A	5	1836.6±199.9			
	rs2070699	T/G	TT	80	1845.0±367.1	0.4673	0.7631	0.7183
			TG	139	1816.2±305.3			
			GG	69	1810.8±289.1			
	rs5370	G (Lys)/T(Asn)	GG	147	1843.9±321.8	0.2519	0.4711	0.5298
			GT	116	1795.2±316.4			
			TT	26	1825.8±319.9			
EDNRA	rs5333	T/C	TT	153	1796.2±306.1	0.1163	0.1601	0.5298
			TC	116	1867.4±331.3			
			CC	17	1776.6±342.9			
EDNRB	rs 5351	A/G	AA	85	1859.2±315.1	0.2257	0.3921	0.3211
			AG	145	1817.9±339.8			
			GG	56	1785.8±268.3			
	rs3818416	G/T	GG	255	1822.4±325.2	0.8168	0.3676	(–)
			GT	29	1821.6±270.6			
			TT	1	2277.0			
ECE1	rs212526	C/T	CC	208	1827.0±308.5	0.7909	0.4074	0.1873
			CT	67	1835.0±360.3			
			TT	11	1698.1±257.9			
	rs212528	T/C	TT	184	1833.1±320.8	0.5150	0.4206	0.3855
			TC	86	1791.7±307.6			
			CC	16	1891.4±371.4			
	rs213045	G/T	GG	93	1834.6±369.1	0.6899	0.4138	0.2837
			GT	142	1801.0±281.4			
			TT	50	1867.8±326.9			
	rs2038089	A/G	AA	124	1821.2±322.9	0.8902	0.9691	0.8109
			AG	131	1824.3±321.9			
			GG	24	1839.2±323.7			
ECE2	rs2272471	C/T	CC	73	1795.8±343.4	0.3612	0.5926	0.4611
			CT	144	1828.5±314.6			
			TT	68	1850.3±304.4			

Abbreviations: baPWV, brachial-ankle pulse wave velocity; SNPs, single nucleotide polymorphisms.
P-value (dominant); major vs hetero+minor, *P*-value (additive); major vs heterozygote vs minor, *P*-value (recessive); minor+hetero vs major.

Screening of genetic variations in EDN1 EDNRA, EDNRB, ECE1 and ECE2

We isolated genomic DNA from the peripheral blood leukocytes of 630 subjects and directly sequenced the entire coding region of the endothelin-1 gene (*EDN1*). The results of the *EDN1* screening are shown in Table 1. Finally, we selected three SNPs in the *EDN1*. We selected SNPs of the endothelin type A receptor gene (*EDNRA* rs5333), endothelin type B receptor gene (*EDNRB* rs5351, rs3818416), endothelin converting enzyme-1 gene (*ECE1* rs212526, rs212528, rs213045, rs2038089) and endothelin converting enzyme-2 gene (*ECE2* rs2272471) from a public database (dbSNP http://www.ncbi.nlm.nih.gov/SNP/). SNPs with a minor allele frequency of greater than 5% were genotyped using the TaqMan-PCR method described previously.[16] The representative SNPs were genotyped when they were linkage disequilibrium (LD: r^2 over 0.5). The LD was calculated between each SNP. The primers and probes used in the TaqMan-PCR system are available upon request.

Statistical analysis

Values are expressed as means ±s.d. and were analyzed using a Student's *t*-test and a χ^2-test where appropriate. Hardy–Weinberg equilibrium was assessed by χ^2analysis, and we considered *P*-values less than 0.05 to be statistically significant. The levels of the *P*-values were adjusted by Bonferroni correction). The LD between each SNP was checked using Haploview version 4 (http://www.broad.mit.edu/mpg/haploview/). The association of genotypes with blood pressure, IMT and PS of carotid arteries and baPWV was examined by simple regression analysis and then investigated using a logistic regression model that adjusted for confounding factors. The distribution of plaque score (PS) was not normal, so we compared the prevalence of severe PS ($\geqslant$10.1)[17] for each allele. All statistical analyses were performed using Stat-View version 5.0 (SAS Institute Inc., Cary, NC, USA).

Results

Patient Characteristics and the Correlation between baPWV and Clinical Parameters

The characteristics of the subjects at baseline are summarized in Table 2. Significant differences were apparent between men and women in age, height, weight, heart rate (HR), systolic blood pressure (SBP), plaque score (PS), baPWV and ABI and lipid

Table 4a Comparisons between ET-1 gene SNPs and mean IMT in male subjects

Genes	*SNPs*	*Allele1/Allele2*		n	*Mean IMT*	P-*dominant*	P-*additive*	P-*recessive*
EDN1	A201- (4A/3A)	3A/4A	3A3A	250	0.824±0.160	0.7936	0.1199	0.0400 (0.4400)*
			3A4A	81	0.821±0.152			
			4A4A	5	0.970±0.148			
	rs2070699	T/G	TT	104	0.815±0.143	0.3957	0.6548	0.5235
			TG	157	0.829±0.163			
			GG	76	0.837±0.170			
	rs5370	G (Lys)/T(Asn)	GG	181	0.832±0.154	0.4286	0.5953	0.7007
			GT	134	0.815±0.162			
			TT	23	0.838±0.174			
EDNRA	rs5333	T/C	TT	181	0.821±0.160	0.4330	0.0023 (0.0253)*	0.0005 (0.0055)*
			TC	130	0.816±0.146			
			CC	23	0.937±0.179			
EDNRB	rs 5351	A/G	AA	107	0.830±0.164	0.8131	0.0104 (0.1144)*	0.0059 (0.0649)*
			AG	161	0.805±0.147			
			GG	65	0.875±0.168			
	rs3818416	G/T	GG	304	0.828±0.161	0.5352	0.7307	0.5119
			GT	28	0.814±0.130			
			TT	3	0.767±0.161			
ECE1	rs212526	C/T	CC	246	0.832±0.161	0.3202	0.5493	0.4919
			CT	82	0.786±0.157			
			TT	7	0.814±0.152			
	rs212528	T/C	TT	198	0.826±0.156	0.9406	0.9714	0.8410
			TC	121	0.828±0.165			
			CC	16	0.819±0.153			
	rs213045	G/T	GG	102	0.831±0.179	0.7299	0.6596	0.3631
			GT	174	0.829±0.154			
			TT	58	0.809±0.134			
	rs2038089	A/G	AA	152	0.830±0.161	0.7842	0.8860	0.6774
			AG	138	0.828±0.144			
			GG	43	0.816±0.195			
ECE2	rs2272471	C/T	CC	93	0.820±0.160	0.6244	0.8500	0.9127
			CT	164	0.832±0.161			
			TT	76	0.826±0.153			

Abbreviations: SNPs, single nucleotide polymorphisms; IMT, intima-media thickness.
P-value (dominant), major vs hetero+minor; *P*-value (additive), major vs heterozygote vs minor; *P*-value (recessive), minor+hetero vs major.
*Bonferroni correction (× 11).

profiles. Almost all subjects were treated with antihypertensive agents such that the ratio of treated patients did not differ between males and females.

We analyzed the correlations between baPWV, mean IMT, max IMT, PS of carotid arteries and the clinical parameters of male and female patients. BaPWV significantly correlated with age, height, weight, SBP, DBP, mean BP, HR and HbA_{1c}. In contrast, baPWV was not associated with serum creatinine, C-reactive protein, or ABI. The mean IMT and PS of carotid arteries significantly correlated with age, height, HbA_{1c} and HDL-Chol. All indices of atherosclerosis strongly associated with age, height and BP. Among these indices, IMT and PS showed weaker association with weight and BP than baPWV.

Correlation between baPWV and SNPs in ET-1 family genes

We studied 11 SNPs in total, including three of *EDN1*, one of *EDNRA*, two of *EDNRB*, four of *ECE1* and one of *ECE2*. We found no tight LD between the 11 analyzed SNPs. We analyzed the association of baPWV with ET-1 SNPs in all subjects, both male and female. As shown in Table 3a, we detected significant differences in baPWV in comparing additive, dominant, or recessive models in *EDNRB*-rs5351 (exon 6), *ECE1*- rs212528 (intron 3) and rs2038089 (intron 17) in male subjects. Finally, only *EDNRB* -rs5351 positively associated with baPWV after performing a Bonferroni correction. No SNPs were significantly associated with baPWV in female subjects (Table 3b).

Mean IMT, max IMT, plaque score of carotid arteries and ET-1 SNPs

The results of comparing additive, dominant, or recessive models for mean IMT in each SNP are shown in Tables 4a and 4b. Only *EDNRA*-rs5333 positively associated with mean IMT after performing a Bonferroni correction, and this association was only apparent in male subjects. With regard to max-IMT, *EDNRA*-rs5333, *EDNRB* -rs5351 and *ECE1*- rs2038089 showed a positive association, but the association was not significant after Bonferroni correction (data not shown). In comparing the prevalence of severe PS ($\geqslant$10.1) for each allele, no

concentrations can be reduced by resistance training and aerobic exercise.[11]

Pulse wave velocity (PWV) is generally recognized as a surrogate marker for atherosclerosis.[12] Using sheep, McEniery *et al.*[13] showed that endogenous ET-1 production regulates large artery PWV *in vivo*. They also revealed that exogenous ET-1 increases PWV and that this increase can be blocked by ET type A receptor blockers. Vuurmans *et al.*[14] examined whether ET-1 increases central aortic systolic blood pressure, pulse pressure and PWV in healthy men, and the effect of ET-1 is prevented by ET-1 receptor blockers.

It remains unclear, however, whether gene polymorphisms of the ET-1 family (including the ET-1 gene (*EDN1*), *EDNRA*, *EDNRB* and the genes for endothelin converting enzymes 1 and 2 (*ECE1* and *ECE2*) are associated with the progression of atherosclerosis. Therefore, we investigated the relationship between single nucleotide polymorphisms (SNPs) of ET-1 family genes and atherosclerotic changes assessed by PWV and carotid echo ultrasonography in patients with essential hypertension (EHT).

Materials and methods

Subjects

This study included 630 outpatients (340 men and 290 women) with EHT at the Division of Hypertension and Nephrology of the National Cardiovascular Centre (NCVC). All subjects provided written informed consent and the protocol was approved by the ethics committee of NCVC. Hypertension was defined as a systolic blood pressure (SBP) of 140 mm Hg or greater and/or a diastolic blood pressure (DBP) of 90 mm Hg or greater, or the current use of antihypertensive medication. The blood pressure used was the average of at least three measurements made during each visit. We also measured brachial-ankle PWV (baPWV) using Form ABI (Colin Medical Technology) and examined carotid arteries using a commercially available ultrasound system (SSA-390A; Toshiba Medical, Japan).[4] We measured the mean intima-media thickness (IMT) and maximum-IMT (max-IMT) of common carotid arteries and the sum of the plaque score (PS) of bilateral common and internal carotid arteries, as reported previously.[15] Blood samples were also taken at the clinic, and diabetes mellitus was defined as a fasting blood sugar level greater than 126 mg/dl, an HbA_{1c} level greater than 6.5%, or the use of anti-hyperglycemic medications. Hyperlipidemia was defined as a total-cholesterol concentration of 220 mg/dl or greater, a triglyceride (TG) concentration of 150 mg/dl or greater, or the use of lipid-lowering medication at the time of the first examination. Subjects who had ankle-brachial indices (ABI) lower than 0.9 were excluded because their baPWV readings were unreliable.

Table 1 The entire coding region of the endothelin-1 gene

		Allele 1/Allele 2			*Allele 1*			*Allele 2*		*Allele frequency*		*Flanking sequence*	*dbSNP ID*
Gene name	*Locus*	*SNPs*	*Aa info.*	*Region*	*Homo*	*Hetero*	*Homo*	*Total*		*Allele 1*	*Allele 2*		
EDN1	6p24-p23	10bp del.(−173)		promoter	47	1	0	48		0.990	0.010	AGGTTTAGCAAA[GGTCTCTAAT/-]GGGTATTTTCTT	
		A201- (4A/3A)	5′-UTR	exon1	1	8	39	48		0.104	0.896	TTTCTCCCCGTT[A/-]AAAGGGCACTTG	
		G2087A	Gly36Arg	exon2	47	1	0	48		0.990	0.010	GGTGAGAACGGC[G/A]GGGAGAAACCCA	
		G2244T		intron2	8	18	21	47		0.362	0.638	ATTGTAACCCTA[G/T]TCATTCATTAGC	rs2070699
		T2252A		intron2	46	1	0	47		0.989	0.011	CCTAGTCATTCA[T/A]TAGCGCTGGCTC	
		T3609C		intron2	33	12	2	47		0.830	0.170	AAGACTATTAAT[T/C]ACACTAATATAG	rs1800543
		A3730G	Glu106Glu	exon3	0	1	46	47		0.011	0.989	AACAGACCGTGA[A/G]AATAGATGCCAA	rs 5369
		T5629A		intron4	47	1	0	48		0.990	0.010	GGGTGATTTTTT[T/A]AAAATAACATTT	
		G5727T	Lys198Asn	exon5	31	14	2	47		0.809	0.191	GCTGAAAGGCAA[G/T]CCCTCCAGAGAG	rs 5370

Abbreviation: SNPs, single nucleotide polymorphisms.
By Gene Cards. Version: 2.25, released 3 July, 2002.

Table 2 Subject characteristics

	All (n = *630*)	*Male* (n = *340*)	*Female* (n = *290*)	P-*value*
Age (years)	64.6±10.6	63.3±11.3	66.0±9.6	0.0015
Height (cm)	160.0±8.7	165.8±6.4	153.1±5.5	<0.0001
Weight (kg)	62.9±11.6	68.5±10.6	56.4±9.1	<0.0001
Heart rate (b.p.m.)	64.0±10.7	62.0±9.4	66.2±11.8	<0.0001
Systolic blood pressure (mm Hg)	138.8±17.1	137.0±15.8	140.9±18.3	0.0042
Diastolic blood pressure (mm Hg)	82.7±10.3	83.2±10.2	82.1±10.5	0.1799
Mean IMT (mm)	0.83±0.16	0.83±0.16	0.84±0.17	0.4634
Plaque score	3.13±4.76	3.57±5.18	2.61±4.17	0.0131
baPWV (cm/s)	1786.2±309.1	1755.7±297.7	1822.0±318.8	0.0071
ABI	1.12±0.08	1.13±0.09	1.11±0.07	0.0018
CRP (mg/dl)	0.15±0.28	0.17±0.20	0.14±0.30	0.1728
HbA_{1c} (%)	5.63±0.80	5.66±0.77	5.58±0.83	0.2259
Total cholesterol (mg/dl)	203.0±35.2	196.7±30.4	210.4±39.0	<0.0001
Triglyceride (mg/dl)	138.3±125.3	152.4±149.7	121.5±85.3	0.0020
HDL-cholesterol (mg/dl)	52.7±15.2	48.7±13.0	57.4±16.3	<0.0001
Smoking (current/past/never)	69/211/339	59/183/89	10/28/250	<0.0001
Anti-hypertensive medication (%)	570/630 (90.5%)	308/340 (90.6%)	262/290 (90.3%)	0.9174

Abbreviations: ABI, ankle brachial index; baPWV, brachial-ankle pulse wave velocity; CRP, C-reactive protein; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; Mean IMT, mean intima-media thickness.
Values are expressed as the means±s.d. *P* ; Student's *t*-test (male vs female).

Table 3a Comparison between SNPs of ET-1 genes and baPWV in male subjects

Genes	*SNPs*	*Allele1/Allele2*		n	*baPWV (cm/s)*	P-*dominamt*	P-*additive*	P-*recessive*
EDN1	A201- (4A/3A)	3A/4A	3A3A	251	1763.8±301.0	0.4250	0.6509	0.7682
			3A4A	81	1730.3±291.5			
			4A4A	5	1795.3±271.3			
	rs2070699	T/G	TT	104	1768.1±341.2	0.6029	0.3509	0.2902
			TG	158	1731.8±265.6			
			GG	76	1787.3±297.1			
	rs5370	G(Lys)/T(Asn)	GG	182	1759.6±308.5	0.8425	0.8318	0.5438
			GT	134	1758.8±286.2			
			TT	23	1720.2±284.5			
EDNRA	rs5333	T/C	TT	182	1746.8±307.0	0.5958	0.4479	0.2086
			TC	130	1752.5±269.4			
			CC	23	1830.1±369.9			
EDNRB	rs 5351	A/G	AA	107	1706.6±285.1	0.0409 (0.4499)*	0.0004 (0.0044)*	0.0001 (0.0011)*
			AG	162	1736.1±277.7			
			GG	65	1882.2±332.7			
	rs3818416	G/T	GG	305	1759.9±301.1	0.2393	0.3593	0.2593
			GT	28	1708.0±260.2			
			TT	3	1560.5±241.2			
ECE1	rs212526	C/T	CC	247	1746.9±294.9	0.4798	0.7583	0.9557
			CT	82	1775.1±298.4			
			TT	7	1747.6±415.2			
	rs212528	T/C	TT	198	1724.6±292.4	0.0311 (0.3421)*	0.0246	0.3099
			TC	122	1810.8±298.3			
			CC	16	1679.9±308.2			
	rs213045	G/T	GG	102	1732.0±282.7	0.3865	0.3293	0.3737
			GT	174	1776.5±305.3			
			TT	59	1722.0±301.3			
	rs2038089	A/G	AA	153	1773.4±300.4	0.3051	0.0821	0.0262 (0.2882)*
			AG	138	1764.3±304.3			
			GG	43	1661.1±253.9			
ECE2	rs2272471	C/T	CC	94	1778.0±303.1	0.3573	0.6116	0.9717
			CT	164	1739.8±282.3			
			TT	76	1755.1±324.4			

Abbreviations: baPWV, brachial-ankle pulse wave velocity; SNPs, single nucleotide polymorphisms.
P-value (dominant), major vs hetero+minor; *P*-value (additive), major vs heterozygote vs minor; *P*-value (recessive), minor+hetero vs major.
*Bonferroni correction (× 11).

Table 4b Comparisons between ET-1 gene SNPs and mean IMT in female subjects

Genes	*SNPs*	*Allele1/Allele2*		n	*Mean IMT*	P-*dominant*	P-*additive*	P-*recessive*
EDN1	A201- (4A/3A)	3A/4A	3A3A	196	0.841±0.175	0.6400	0.1625	0.0706
			3A4A	83	0.828±0.152			
			4A4A	5	0.700±0.079			
	rs2070699	T/G	TT	79	0.839±0.170	0.8018	0.1429	0.0546
			TG	137	0.849±0.174			
			GG	69	0.801±0.152			
	rs5370	G (Lys)/T(Asn)	GG	144	0.835±0.168	0.9802	0.3176	0.1531
			GT	116	0.846±0.172			
			TT	26	0.835±0.168			
EDNRA	rs5333	T/C	TT	152	0.826±0.156	0.3386	0.0463 (0.5093)*	0.1527
			TC	115	0.858±0.182			
			CC	17	0.759±0.139			
EDNRB	rs 5351	A/G	AA	84	0.836±0.152	0.9740	0.9909	0.9095
			AG	144	0.834±0.171			
			GG	56	0.837±0.186			
	rs3818416	G/T	GG	253	0.830±0.167	0.1547	0.2753	(–)
			GT	29	0.872±0.178			
			TT	1	1.000			
ECE1	rs212526	C/T	CC	206	0.834±0.168	0.9034	0.6696	0.4238
			CT	67	0.844±0.169			
			TT	11	0.795±0.159			
	rs212528	T/C	TT	183	0.831±0.158	0.5586	0.5997	0.5508
			TC	86	0.849±0.182			
			CC	15	0.810±0.205			
	rs213045	G/T	GG	92	0.826±0.162	0.5215	0.7194	0.4975
			GT	141	0.836±0.162			
			TT	50	0.850±0.196			
	rs2038089	A/G	AA	126	0.818±0.166	0.1171	0.2132	0.2300
			AG	130	0.845±0.170			
			GG	24	0.875±0.174			
ECE2	rs2272471	C/T	CC	71	0.847±0.186	0.5061	0.0360 (0.3960)*	0.0327 (0.3597)*
			CT	144	0.812±0.154			
			TT	68	0.874±0.171			

Abbreviations: SNPs, single nucleotide polymorphisms; IMT, intima-media thickness.
P-value (dominant), major vs hetero+minor; *P*-value (additive), major vs heterozygote vs minor, *P*-value (recessive), minor+hetero vs major.
*Bonferroni correction (× 11).

SNPs were positive in either male or female subjects after Bonferroni correction (Table 5).

Association of SNPs in ET-1 family genes with severe atherosclerosis

We have compared the atherosclerosis parameters and the background of each genotype of *EDNRA*-rs5333, *EDNRB*-rs5351, *ECE1*-rs212528 and rs2038089. These four SNPs showed significant association with atherosclerotic indices, including baPWV, PS and IMT. They had no association with atherosclerotic risk factors, such as HbA1c, TG, HDL-chol, except *EDNRA*-rs5333 which showed association with TG.

We divided the male subjects in three ways: a rapid or slow group based on the average baPWV (rapid: $\geqslant$1756 cm/s, slow: $<$1756 cm/s), averaged mean-IMT (severe: $\geqslant$0.86 mm, mild: $<$0.86 mm) and a severe or mild atherosclerotic group using plaque scores (severe: $\geqslant$10.1, mild: $<$10.1). We performed logistic regression analysis on the progression of baPWV, mean-IMT and PS. Multiple logistic regression analysis indicated that GG in *EDNRB* -rs5351, with higher baPWV and PS, was an independent risk factor in male subjects (Tables 6a–c).

Discussion

The human ET-1 gene was cloned and sequenced in 1989 by Inoue *et al.*[18] Recent studies have examined the relationship between polymorphisms of ET-1 and BP. Tiret *et al.*[19] indicated that a G/T polymorphism with an amino acid substitution (Lys → Asn) at codon 198 in exon 5 of ET-1 was associated with BP in overweight Europeans, and similar results were obtained in Japanese subjects.[20,21]

In this study, we evaluated the association of 11 SNPs of ET-1 family genes with atherosclerosis in hypertensive patients. We found a significant correlation between baPWV and *EDNRB*-rs5351 and between mean IMT of carotid arteries and *EDNRA*-rs5333 in male, but not female hypertensive patients after Bonferroni correction; however, EDNRA-rs5333 was not significantly associated with severe IMT thickening after multiple logistic regression analysis. Thus, *EDNRB*-rs5351 was the most suscep-

Table 5 Genotype distribution among the subjects with severe (≥10.1) or mild atherosclerosis (by plaque scores)

				Male				*Female*			
Genes	*SNPs*	*Allele (major/minor)*	*Genotype*	*Mild*	*Severe*	χ^2	*P-value*	*Mild*	*Severe*	χ^2	*P-value*
EDN1	A201- (4A/3A)	3A/4A	3A3A	223	22	0.970	0.6156	184	11	2.668	0.2635
			3A4A	71	9			80	3		
			4A4A	4	1			4	1		
	rs2070699	T/G	TT	92	10	0.275	0.8714	74	4	1.660	0.4361
			TG	143	14			131	6		
			GG	64	8			63	6		
	rs5370	G (Lys)/T(Asn)	GG	161	18	0.082	0.9600	136	7	1.733	0.4205
			GT	120	12			109	7		
			TT	19	2			23	3		
EDNRA	rs5333	T/C	TT	160	19	0.906	0.6357	145	7	1.733	0.4204
			TC	116	10			106	8		
			CC	20	3			15	2		
EDNRB	rs 5351	A/G	AA	99	4	9.898	0.0071 (0.0781)*	79	4	2.740	0.2541
			AG	144	16			137	7		
			GG	52	12			50	6		
	rs3818416	G/T	GG	269	29	0.354	0.8376	237	15	0.105	0.9487
			GT	25	3			27	2		
			TT	3	0			1	0		
ECE1	rs212526	C/T	CC	217	23	0.171	0.9179	192	13	0.744	0.6894
			CT	74	8			63	4		
			TT	6	1			11	0		
	rs212528	T/C	TT	180	16	1.354	0.5082	171	11	1.102	0.5763
			TC	103	14			80	6		
			CC	14	2			15	0		
	rs213045	G/T	GG	92	8	1.447	0.4851	88	4	1.569	0.4564
			GT	154	16			130	11		
			TT	50	8			47	2		
	rs2038089	A/G	AA	126	23	9.901	0.0071 (0.0781)*	121	4	3.383	0.1843
			AG	130	7			119	11		
			GG	39	2			23	1		
ECE2	rs2272471	C/T	CC	82	7	4.258	0.1190	66	5	1.914	0.3840
			CT	150	13			137	6		
			TT	63	12			62	6		

Abbreviation: SNPs, single nucleotide polymorphisms.
Men and women were divided into three groups for each genotype.
*Bonferroni correction (× 11).

tible endothelin-related SNP associated with atherosclerosis in male hypertensives. With regard to the gender differences between baPWV and/or arteriosclerosis and ET-1 family gene polymorphisms, one possible explanation is that the effect of ET-1 on vasoconstriction and atherosclerosis may differ between males and females. Tatchum-Talom *et al.*[22] described the vasoconstrictive effect of ET-1 as much greater in male rats than in female rats. Alternatively, oestrogen may reduce the vasoconstriction induced by ET-1.[23] Our current findings indicate that there are differences in the progression of atherosclerotic changes among hypertensive patients that depend on the genotypes of ET-1 family genes. Therefore, our findings provide important information regarding the use of hypertensive agents. Hypertensive agents should perhaps be prescribed after taking the polymorphisms in specific patients into consideration.

Lajemi *et al.*[24] showed that the *EDNRA* -231A/G and *EDNRB* 30G/A gene polymorphisms influence PWV in women, and the *EDNRB* 30G/A genotype related to the level of radial artery parameters in men. They suggested that these genes were involved in arterial stiffness. Funalot *et al.*[25] showed that *ECE 1B* C338A and *EDN1* Lys198Asn work together to modulate BP levels in women. Of the three SNPs tested in this study, only *EDNRB*-rs5351 was associated with baPWV and PS. This may be because baPWV reveals a more functional change, while PS indicates a more structural change.

In this study, most patients were treated with antihypertensive agents, some of which might affect PWV either directly or indirectly. We did not have detailed information on the drugs being taken by each subject, which could be seen as a limitation on our study. However, it has been reported that evaluations of PWV for monitoring arterial stiffness and in developing risk assessment strategies for hypertensive patients are useful.[26]

It will be important to determine serum ET-1 levels in patients to examine whether the cause of differences in baPWV or carotid arteriosclerosis between genotypes is dependent on only the