Original Article

Relationship between the Thromboxane A_2 Receptor Gene and Susceptibility to Cerebral Infarction

Yoshiyuki KANEKO[1], Tomohiro NAKAYAMA[2], Kosuke SAITO[2,3], Akihiko MORITA[4], Ichiro SATO[5], Aya MARUYAMA[5], Masayoshi SOMA[6], Teruyuki TAKAHASHI[7], and Naoyuki SATO[2]

The risk of cerebral infarction (CI) in an individual is dependent on the interplay between genetic risk factors and environmental influences. Binding of thromboxane A_2 (TXA_2) to its receptor (TP) modulates thrombosis/hemostasis and plays a significant role in the pathogenesis of CI. The aim of the present study was to investigate the relationship between human TP gene single nucleotide polymorphisms (SNPs) and haplotypes and CI in a Japanese population. A genetic association study was performed in 194 CI patients and 365 non-CI subjects by specifically characterizing 6 SNPs in the human TP gene (rs2271875, rs768963, rs2238634, rs11085026, rs4523 and rs4806942). Analysis demonstrated that there were significant differences in the overall distribution of genotypes and dominant or recessive models of rs2271875 and rs768963 between the CI and the non-CI groups. Multiple logistic regression analysis revealed that the C allele of rs768963 was significantly associated with CI ($p=0.029$), even after adjusting for confounding factors (odds ratio: 2.41). Further, the C-T-C haplotype of rs768963-rs2238634-rs4806942 was significantly more frequent in the CI group (23.0%) than in the non-CI group (17.7%). These results suggest that specific SNPs and haplotypes may have utility as genetic markers for the risk of CI and that TP or a neighboring gene is associated with the increased susceptibility to CI. (*Hypertens Res* 2006; 29: 665–671)

***Key Words*: thromboxane A_2 receptors, cerebral infarction, case-control studies, single nucleotide polymorphism, haplotypes**

Introduction

Cerebral infarction (CI) is a major cause of death and disability worldwide, but the genetic basis of this disease has not been elucidated (*1*). The risk of CI may vary according to the presence of various medical conditions and lifestyle factors, including atrial fibrillation, diabetes mellitus, and serum lipid profile (*2*, *3*). Further, investigators have hypothesized that specific genetic variances may contribute to the pathogenesis and risk of CI (*3*).

Thromboxane A_2 (TXA_2) is generated through sequential

From [1]Nihon University School of Medicine, Tokyo, Japan; [2]Division of Molecular Diagnostics, Advanced Medical Research Center, [4]Division of Neurology, Department of Medicine, [5]Department of Obstetrics and Gynecology, and [6]Division of Nephrology and Endocrinology, Department of Medicine, Nihon University School of Medicine,Tokyo, Japan; [3]Department of Applied Chemistry, Toyo University School of Engineering, Kawagoe, Japan; and [7]Department of Neurology, Graduate School of Medicine, Nihon University, Tokyo, Japan.

[1]5th grade student.

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Address for Reprints: Tomohiro Nakayama, M.D., Division of Molecular Diagnostics, Advanced Medical Research Center, Nihon University School of Medicine, Ooyaguchi-kamimachi, 30–1 Itabashi-ku, Tokyo 173–8610, Japan. E-mail: tnakayam@med.nihon-u.ac.jp

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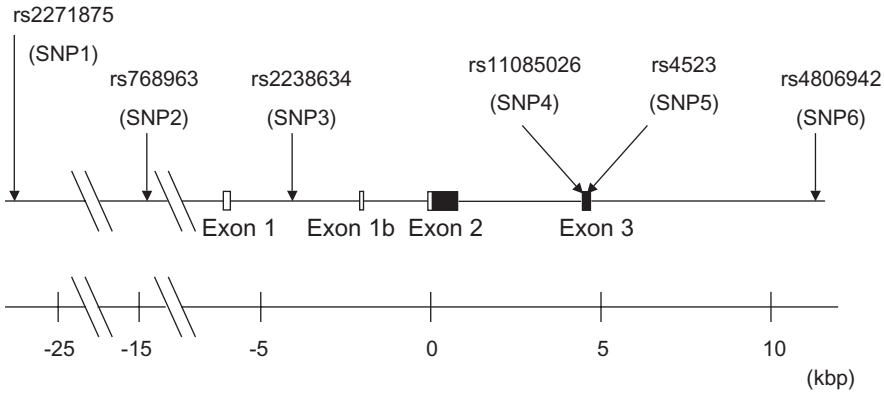

Fig. 1. *Organization of the human TP gene, and location of SNPs. Boxes indicate exons, and lines indicate introns. The white and black boxes show untranslated regions and coding regions, respectively.*

metabolism of arachidonic acid by cyclooxygenases 1 or 2 and TXA_2 synthase (*4*). TXA_2 stimulates smooth muscle constriction and platelet aggregation and promotes intravascular hemostasis. Therefore, TXA_2 is thought to be a mediator in myocardial infarction, atherosclerosis, and CI (*5*).

The TXA_2 receptor (TP) is a member of the superfamily of G-protein–coupled seven-transmembrane receptors (*6*). TXA_2 exerts its physiologic effects by binding to TP (*7*). The human TP gene is a single copy gene that spans over 15 kilobasepairs (kbp) and is composed of 4 exons interrupted by 3 introns (Fig. 1). The gene is located on 19p13.3 (*5*).

The aim of the present study was to investigate the relationship between human TP gene single nucleotide polymorphisms (SNPs) and haplotypes and CI in a Japanese population.

Methods

Subjects

Patients and control subjects from the northern area of Tokyo were recruited for this case-control study. Patients were selected from those that had been admitted to our hospital (Nihon University Hospital in Tokyo) or community hospitals (in Tokyo) between 1995 and April 2005 (*8*). Control subjects were selected from outpatients who were present at the hospital during the same period. The study group consisted of 194 Japanese patients with CI (mean age, 65.3±12.3 years) that had been diagnosed either by CT or MRI. All patients had neurological deficits that persisted for at least 1 month, and none of the patients were diagnosed with antiphospholipid antibody syndrome. Patients with potential sources of cardioembolism (*e.g.*, recent myocardial infarction, valvular heart disease, and arrhythmia including atrial fibrillation) were excluded from study. Thus, the CI group consisted of patients with non-cardioembolic CI, including atherothrombotic infarction and lacunar infarction.

The control group consisted of 365 Japanese subjects without CI (mean age, 74.4±9.8 years). Because the mean age of the control group was older than that of the CI group, the control group was regarded as a super-control group (*9*). Healthy elderly subjects are more suitable than young or middle-aged subjects for the determination of phenotypes of cardiovascular and cerebrovascular diseases related to aging, because many such diseases occur late in life. Ischemic stroke is an age-influenced disease; therefore, it was better to use a super-control group than an age-matched control group. The control subjects had vascular risk factors, such as hypertension, hypercholesterolemia, or diabetes mellitus, but did not have ischemic cerebrovascular disease. Informed consent was obtained from each subject according to a protocol approved by the Human Studies Committee of Nihon University.

Biochemical Analysis

Plasma concentrations of total cholesterol, high-density lipoprotein (HDL)–cholesterol, creatinine, and uric acid were measured using the methods of the Clinical Laboratory Department of Nihon University Hospital (*10*).

Genotyping

Based on the allelic frequency data for registered SNPs from the National Center for Biotechnology Information (NCBI) website and from the Applied Biosystems (Foster City, USA)–Celera (Rockville, USA) Discovery System, SNPs with minor allele frequencies >20% were chosen for study. This criterion was selected because SNPs with a high frequency of minor alleles are very useful genetic markers in genetic association studies. Thus, 6 SNPs in the human TP gene were selected with accession numbers as follows: rs2271875 (C__2589825_1), rs768963 (C__2576338_10), rs2238634 (C__11698607_1), rs11085026 (C__2576300_10), rs4523 (C__2576299_10), and rs4806942 (C__2576290_10) (Fig. 1). These SNPs were designated SNP1, SNP2, SNP3, SNP4, SNP5, and SNP6, respectively,

Table 1. Characteristics of Study Participants

	Total		*p* value	Male		*p* value	Female		*p* value
	Non-CI	CI		Non-CI	CI		Non-CI	CI	
Number of subjects	365	194		192	117		173	77	
Age (years)	74.4±9.8	65.3±12.3	<0.001	72.6±11.9	63.6±11.6	<0.001	76.4±6.4	67.9±13.0	<0.001
BMI (kg/m^2)	22.6±2.8	23.5±3.6	0.005	22.8±2.8	23.4±3.0	0.159	22.4±2.8	23.9±4.5	0.011
SBP (mmHg)	138.9±20.3	152.7±27.5	<0.001	140.7±21.6	151.1±26.9	<0.001	136.8±18.7	155.3±28.3	<0.001
DBP (mmHg)	81.2±14.1	86.7±16.3	<0.001	82.7±15.0	87.9±16.5	0.005	79.5±12.9	84.8±16.1	0.007
Pulse (beats/min)	71.4±12.1	76.6±14.3	<0.001	70.8±13.3	75.8±14.0	0.003	72.1±10.6	78.0±14.7	<0.001
Creatinine (mg/dl)	0.90±0.55	1.16±1.23	0.001	1.04±0.71	1.24±1.17	0.071	0.75±0.19	1.05±1.31	0.006
Total cholesterol (mg/dl)	212.9±44.5	201.8±48.3	0.008	201.4±35.9	195.1±49.7	0.215	225.5±49.5	211.9±44.6	0.049
HDL cholesterol (mg/dl)	51.8±17.8	49.6±19.9	0.451	48.6±13.7	47.1±19.5	0.658	60.1±24.3	53.6±20.1	0.282
Uric acid (mg/dl)	5.6±1.7	5.6±2.3	0.889	5.9±1.7	5.9±2.0	0.969	4.5±1.5	5.1±2.6	0.379
EH(%)	14.8	36.1	<0.001	18.8	35.0	0.001	10.4	37.7	<0.001
DM(%)	4.7	20.6	<0.001	5.7	19.7	<0.001	3.5	22.1	<0.001
HL(%)	33.7	39.7	0.160	25.5	32.5	0.187	42.8	50.6	0.248

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; EH, essential hypertension; DM, diabetes mellitus; HL, hyperlipidemia; CI, cerebral infarction.

for the purposes of this study. Although SNP4 and SNP5 were located in the exon region, neither SNP produces amino acid changes (*e.g.*, silent mutations).

Genotypes were determined using Assays-on-Demand kits (Applied Biosystems) together with TaqMan® PCR as previously described (*11*, *12*).

Linkage Disequilibrium Analysis and the Haplotype-Based Case Control Study

Based on the genotype data of the genetic variations, a linkage disequilibrium (LD) analysis and a haplotype-based case control study were performed by applying SNPAlyze version 3.2 (Dynacom Co., Ltd., Yokohama, Japan) of the expectation-maximization (EM) algorithm (*13*, *14*). D' values >0.5 were used to assign SNP locations to one haplotype block. One SNP was selected by omitting one SNP in SNP pairs showing $r^2>0.25$ in all the combinations of the haplotype-based case control study.

Statistical Analysis

Data are shown as the mean±SD. Differences in clinical data between the CI and non-CI groups were assessed by analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test.

The overall distributions of the genotypes and dominant or recessive models were examined with Fisher's exact test. In addition, multiple logistic regression analyses were performed to assess the contribution of confounders. Statistical analyses were performed with SPSS™ software for Windows, version 12 (SPSS Inc., Chicago, USA). Statistical significance was established at $p<0.05$.

Pair-wise LD patterns for the TP gene were evaluated using $|D'|$ and r^2. The threshold value of the frequencies of the haplotypes included in the analysis was set to 2%. All haplotypes below the threshold value were excluded from the analysis. The overall distribution of haplotypes was analyzed using $2\times m$ contingency tables, with a p value of <0.05 considered to indicate statistical significance. The p value of each haplotype was determined using χ^2 analysis, a permutation method, and SNPAlyze version 3.2 (Dynacom Co., Ltd., Yokohama, Japan) (*11*).

Results

Clinical characteristics of the CI and non-CI subjects are summarized in Table 1. Age, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse rate, serum concentrations of creatinine, plasma concentrations of total cholesterol and frequencies of essential hypertension (EH) and diabetes mellitus (DM) were significantly different when comparing the two groups. However, no significant differences were noted in plasma concentrations of HDL cholesterol, uric acid, and hyperlipidemia (HL) when comparing the two groups. When performing subgroup analysis on male patients, age, SBP, DBP, pulse rate, and the frequency of EH and DM were significantly different when comparing the two groups, but there was no significant difference in BMI, serum concentrations of creatinine, plasma concentrations of total cholesterol, plasma concentrations of HDL cholesterol, uric acid, and HL. When performing subgroup analysis on female patients, age, BMI, SBP, DBP, pulse rate, serum concentrations of creatinine, plasma concentrations of total cholesterol, and the frequency of EH and DM were significantly different when comparing the two groups, but there was no difference in BMI, plasma concentrations of HDL cholesterol, uric acid, and HL.

Table 2. Genotype Distribution in Non-Cerebral Infarction (Non-CI) and Cerebral Infarction (CI)

Variants	Non-CI (365)	CI (194)	*p* value
rs2271875 (SNP1)			
Genotype			
G/G	148 (40.6)	61 (31.4)	
G/A	153 (41.9)	102 (52.6)	
A/A	64 (17.5)	31 (16.0)	0.046*
Dominant or recessive model			
G/A, A/A	217 (59.4)	133 (68.6)	
G/G	148 (40.6)	61 (31.4)	0.046*
rs768963 (SNP2)			
Genotype			
C/C	145 (39.7)	73 (37.6)	
C/T	157 (43.0)	103 (53.1)	
T/T	63 (17.3)	18 (9.3)	0.014*
Dominant or recessive model			
C/C, C/T	302 (82.7)	176 (91.7)	
T/T	63 (17.3)	18 (9.3)	0.011*
rs2238634 (SNP3)			
Genotype			
G/G	214 (58.6)	107 (55.2)	
G/T	133 (36.4)	79 (40.7)	
T/T	18 (4.9)	8 (4.1)	0.590
Dominant or recessive model			
G/G	214 (58.6)	107 (55.2)	
G/T, T/T	151 (41.4)	87 (44.8)	0.429
rs11085026 (SNP4)			
Genotype			
T/T	154 (42.2)	79 (40.7)	
T/C	167 (45.8)	98 (50.5)	
C/C	44 (12.1)	17 (8.8)	0.381
Dominant or recessive model			
T/T, T/C	321 (87.9)	177 (91.2)	
C/C	44 (12.1)	17 (8.8)	0.235
rs4523 (SNP5)			
Genotype			
T/T	261 (71.5)	142 (73.2)	
T/C	88 (24.1)	47 (24.2)	
C/C	16 (4.4)	5 (2.6)	0.562
Dominant or recessive model			
T/T, T/C	349 (95.6)	189 (97.4)	
C/C	16 (4.4)	5 (2.6)	0.285
rs4806942 (SNP6)			
Genotype			
C/C	113 (31.0)	67 (34.5)	
C/T	180 (49.3)	94 (48.5)	
T/T	72 (19.7)	33 (17.0)	0.601
Dominant or recessive model			
C/C, C/T	293 (80.3)	161 (83.0)	
T/T	72 (19.7)	33 (17.0)	0.434

SNP, single nucleotide polymorphism. *Significant difference in distribution.

The distributions of the genotypes and dominant or recessive models of the 6 SNPs in the 194 CI patients and the 365 non-CI controls are summarized in Table 2. There were significant differences in the genotype and dominant or recessive models of SNP1 and SNP2 when comparing the CI and the non-CI groups.

Multiple logistic regression analysis revealed a significant association between C/C, C/T and CI ($p=0.029$), even after adjustment for confounding factors; the calculated odds ratio was 2.41 (95% confidence interval: 1.10–5.30) (Table 3).

Figure 2 shows pair-wise LD patterns for the TP gene. All SNPs were located in 1 haplotype block. The r^2 values indicated that the combinations of SNP1-SNP2, SNP3-SNP4, and SNP4-SNP5 were not useful for haplotype-based case-control studies. Next, a haplotype-based case-control study was performed using 23 useful combinations of SNPs that did not include the combinations of SNP1-SNP2, SNP3-SNP4, and SNP4-SNP5 in all 57 combinations using 6 SNPs. Of the 23 combinations, three combinations (SNP2-SNP3-SNP6, SNP2-SNP4-SNP6, and SNP2-SNP3-SNP5-SNP6) showed significant differences in overall distributions when comparing the CI and non-CI groups (Table 4). One haplotype (C-T-C of SNP2-SNP3-SNP6) was significantly more frequent in the CI group (23.0%) than in the non-CI group (17.7%).

Discussion

Metabolism of arachidonic acid produces prostaglandin (PG) G_2 and PGH_2, and thromboxane synthase catalyzes the reaction that converts PGH_2 to TXA_2, while PGIS catalyzes the reaction that converts PGH_2 to prostacyclin. The balance between production of TXA_2 and prostacyclin determines the vasoconstriction/vasodilatory state of the vasculature. We previously reported that mutations of the human PGIS gene are associated with CI. Specifically, a nonsense mutation in exon 2 was found in one female patient in the screening using 300 people (150 with essential hypertension subjects and 150 healthy controls). Three of her five living siblings had the mutation; all were hypertensives, and one had had a CI (*15*). Furthermore, a tandem repeat polymorphism varied with 3 to 7 repeats of nine nucleotides in the Sp1 site of the core promoter element, with transcriptional activity increasing in proportion to the number of repeats. Finally, a small number repeat alleles was found more frequently in patients with CI (odds ratio: 1.38, 95% confidence interval: 1.11–1.71) (*16*). These observations suggest that the risk of CI may vary with prostacyclin activity. Therefore, genes related to TXA_2 may represent another mechanism by which the susceptibility for CI may be modulated.

SNPs of the human TP gene are associated with various diseases. For example, Unoki *et al*. reported that a silent mutation (T924C; rs4523) in the human TP gene was associated with bronchial asthma (*17*). However, other investigators have questioned the statistical analysis used in that study (*18*, *19*). Hirata *et al*. identified a missense mutation (Arg60 to

Table 3. Logistic Regression Analysis by Confounding Factors

	rs2271875				rs768963			
	p	Odds ratio	95% confidence interval		*p*	Odds ratio	95% confidence interval	
			Lower limit	Upper limit			Lower limit	Upper limit
BMI	0.102	1.066	0.987	1.151	0.120	1.062	0.984	1.146
Creatinine	0.010*	1.624	1.122	2.321	0.010*	1.617	1.120	2.334
EH	0.000*	3.967	2.359	6.670	0.000*	4.110	2.468	7.047
DM	0.000*	3.945	1.867	8.335	0.000*	2.947	1.855	8.397
HL	0.087	1.509	0.943	2.415	0.096	1.493	0.931	2.396
Dominant or recessive model	0.166	1.423	0.864	2.345	0.029*	2.410	1.096	5.299

BMI, body mass index; EH, essential hypertension; DM, diabetes mellitus; HL, hyperlipidemia. *Significant difference.

		SNP1	SNP2	SNP3	SNP4	SNP5	SNP6
SNP1	|D'|		1.000	0.069	0.146	0.461	0.182
	r^2		0.396	0.002	0.007	0.026	0.017
	SNP2	|D'|		0.829	0.719	0.714	0.272
		r^2		0.131	0.175	0.064	0.059
		SNP3	|D'|		0.850	0.052	0.498
			r^2		0.405	0.000	0.060
			SNP4	|D'|		1.000	0.517
				r^2		0.366	0.114
				SNP5	|D'|		0.516
					r^2		0.042

\|D'\| >0.5
r^2 >0.25

Fig. 2. *Pair-wise linkage disequilibrium (LD) analysis. Pair-wise LD among the 6 marker pairs studied in the TP gene were computed, and LD pairs (*$|D'| > 0.5$ *or* $r^2 > 0.25$*) are shown as gray-shaded values.*

Leu) in patients with a dominantly inherited bleeding disorder characterized by defective platelet response to TXA_2 (*20*). The substitution of amino acid is located in the first cytoplasmic loop of the TP protein. This mutation is thought to be a very rare mutation, since it is observed only in inherited family members.

Studies with TP knockout mice have demonstrated that the absence of functional TP receptors has no effect on normal development or survival (*21*, *22*). Specifically, TP−/− mice have a mild bleeding disorder and altered vascular response to TXA_2 and arachidonic acid but have normal major organ systems. Further, there have not been any reports of cerebral damage or stroke in TP knockout mice or in TP-overexpressing mice.

Several studies suggest there is a link between hypercoagulability and thromboxane, particularly in patients with CI. For example thromboxane biosynthesis, as assessed by measurement of plasma levels and urinary excretion of a thromboxane metabolite, 11-dehydro-thromboxane B_2, is enhanced in patients with CI (*23*, *24*). Administration of low-dose aspirin (50 mg/day for 7 days) reduced the urinary excretion of 11-dehydro-thromboxane B_2 by approximately 85% in patients with CI, and the TXA_2-synthetase inhibitor, ozagrel, may have clinical efficacy in the treatment of early- and chronic-stage CI (*25*). Further study to determine the efficacy of prophylactic administration of aspirin or TXA_2-synthetase inhibitors in patients with genetic susceptibility markers for CI would be of benefit.

In the phenomenon known as aspirin resistance, platelet production of TXA_2 is refractory to the inhibitory effect of aspirin treatment, and the risk of adverse cardiovascular events may increase. Potential causes of aspirin resistance include inadequate dosing, drug interactions, upregulation of non-platelet sources of thromboxane biosynthesis, and increased platelet turnover. Further, aspirin resistance may result from certain genetic polymorphisms involved in thromboxane biosynthesis, including a polymorphism of the cyclooxygenase-1 (COX-1) gene affecting Ser529; polymorphism PLA1/A2 of the gene encoding glycoprotein IIIa (GPIIIa); and the homozygous 807T (873A) polymorphism associated with increased density of the platelet GPIa/IIa collagen-receptor gene (*26*). Because of the possible increased

Table 4. All Haplotypes Showing Significant Differences in Overall Distribution between NT Controls and EH Patients

Combination of SNPs	Overall distribution		Distribution of individual haplotypes					
	χ^2	*p* value	Haplotype	Non-CI (400)	CI (416)	χ^2	χ^2 *p*-value	Permutation *p*-value
SNP2-SNP3-SNP6	22.0	<0.001*	C-G-C	0.230	0.241	0.185	0.667	0.687
			T-G-T	0.227	0.220	0.069	0.792	0.816
			C-T-C	0.177	0.230	4.319	0.038*	0.035*
			C-G-T	0.169	0.176	0.097	0.756	0.794
			T-G-C	0.151	0.133	0.583	0.445	0.499
			C-T-T	0.048	0.000	18.332	<0.001*	<0.001*
SNP2-SNP4-SNP6	18.6	0.005	C-C-C	0.284	0.244	2.078	0.150	0.159
			T-T-T	0.229	0.222	0.057	0.811	0.827
			C-T-C	0.185	0.156	1.616	0.204	0.239
			C-T-T	0.142	0.145	0.033	0.855	0.937
			T-T-C	0.117	0.128	0.297	0.586	0.624
			C-C-T	0.044	0.072	3.656	0.056	0.069
			T-C-C	0.000	0.032	12.200	<0.001*	<0.001*
SNP2-SNP3-SNP5-SNP6	19.4	0.013	T-G-T-T	0.222	0.227	0.005	0.945	1.000
			C-T-T-C	0.185	0.152	2.072	0.150	0.163
			C-G-T-C	0.165	0.144	0.747	0.387	0.407
			C-G-T-T	0.150	0.146	0.048	0.826	0.850
			T-G-T-C	0.127	0.142	0.455	0.500	0.474
			C-G-C-C	0.079	0.087	0.229	0.633	0.739
			C-T-C-C	0.046	0.033	1.058	0.304	0.292
			C-G-C-T	0.025	0.030	0.326	0.568	0.696
			C-T-T-T	0.000	0.040	15.414	<0.001*	<0.001*

NT, normotensive; EH, essential hypertensive; SNP, single nucleotide polymorphism. *Significant difference.

risk of ischemic vascular events, the use of non-aspirin antiplatelet agents should be considered in patients with these genetic polymorphisms. Further study to determine whether polymorphisms of the TP gene are also associated with aspirin resistance would be of benefit.

Genetic analysis of complex traits and diseases and population-based gene identification studies are more easily performed with SNPs than with other polymorphisms, such as microsatellite markers. In fact, SNPs with high genomic frequency are particularly useful for susceptible gene discovery purposes. Moreover, because new SNP alleles arise as mutations at different loci and different points in time, and because they occur in such great abundance compared to genomes, groups of neighboring SNPs may create a haplotypic diversity that can be exploited in direct association studies (*27*). Morris and Kaplan found that an analysis based on haplotypes has advantages over an analysis based on individual SNPs in genes with multiple susceptibilities, particularly when LDs between SNPs are weak (*28*). Based on such findings, we hypothesized that haplotype analysis would be useful for the assessment of the association between haplotypes and CI, thus resulting in the present attempt to establish haplotypes of the TP gene consisting of 6 SNPs. However, there are some limitations associated with our study. For example, case-control studies sometimes exhibit pseudo-positive results due to sample scales or selection of the genetic markers. Familial linkage studies and transmission disequilibrium tests should be performed to confirm the reliability of the present data.

In conclusion, SNPs and haplotypes in the TP gene showed significant differences between CI and non-CI patients. The results indicate that these polymorphisms and haplotypes could be genetic markers for CI and suggest that TP or a neighboring gene may act as a susceptibility gene for CI.

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References

1. Hassan A, Markus HS: Genetics and ischaemic stroke. *Brain* 2000; **123**: 1784–1812.
2. Gorelick PB, Sacco RL, Smith DB, *et al*: Prevention of a first stroke: a review of guidelines and a multidisciplinary consensus statement from the National Stroke Association. *JAMA* 1999; **281**: 1112–1120.
3. Yamagishi K, Iso H, Kitamura A, *et al*: Smoking raises the risk of total and ischemic strokes in hypertensive men. *Hypertens Res* 2003; **26**: 209–217.

4. Coyle AT, Miggin SM, Kinsella BT: Characterization of the 5′ untranslated region of alpha and beta isoforms of the human thromboxane A2 receptor (TP). Differential promoter utilization by the TP isoforms. *Eur J Biochem* 2002; **269**: 4058–4073.
5. Nusing RM, Hirata M, Kakizuka A, Eki T, Ozawa K, Narumiya S: Characterization and chromosomal mapping of the human thromboxane A2 receptor gene. *J Biol Chem* 1993; **268**: 25253–25259.
6. Turek JW, Halmos T, Sullivan NL, Antonakis K, Le Breton GC: Mapping of a ligand-binding site for the human thromboxane A_2 receptor protein. *J Biol Chem* 2002; **277**: 16791–16797.
7. Huang JS, Ramamurthy SK, Lin X, Le Breton GC: Cell signalling through thromboxane A2 receptors. *Cell Signal* 2004; **16**: 521–533.
8. Aoi N, Soma M, Nakayama T, *et al*: Variable number of tandem repeat of the 5′-flanking region of Type-C human natriuretic peptide receptor gene influences blood pressure levels in obesity-associated hypertension. *Hypertens Res* 2004; **27**: 711–716.
9. Baird PN, Richardson AJ, Craig JE, Mackey DA, Rochtchina E, Mitchell P: Analysis of optineurin (OPTN) gene mutations in subjects with and without glaucoma: the Blue Mountains Eye Study. *Clin Exp Ophthalmol* 2004; **32**: 518–522.
10. Nakayama T, Soma M, Kanmatsuse K, Kokubun S: The microsatellite alleles on chromosome 1 associated with essential hypertension and blood pressure levels. *J Hum Hypertens* 2004; **18**: 823–828.
11. Nakayama T, Soma M, Sato N, *et al*: An association study in essential hypertension using functional polymorphisms in lymphotoxin-α gene. *Am J Hypertens* 2004; **17**: 1045–1049.
12. Maruyama A, Nakayama T, Sato N, Mizutani Y, Furuya K, Yamamoto T: Association study using single nucleotide polymorphisms in the estrogen receptor β (ESR2) gene for preeclampsia. *Hypertens Res* 2004; **27**: 903–909.
13. Dempster AP, Laird NM, Rubin DB: Maximum likelihood from in complete data *via* the EM algorithm. *J R Stat Soc* 1977; **39**: 1–22.
14. Kobayashi Y, Nakayama T, Sato N, *et al*: Haplotype-based case-control study of adrenomedullin genes on proteinuria in the subjects with essential hypertension. *Hypertens Res* 2005; **28**: 229–236.
15. Nakayama T, Soma M, Rahmutula D, Izumi Y, Kanmatsuse K: Nonsense mutation of prostacyclin synthase gene in a family. *Lancet* 1997; **349**: 1887–1888.
16. Nakayama T, Soma M, Rehemudula D, *et al*: Association of 5′ upstream promoter region of prostacyclin synthase gene variant with cerebral infarction. *Am J Hypertens* 2000; **13**: 1263–1267.
17. Unoki M, Furuta S, Onouchi Y, *et al*: Association studies of 33 single nucleotide polymorphisms (SNPs) in 29 candidate genes for bronchial asthma: positive association a T924C polymorphism in the thromboxane A2 receptor gene. *Hum Genet* 2000; **106**: 440–446.
18. Boehringer S, Epplen JT, Krawczak M: Genetic association studies of bronchial asthma—a need for Bonferroni correction? *Hum Genet* 2000; **107**: 197.
19. Nyholt DR: Genetic case-control association studies—correcting for multiple testing. *Hum Genet* 2001; **109**: 564–567.
20. Hirata T, Kakizuka A, Ushikubi F, Fuse I, Okuma M, Narumiya S: Arg60 to Leu mutation of the human thromboxane A2 receptor in a dominantly inherited bleeding disorder. *J Clin Invest* 1994; **94**: 1662–1667.
21. Francois H, Athirakul K, Mao L, Rockman H, Coffman TM: Role for thromboxane receptors in angiotensin-II–induced hypertension. *Hypertension* 2004; **43**: 364–369.
22. Thomas DW, Mannon RB, Mannon PJ, *et al*: Coagulation defects and altered hemodynamic responses in mice lacking receptors for thromboxane A2. *J Clin Invest* 1998; **102**: 1994–2001.
23. Satoh K, Imaizumi T, Yoshida H, Hiramoto M, Konta A, Takamatsu S: Plasma 11-dehydrothromboxane B2: a reliable indicator of platelet hyperfunction in patients with ischemic stroke. *Acta Neurol Scand* 1991; **83**: 99–102.
24. Koudstaal PJ, Ciabattoni G, van Gijn J, *et al*: Increased thromboxane biosynthesis in patients with acute cerebral ischemia. *Stroke* 1993; **24**: 219–223.
25. Imamura T, Kiguchi S, Kobayashi K, Ichikawa K, Yamazaki Y, Kojima M: Effect of ozagrel, a selective thromboxane A2–synthetase inhibitor, on cerebral infarction in rats. Comparative study with norphenazone, a free-radical scavenger. *Arzneimittelforschung* 2003; **53**: 688–694.
26. Cambria-Kiely JA, Gandhi PJ: Aspirin resistance and genetic polymorphisms. *J Thromb Thrombolysis* 2002; **14**: 51–58.
27. Schork NJ, Fallin D, Lanchbury JS: Single nucleotide polymorphisms and the future of genetic epidemiology. *Clin Genet* 2000; **58**: 250–264.
28. Morris RW, Kaplan NL: On the advantage of haplotype analysis in the presence of multiple disease susceptibility alleles. *Genet Epidemiol* 2002; **23**: 221–233.