Association of polymorphisms of *ABCA1* and *ROS1* with hypertension in Japanese individuals

YOSHIJI YAMADA[1], KIMIHIKO KATO[2], TETSURO YOSHIDA[2], KIYOSHI YOKOI[2], HITOSHI MATSUO[3], SACHIRO WATANABE[3], SAHOKO ICHIHARA[1], NORIFUMI METOKI[4], HIDEMI YOSHIDA[5], KEI SATOH[5], YUKITOSHI AOYAGI[6], AKITOMO YASUNAGA[6], HYUNTAE PARK[6], MASASHI TANAKA[6] and YOSHINORI NOZAWA[7]

[1]Department of Human Functional Genomics, Life Science Research Center, Mie University, Tsu; [2]Department of Cardiovascular Medicine, Gifu Prefectural Tajimi Hospital, Tajimi; [3]Department of Cardiology, Gifu Prefectural General Medical Center, Gifu; [4]Department of Internal Medicine, Hirosaki Stroke Center, Hirosaki; [5]Department of Vascular Biology, Institute of Brain Science, Hirosaki University School of Medicine, Hirosaki; [6]Department of Genomics for Longevity and Health, Tokyo Metropolitan Institute of Gerontology, Tokyo; [7]Gifu International Institute of Biotechnology, Kakamigahara, Japan

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Abstract. Although various environmental factors, such as a high-salt diet, a smoking habit, excessive alcohol intake, and physical inactivity, influence the development of hypertension, genetic variation also contributes to an individual's susceptibility to this condition. The purpose of the present study was to identify gene polymorphisms that confer susceptibility or resistance to hypertension, and thereby contribute to the prediction of the genetic risk for this condition. The study population comprised 2752 unrelated Japanese individuals (1370 men, 1382 women), including 1276 subjects with hypertension (774 men, 502 women) and 1476 controls (596 men, 880 women). The genotypes for 50 polymorphisms of 35 candidate genes were determined by a method that combines polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology. Evaluation of genotype distributions by the Chi-square test and subsequent multivariable logistic regression analysis with adjustment for age, sex, body mass index, smoking status, and the prevalence of diabetes mellitus and hypercholesterolemia revealed that the -14C→T polymorphism of *ABCA1*, the C→G (Ser2229Cys) polymorphism of *ROS1*, the C→T (Asn591Asn) polymorphism of *LDLR*, the 13989A→G (Ile118Val) polymorphism of *CYP3A4*, the C→G and A→C polymorphisms of *ADIPOR1*, and the -519A→G polymorphism of *MMP1* were significantly ($P<0.05$) associated with the prevalence of hypertension. Systolic and diastolic blood pressure differed significantly among genotypes for the -14C→T polymorphism of *ABCA1* and the C→G (Ser2229Cys) polymorphism of *ROS1*, with the variant *T* and *G* alleles, respectively, being related to increased blood pressure. These results suggest that polymorphisms of *ABCA1* and *ROS1* are determinants of blood pressure and the development of hypertension in Japanese individuals. Determination of genotypes for *ABCA1* and *ROS1* may thus prove informative for the prediction of the genetic risk for hypertension.

Correspondence to: Dr Yoshiji Yamada, Department of Human Functional Genomics, Life Science Research Center, Mie University, 1577 Kurima-machiya, Tsu, Mie 514-8507, Japan
E-mail: yamada@gene.mie-u.ac.jp

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Introduction

Hypertension is a complex multifactorial disorder that is thought to result from an interaction between an individual's genetic background (1) and various environmental factors, including a high-salt diet, a smoking habit, excessive alcohol intake, and physical inactivity. Given that hypertension is a major risk factor for stroke, coronary heart disease, heart failure, and end-stage renal disease, personalized prevention of hypertension is an important public health goal. One approach to personalized prevention of, and selection of the most appropriate treatment for, hypertension is to identify genes that confer susceptibility to this condition (2). Although genetic linkage analyses (3-6) and candidate gene association studies (7-12) have implicated various loci and genes in predisposition to hypertension, the genes that confer susceptibility to this condition remain to be identified definitively. In addition, because of ethnic differences in genetic variants as well as in environmental factors and lifestyle, it is important to examine polymorphisms related to hypertension in each ethnic group.

We performed a large-scale association study of 50 candidate gene polymorphisms and hypertension in 2752 Japanese individuals. The purpose of the present study was to

identify genetic variants that confer susceptibility to hypertension thereby contributing to the prediction of the genetic risk for this condition.

Materials and methods

Study population. The study population comprised 2752 unrelated Japanese individuals (1370 men, 1382 women) who either visited outpatient clinics of or were admitted to one of the participating hospitals (Gifu Prefectural General Medical Center and Gifu Prefectural Tajimi Hospital in Gifu Prefecture, Japan; Hirosaki University Hospital, Reimeikyo Rehabilitation Hospital, and Hirosaki Stroke Center in Aomori Prefecture, Japan) between October 2002 and March 2007 because of various symptoms or for an annual health checkup, or who were recruited to a population-based prospective cohort study of aging and age-related diseases in Gunma Prefecture, Japan. The 1276 subjects (774 men, 502 women) with hypertension had a systolic blood pressure of ≥160 mmHg or a diastolic blood pressure of ≥95 mmHg (or both) before medical treatment. Individuals with valvular heart disease, congenital malformations of the heart or vessels, or renal or endocrinologic diseases that cause secondary hypertension were excluded from the study. The control subjects comprised a total of 1476 individuals (596 men, 880 women) who either visited the outpatient clinics of the participating hospitals for an annual health checkup or who were community-dwelling individuals recruited to the prospective cohort study. They had normal blood pressure (systolic blood pressure of <140 mmHg and diastolic blood pressure of <90 mmHg) and no history of hypertension or of taking antihypertensive medication. The hypertensive and control individuals either had or did not have diabetes mellitus, hypercholesterolemia, or obesity. Blood pressure was measured at least twice with subjects having rested in a sitting position for >5 min; the measurements were taken by a skilled physician according to the guidelines of the American Heart Association (13). The study protocol complied with the Declaration of Helsinki and was approved by the Committees on the Ethics of Human Research of Mie University School of Medicine, Hirosaki University School of Medicine, Gifu International Institute of Biotechnology, Tokyo Metropolitan Institute of Gerontology, and the participating hospitals, and written informed consent was obtained from each participant.

Selection and genotyping of polymorphisms. Our aim was to identify gene polymorphisms associated with hypertension in the Japanese population in a case-control association study by examining the relations of one to three polymorphisms of each candidate gene to hypertension. With the use of public databases, including PubMed (NCBI), Online Mendelian Inheritance in Man (NCBI), and dbSNP (NCBI), we selected 50 polymorphisms of 35 candidate genes (data not shown) that we had not examined previously (9-12).

Venous blood (7 ml) was collected into tubes containing 50 mmol/l EDTA (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 50 polymorphisms were determined at G&G Science (Fukushima, Japan) by a method that combines polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX, USA). Primers, probes, and other PCR conditions for genotyping polymorphisms found to be related ($P<0.05$) to hypertension by the Chi-square test are shown in Table I. Detailed genotyping methodology was described previously (14).

Statistical analysis. Quantitative data were compared between subjects with hypertension and controls by the unpaired Student's t-test. Categorical data were compared by the Chi-square test. Allele frequencies were estimated by the gene counting method, and the Chi-square test was used to identify departure from Hardy-Weinberg equilibrium. In the initial screen, genotype distributions for each polymorphism were compared between subjects with hypertension and controls with the Chi-square test. Polymorphisms with a P value of <0.05 were further examined in a more rigorous evaluation of association by multivariable logistic regression analysis with adjustment for covariates, with hypertension as a dependent variable and independent variables including age, sex (0 = woman, 1 = man), body mass index (BMI), smoking status (0 = nonsmoker, 1 = smoker), metabolic variables (0 = no history of diabetes mellitus or hypercholesterolemia; 1 = positive history), and genotype of each polymorphism, and the P value, odds ratio, and 95% confidence interval were calculated. Genotypes were assessed according to dominant, recessive, and two additive (additive 1 and 2) genetic models. Each genetic model comprised two groups: the combined group of variant homozygotes and heterozygotes versus wild-type homozygotes for the dominant model; variant homozygotes versus the combined group of wild-type homozygotes and heterozygotes for the recessive model; heterozygotes versus wild-type homozygotes for the additive 1 model; and variant homozygotes versus wild-type homozygotes for the additive 2 model. Systolic or diastolic blood pressure was compared among three genotype groups for selected polymorphisms by the nonparametric Kruskal-Wallis test or between two groups by the Wilcoxon rank sum test, given that these data were not distributed normally ($P<0.01$ by the Kolmogorov-Smirnov Lilliefors test). For statistical analyses, a $P<0.05$ was considered significant. Statistical significance was examined by the two-sided tests, and statistical analysis was performed with JMP version 5.1 software (SAS Institute, Cary, NC, USA).

Results

The characteristics of the 2752 study subjects are shown in Table II. The frequency of male subjects, BMI, the prevalence of diabetes mellitus and hypercholesterolemia, as well as systolic and diastolic blood pressure was greater in subjects with hypertension than in controls. Comparisons of genotype distributions with the Chi-square test revealed that the -14C→T polymorphism of the ATP-binding cassette, subfamily A, member 1 gene (*ABCA1*), the C→G (Ser2229Cys) polymorphism of the v-ros avian UR2 sarcoma virus oncogene homolog 1 gene (*ROS1*), the C→T (Asn591Asn) polymorphism of the low density lipoprotein receptor gene (*LDLR*), the 13989A→G (Ile118Val) polymorphism of the cytochrome P450, family 3, subfamily A, polypeptide 4 gene

Table I. Primers, probes, and other PCR conditions for genotyping of polymorphisms related to hypertension by the Chi-square test.

Gene symbol	Polymorphism	Sense primer 5'→3'	Antisense primer 5'→3'
ABCA1	-14C→T	GGCTTTGACCGATAGTAACCTCTGC	CCACTCACTCTCGCTCGCAAT
ROS1	C→G (Ser2229Cys)	TCAGAACCAACTTCAGTTATTCAGAA	AGCTTTCATTTATGACTCCACTGTTG
LDLR	C→T (Asn591Asn)	CTTCACTCCATCTCAAGCATCG	CACCTCAAAGACGGCCAAGG
CYP3A4	13989A→G (Ile118Val)	CAACCATGGAGACCTCCACAA	TGGTGAAGGTTGGAGACAGCA
ADIPOR1	C→G	AACCTGCTATCATTGCTATGTATCT	GCAAATAATCAAGACCATACATGTG
ADIPOR1	A→C	TCTTCCATTGTAGAAACTTGACTC	AATCACTTCTCAGCTAATGCAGGC
MMP1	-519A→G	CTGGCTCTGAGTAAAGATTAAG	GTTCTCTGAGGTTCCCTTCTGCCT

Gene symbol	Polymorphism	Probe 1 5'→3'	Probe 2 5'→3'	Annealing (°C)	Cycles
ABCA1	-14C→T	TTGCCGGGACTAGTTCCTT	TTGCCGAGACTAGTTCCTTT	60	50
ROS1	C→G (Ser2229Cys)	GCATTTATIAGTGCAGAGATGA	GCATTTATIAGTCCAGAGATGAAGC	60	50
LDLR	C→T (Asn591Asn)	CCCCGTTGACATCGATGC	CCCCATTGACATCGATGCT	60	50
CYP3A4	13989A→G (Ile118Val)	TGCCATCTCTATAGCTGAG	TGCCGTCTCTATAGCTGA	60	50
ADIPOR1	C→G	GCCAAGTGTCTTCTGTACTT	GCCAAGTCTCTTCTGTACTTTC	60	50
ADIPOR1	A→C	GAGTTAAAGTTGGGTTCATGTC	TTGAGTTAAAGGTGGGTTC	60	50
MMP1	-519A→G	CAATAGGGTACCAGGCAGC	AGCTGCCTGGCACCCTATTG	60	50

Table II. Characteristics of the 2752 study subjects.

Characteristic	Hypertension	Controls	P
No. of subjects	1276	1476	
Age (years)	64.0±11.8	63.9±11.0	0.8494
Sex (male/female, %)	60.7/39.3	40.4/59.6	<0.0001
BMI (kg/m^2)	23.6±3.5	23.3±3.2	0.0065
Current or former smoker (%)	25.1	23.3	0.2689
Diabetes mellitus (%)	41.1	7.3	<0.0001
Hypercholesterolemia (%)	45.7	25.3	<0.0001
Systolic blood pressure (mmHg)	168±23	124±11	<0.0001
Diastolic blood pressure (mmHg)	88±16	74±8	<0.0001

Data for age, BMI, and blood pressure are the means ± SD. Smoker, ≥10 cigarettes daily. Diabetes mellitus, fasting blood glucose of ≥6.93 mmol/l (126 mg/dl) or glycosylated hemoglobin of ≥6.5% (or both), or taking antidiabetes medication. Hypercholesterolemia, serum total cholesterol of ≥5.72 mmol/l (220 mg/dl) or taking lipid-lowering medication.

(*CYP3A4*), the C→G and A→C polymorphisms of the adiponectin receptor 1 gene (*ADIPOR1*), and the -519A→G polymorphism of the matrix metallopeptidase 1 gene (*MMP1*) were related to hypertension on the basis of a P value of <0.05 (Table III). The genotype distributions of these seven polymorphisms in control subjects were all in Hardy-Weinberg equilibrium (Table III).

Multivariable logistic regression analysis with adjustment for age, sex, BMI, smoking status, and the prevalence of diabetes mellitus and hypercholesterolemia revealed that the -14C→T polymorphism of *ABCA1* (dominant and additive 2 models), the C→G (Ser2229Cys) polymorphism of *ROS1* (recessive and additive 2 models), the C→T (Asn591Asn) polymorphism of *LDLR* (dominant and additive 1 models), the 13989A→G (Ile118Val) polymorphism of *CYP3A4* (dominant and additive 1 models), the C→G and A→C polymorphisms of *ADIPOR1* (all genetic models), and the -519A→G polymorphism of *MMP1* (recessive and additive 2 models) were significantly (P<0.05) associated with the prevalence of hypertension (Table IV). The variant *T* allele of *ABCA1*, *G* allele of *ROS1*, and *G* and *C* alleles of *ADIPOR1* were risk factors for hypertension, whereas the variant *T* allele of *LDLR*, *G* allele of *CYP3A4*, and *G* allele of *MMP1* were protective against this condition.

Systolic or diastolic blood pressure of all subjects was compared among (or between) genotypes of the seven identified polymorphisms (Table V). Systolic and diastolic blood pressure differed significantly among genotypes of the -14C→T polymorphism of *ABCA1* and the C→G (Ser2229Cys) polymorphism of *ROS1*, with the variant *T* and the *G* alleles, respectively, being related to increased blood pressure. Systolic blood pressure also differed significantly among genotypes of the C→T (Asn591Asn) polymorphism of *LDLR*, but a gene-dosage effect was not observed.

Discussion

We examined the relations of 50 candidate gene polymorphisms to hypertension in 2752 Japanese individuals. Our results showed that seven polymorphisms of *ABCA1*, *ROS1*, *LDLR*, *CYP3A4*, *ADIPOR1*, and *MMP1* were related to the prevalence of hypertension. Among these polymorphisms, the -14C→T polymorphism of *ABCA1* and the C→G (Ser2229Cys) polymorphism of *ROS1* were associated with

Table III. Genotype distributions of polymorphisms related to hypertension as determined by the Chi-square test.

Gene symbol	Polymorphism	dbSNP[a]	Hypertension	Controls	P
ABCA1	-14C→T	rs1800977			0.0079
	CC		688 (53.9)	866 (58.7)	
	CT		495 (38.8)	536 (36.3)	
	TT		93 (7.3)	74 (5.0)	
ROS1	C→G (Ser2229Cys)	rs619203			0.0083
	CC		858 (75.4)	1119 (78.6)	
	CG		244 (21.4)	284 (19.9)	
	GG		36 (3.2)	21 (1.5)	
LDLR	C→T (Asn591Asn)	rs688			0.0085
	CC		1033 (81.0)	1125 (76.2)	
	CT		223 (17.5)	327 (22.2)	
	TT		20 (1.6)	24 (1.6)	
CYP3A4	13989A→G (Ile118Val)	NC_000007.12			0.0086
	AA		1269 (99.5)	1452 (98.4)	
	AG		7 (0.5)	23 (1.6)	
	GG		0 (0)	0 (0)	
ADIPOR1	C→G	rs1139646			0.0132
	CC		751 (58.9)	931 (63.2)	
	CG		443 (34.7)	479 (32.5)	
	GG		82 (6.4)	64 (4.3)	
ADIPOR1	A→C	rs10920531			0.0250
	AA		677 (53.1)	849 (57.5)	
	AC		495 (38.8)	536 (36.3)	
	CC		104 (8.2)	91 (6.2)	
MMP1	-519A→G	AY769434			0.0465
	AA		1056 (82.8)	1181 (80.0)	
	AG		210 (16.5)	271 (18.4)	
	GG		10 (0.8)	24 (1.6)	

Numbers in parentheses are percentages. [a]In instances where which rs numbers in dbSNP were not detected, NCBI GenBank accession nos. are shown.

Table IV. Multivariable logistic regression analysis of polymorphisms related to hypertension by the Chi-square test.

Gene symbol	Polymorphism	Dominant		Recessive		Additive 1		Additive 2	
		P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95%CI)
ABCA1	-14C→T	0.0391	1.20 (1.01-1.42)	0.0681		0.1080		0.0352	1.48 (1.03-2.13)
ROS1	C→G (Ser2229Cys)	0.1150		0.0165	2.11 (1.15-3.92)	0.3412		0.0137	2.16 (1.18-4.02)
LDLR	C→T (Asn591Asn)	0.0019	0.72 (0.58-0.88)	0.8637		0.0016	0.70 (0.56-0.87)	0.7083	
CYP3A4	13989A→G (Ile118Val)	0.0116	0.22 (0.06-0.64)			0.0116	0.22 (0.06-0.64)		
ADIPOR1	C→G	0.0042	1.29 (1.08-1.54)	0.0140	1.60 (1.10-2.32)	0.0258	1.23 (1.03-1.48)	0.0050	1.72 (1.18-2.52)
ADIPOR1	A→C	0.0013	1.33 (1.12-1.57)	0.0183	1.48 (1.07-2.05)	0.0090	1.27 (1.06-1.52)	0.0040	1.63 (1.17-2.28)
MMPI	-519A→G	0.3590		0.0233	0.36 (0.14-0.84)	0.6980		0.0233	0.36 (0.14-0.83)

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, BMI, and the prevalence of smoking, diabetes mellitus, and hypercholesterolemia.

Table V. Systolic and diastolic blood pressure of subjects according to genotypes of polymorphisms related to hypertension by the Chi-square test.

Blood pressure (mmHg)		Genotype			
		Wild-type homozygote	Heterozygote	Variant homozygote	P
ABCA1	-14C→T				
Systolic		148±29	150±29	154±30	0.0403
Diastolic		81±15	83±14	84±14	0.0214
ROS1	C→G (Ser2229Cys)				
Systolic		147±29	151±29	159±30	0.0041
Diastolic		81±14	82±14	84±13	0.0058
LDLR	C→T (Asn591Asn)				
Systolic		150±29	146±28	153±33	0.0082
Diastolic		82±15	81±15	83±14	0.1043
CYP3A4	13989A→G (Ile118Val)				
Systolic		149±29	147±28		0.6454
Diastolic		82±15	87±15		0.2398
ADIPOR1	C→G				
Systolic		149±29	150±29	152±28	0.1398
Diastolic		82±15	82±14	83±14	0.4215
ADIPOR1	A→C				
Systolic		149±29	150±29	152±28	0.1851
Diastolic		82±15	82±14	82±14	0.5987
MMP1	-519A→G				
Systolic		150±29	148±29	142±24	0.2400
Diastolic		82±15	82±15	79±11	0.6550

both systolic and diastolic blood pressure. These observations thus suggest that polymorphisms of *ABCA1* and *ROS1* are determinants of blood pressure and the development of hypertension in Japanese individuals.

ABCA1 mediates transport of intracellular cholesterol and phospholipids across the plasma membrane of cells, especially macrophages, from which these lipid molecules are removed by apolipoprotein AI and other apolipoproteins of nascent high-density lipoprotein (HDL)-cholesterol in a process that is crucial for the initial step of reverse cholesterol transport (15). This process plays an important role in maintaining cellular cholesterol homeostasis and exerts a protective effect against atherosclerosis (15,16). Loss-of-function mutations in *ABCA1* are responsible for Tangier disease, a rare genetic disorder characterized by the near absence of HDL-cholesterol and the accumulation of lipids within cells of various tissues including the vascular wall (17-19). In families affected by Tangier disease, the onset of coronary heart disease occurs substantially earlier in mutation carriers than in noncarriers (20,21). The increased incidence of early-onset coronary heart disease in *ABCA1* mutation carriers is likely attributable to the accumulation of lipid-laden macrophage foam cells in the vascular wall, which promotes the development and progression of atherosclerosis (15,16). Common polymorphisms of *ABCA1* have been associated with susceptibility to coronary heart disease in the general population (22-27), or with the severity of atherosclerosis without an effect on plasma lipid levels (23,25). The -14C→T polymorphism in the promoter of *ABCA1* was shown to be associated with the plasma concentration of HDL-cholesterol, with the *T* allele being related to an increased HDL-cholesterol level (28,29). We have now shown that the -14C→T polymorphism of *ABCA1* is associated with systolic and diastolic blood pressure and the prevalence of hypertension, with the *T* allele representing a risk factor for this condition. The association of this polymorphism with hypertension was independent of hypercholesterolemia, given that the association was significant after adjustment for this factor in the multivariable logistic regression analysis. As far as we are aware, this is the first demonstration of an association of the -14C→T polymorphism of *ABCA1* with hypertension, although the mechanism responsible for the association of the *T* variant with both an increased plasma concentration of HDL-cholesterol (28,29) and an increased risk of hypertension (our study) remains to be elucidated.

The protein encoded by *ROS1* located on 6q22 is a type I integral membrane protein with tyrosine kinase activity that may function as a receptor for a growth or differentiation factor and thereby may play an important role in the regulation of cell proliferation, differentiation, migration, metabolism, or apoptosis (30,31). Human *ROS1* is the closest homolog of the v-*Ros* oncogene of the avian sarcoma UR2 retrovirus, a

replication-defective virus that was isolated from a spontaneous chicken tumor (32). The human gene is also an oncogene and is expressed in a large proportion of glioblastomas but not in a normal glial cell line or in normal adult brain tissue (33). In an association study of 11,053 polymorphisms in 6891 genes and myocardial infarction, the G→A (Asp2213Asn) and C→G (Ser2229Cys) polymorphisms of *ROS1*, which were in linkage disequilibrium, were associated with the prevalence of this condition (34). Another study replicated the association of the C→G (Ser2229Cys) polymorphism of *ROS1* with an increased risk of myocardial infarction (35). The thiol groups of two cysteine residues have the potential to form a disulfide bond that can link two peptide chains together, as in insulin, or cause a single peptide chain to fold back on itself in a loop. Such a latter effect of the C→G (Ser2229Cys) substitution may thus alter the structural stability, binding affinity, or catalytic function of *ROS1* (35). Our present results show that the C→G (Ser2229Cys) polymorphism of *ROS1* was associated with systolic and diastolic blood pressure and the prevalence of hypertension, with the *G* allele representing a risk factor for this condition. As far as we are aware, this is the first demonstration of an association of this polymorphism of *ROS1* with hypertension, although the underlying molecular mechanism remains to be elucidated.

It is possible that the polymorphisms of *ABCA1* or *ROS1* are in linkage disequilibrium with other polymorphisms of the same or nearby genes that are actually responsible for the development of hypertension. In addition, the functional relevance of the polymorphisms to gene transcription or to protein structure or function was not determined in the present study. Our present results, however, suggest that *ABCA1* and *ROS1* are susceptibility loci for hypertension in the Japanese population. Determination of the genotypes for these polymorphisms may prove informative for prediction of the genetic risk for hypertension.

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References

1. Lifton RP, Gharavi AG and Geller DS: Molecular mechanisms of human hypertension. Cell 104: 545-556, 2001.
2. Turner ST, Schwartz GL and Boerwinkle E: Personalized medicine for high blood pressure. Hypertension 50: 1-5, 2007.
3. Caulfield M, Munroe P, Pembroke J, *et al*: Genome-wide mapping of human loci for essential hypertension. Lancet 361: 2118-2123, 2003.
4. Gong M, Zhang H, Schulz H, *et al*: Genome-wide linkage reveals a locus for human essential (primary) hypertension on chromosome 12p. Hum Mol Genet 12: 1273-1277, 2003.
5. de Lange M, Spector TD and Andrew T: Genome-wide scan for blood pressure suggests linkage to chromosome 11, and replication of loci on 16, 17, and 22. Hypertension 44: 872-877, 2004.
6. Wallace C, Xue MZ, Newhouse SJ, *et al*: Linkage analysis using co-phenotypes in the BRIGHT study reveals novel potential susceptibility loci for hypertension. Am J Hum Genet 79: 323-331, 2006.
7. Cusi D, Barlassina C, Azzani T, *et al*: Polymorphisms of α-adducin and salt sensitivity in patients with essential hypertension. Lancet 349: 1353-1357, 1997.
8. Siffert W, Rosskopf D, Siffert G, *et al*: Association of a human G-protein ß3 subunit variant with hypertension. Nat Genet 18: 45-48, 1998.
9. Izawa H, Yamada Y, Okada T, Tanaka M, Hirayama H and Yokota M: Prediction of genetic risk for hypertension. Hypertension 41: 1035-1040, 2003.
10. Yamada Y, Matsuo H, Segawa T, *et al*: Assessment of the genetic component of hypertension. Am J Hypertens 19: 1158-1165, 2006.
11. Yamada Y, Ando F and Shimokata H: Association of a microsomal triglyceride transfer protein gene polymorphism with blood pressure in Japanese women. Int J Mol Med 17: 83-88, 2006.
12. Yamada Y, Ando F and Shimokata H: Association of gene polymorphisms with blood pressure and the prevalence of hypertension in community-dwelling Japanese individuals. Int J Mol Med 19: 675-683, 2007.
13. Perloff D, Grim C, Flack J, *et al*: Human blood pressure determination by sphygmomanometry. Circulation 88: 2460-2470, 1993.
14. Itoh Y, Mizuki N, Shimada T, *et al*: High-throughput DNA typing of HLA-A, -B, -C, and -DRB1 loci by a PCR-SSOP-Luminex method in the Japanese population. Immunogenetics 57: 717-729, 2005.
15. Oram JF and Heinecke JW: ATP-binding cassette transporter A1: a cell cholesterol exporter that protects against cardiovascular disease. Physiol Rev 85: 1343-1372, 2005.
16. Lusis AJ: Atherosclerosis. Nature 407: 233-241, 2000.
17. Brooks-Wilson A, Marcil M, Clee SM, *et al*: Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet 22: 336-345, 1999.
18. Bodzioch M, Orso E, Klucken J, *et al*: The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat Genet 22: 347-351, 1999.
19. Rust S, Rosier M, Funke H, *et al*: Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet 22: 352-355, 1999.
20. Clee SM, Kastelein JJ, van Dam M, *et al*: Age and residual cholesterol efflux affect HDL cholesterol levels and coronary artery disease in ABCA1 heterozygotes. J Clin Invest 106: 1263-1270, 2000.
21. van Dam MJ, de Groot GE, Clee SM, *et al*: Association between increased arterial-wall thickness and impairment in ABCA1-driven cholesterol efflux: an observational study. Lancet 359: 37-42, 2002.
22. Wang J, Burnett JR, Near S, *et al*: Common and rare ABCA1 variants affecting plasma HDL cholesterol. Arterioscler Thromb Vasc Biol 20: 1983-1989, 2000.
23. Clee SM, Zwinderman AH, Engert JC, *et al*: Common genetic variation in ABCA1 is associated with altered lipoprotein levels and a modified risk for coronary artery disease. Circulation 103: 1198-1205, 2001.
24. Kyriakou T, Pontefract DE, Viturro E, *et al*: Functional polymorphism in ABCA1 influences age of symptom onset in coronary artery disease patients. Hum Mol Genet 16: 1412-1422, 2007.
25. Tregouet DA, Ricard S, Nicaud V, *et al*: In-depth haplotype analysis of *ABCA1* gene polymorphisms in relation to plasma ApoA1 levels and myocardial infarction. Arterioscler Thromb Vasc Biol 24: 775-781, 2004.
26. Frikke-Schmidt R, Nordestgaard BG, Jensen GB and Tybjaerg-Hansen A: Genetic variation in ABC transporter A1 contributes to HDL cholesterol in the general population. J Clin Invest 114: 1343-1353, 2004.
27. Kyriakou T, Hodgkinson C, Pontefract DE, *et al*: Genotypic effect of the -565C→T polymorphism in the *ABCA1* gene promoter on ABCA1 expression and severity of atherosclerosis. Arterioscler Thromb Vasc Biol 25: 418-423, 2005.

28. Tan JH, Low PS, Tan YS, *et al*: ABCA1 gene polymorphisms and their associations with coronary artery disease and plasma lipids in males from three ethnic populations in Singapore. Hum Genet 113: 106-117, 2003.
29. Hodoglugil U, Williamson DW, Huang Y and Mahley RW: Common polymorphisms of ATP binding cassette transporter A1, including a functional promoter polymorphism, associated with plasma high density lipoprotein cholesterol levels in Turks. Atherosclerosis 183: 199-212, 2005.
30. Schlessinger J: Cell signaling by receptor tyrosine kinases. Cell 103: 211-225, 2000.
31. Zong CS, Chan JL, Yang SK, *et al*: Mutations of Ros differentially effecting signal transduction pathways leading to cell growth versus transformation. J Biol Chem 272: 1500-1506, 1997.
32. Neckameyer WS and Wang LH: Nucleotide sequence of avian sarcoma virus UR2 and comparison of its transforming gene with other members of the tyrosine protein kinase oncogene family. J Virol 53: 879-884, 1985.
33. Birchmeier C, O'Neill K, Riggs M, *et al*: Characterization of ROS1 cDNA from a human glioblastoma cell line. Proc Natl Acad Sci USA 87: 4799-4803, 1990.
34. Shiffman D, Ellis SG, Rowland CM, *et al*: Identification of four gene variants associated with myocardial infarction. Am J Hum Genet 77: 596-605, 2005.
35. Zee RY, Michaud SE, Hegener HH, Diehl KA and Ridker PM: A prospective replication study of five gene variants previously associated with risk of myocardial infarction. J Thromb Haemost 4: 2093-2095, 2006.