Journal of Human Hypertension (2010) 24, 320–326

www.nature.com/jhh

ORIGINAL ARTICLE

Association of insulin-like growth factor-1 receptor gene polymorphisms with left ventricular mass and geometry in essential hypertension

T Horio[1], K Kamide[1], S Takiuchi[1], M Yoshii[1], Y Miwa[1], T Matayoshi[1], F Yoshihara[1], S Nakamura[1], T Tokudome[2], T Miyata[2] and Y Kawano[1]

[1]*Division of Hypertension and Nephrology, Department of Medicine, Suita, Osaka, Japan and* [2]*Research Institute, National Cardiovascular Center, Suita, Osaka, Japan*

Stimulation of insulin-like growth factor (IGF)-1 receptor by IGF-1 and insulin strongly induces cardiomyocyte hypertrophy. In this study, we assessed the hypothesis that genetic variations of the IGF-1 receptor may be linked to the diversity of left ventricular (LV) structure in hypertensive patients. Genotypes in 12 single nucleotide polymorphisms (SNPs) of the IGF-1 receptor gene identified by direct sequencing were determined in 795 Japanese patients with essential hypertension. In echocardiographic examinations, LV mass index (LVMI) and relative wall thickness (RWT) were measured. Among 12 SNPs, promoter −328C>T and intron-13 275124A>C polymorphisms were significantly associated with LV hypertrophy (LVMI $\geqslant$ 125 g m^{-2}) and concentric change (RWT $\geqslant$ 0.44), respectively. In allele frequencies, the C allele of −328C>T was related to LV hypertrophy, and the A allele of 275124A>C was related to LV concentric change. In fact, LVMI and prevalence of LV hypertrophy increased in CC genotype of −328C>T. RWT and prevalence of LV concentric change increased in AA genotype of 275124A>C. A multiple logistic regression analysis revealed that the presence of CC genotype of −328C>T or AA genotype of 275124A>C was an independent determinant for LV hypertrophy or concentric change, respectively. Furthermore, the combination of CC of −328C>T and AA of 275124A>C genotypes was significantly associated with abnormal LV geometry, especially concentric hypertrophy. Our findings show that two SNPs of the IGF-1 receptor gene are related to LV hypertrophy in patients with essential hypertension, suggesting that the genetic variation of the IGF-1 receptor may be involved in the diversity of LV structure in hypertensives.

Journal of Human Hypertension (2010) **24**, 320–326; doi:10.1038/jhh.2009.73; published online 17 September 2009

Keywords: insulin-like growth factor; receptor; gene polymorphism; hypertrophy

Introduction

Left ventricular (LV) hypertrophy, the most common cardiac consequence of hypertension, is considered as a maladaptation to the increased afterload, because it is one of the independent risk factors for cardiovascular morbidity and mortality.[1] However, the haemodynamic load is not the only determinant of LV hypertrophy, because for similar elevations of blood pressure, a wide range of severities and types of LV hypertrophy have been observed.[2,3] Many studies have shown that blood pressure explains only 10 to 25% of the variation in LV mass, supporting the hypothesis that several non-haemodynamic factors, such as genetic and metabolic factors, are involved in the cardiac growth in human hypertension.[4–6]

Hypertension is one of the components of the metabolic syndrome, which is characterized by insulin-resistance and hyperinsulinemia. An earlier study has shown that circulating levels of insulin and insulin-like growth factor (IGF)-1 are associated with LV hypertrophy and geometric change in hypertensive subjects.[7] IGF-1 is a well-known strong promoter of cardiomyocyte growth through its receptors abundantly expressed in myocardium.[8] Insulin may also stimulate cardiomyocyte hypertrophy by binding to the IGF-1 receptors because of the structural similarity between the two molecules.[9,10]

The above considerations prompted us to hypothesize that the genetic variation of the IGF-1 receptor may be linked to the diversity of LV structure in hypertensives. Thus, this study analysed the association of IGF-1 receptor gene polymorphisms with LV mass and geometry in patients with essential hypertension.

Correspondence: Dr T Horio, Division of Hypertension and Nephrology, Department of Medicine, National Cardiovascular Center, 5-7-1, Fujishirodai, Suita, Osaka 565-8565, Japan.
E-mail: thorio@ri.ncvc.go.jp
Received 12 June 2009; accepted 15 July 2009; published online 17 September 2009

Materials and methods

Subjects

A total of 795 Japanese patients with essential hypertension (438 men and 357 women; mean age, 65 ± 11 years) were enroled in this study. Patients with secondary hypertension, myocardial infarction, valvular heart disease, congestive heart failure, atrial fibrillation, or unsatisfactory B-mode and Doppler echocardiograms were excluded from this study. Hypertension was defined as a systolic blood pressure of $\geqslant$140 mm Hg and/or a diastolic blood pressure of $\geqslant$90 mm Hg by repeated measurements or when medication was taken for treatment of hypertension. Diabetes mellitus was diagnosed according to the American Diabetes Association criteria, such as a fasting plasma glucose of $\geqslant$126 mg per 100 ml and/or a plasma glucose level at 2 h after a 75 g oral glucose load of $\geqslant$200 mg per 100 ml, or when medication was taken for treatment of hyperglycaemia.

Among the 795 patients, 726 (91%) were receiving antihypertensive drugs, including combination therapy in some cases. In total, 574 patients (72%) were treated with calcium channel blockers, 407 (51%) with renin angiotensin system inhibitors (that is, angiotensin II receptor blockers and angiotensin converting enzyme inhibitors), 285 (36%) with β-blockers, 181 (23%) with diuretics and 113 (14%) with other classes of agents. In all, 69 patients (9%) were treated with diet and/or exercise therapy (without antihypertensive medication).

All subjects gave their informed consent to participate in this study. The study protocol was approved by the ethical review committee of the National Cardiovascular Center.

Clinical parameters

At the time of the physical examination, blood pressure, heart rate, body mass index and biochemical profiles were determined. Blood pressure and heart rate were measured in the subjects after at least 10 min of rest in a sitting position. Peripheral blood samples were obtained in the morning after an overnight fast. Total cholesterol, triglycerides, fasting plasma glucose, haemoglobin A1c and serum creatinine levels were determined by standard laboratory measurements.

Echocardiographic measurement

A comprehensive two-dimensional echocardiography was performed using a cardiac ultrasound unit (Sonos 5500; Philips Medical Systems, Andover, MA, USA) as described earlier.[11,12] Echocardiographic parameters were measured by the consensus of two experienced investigators who were blinded to the clinical data of the subjects. Measurements, such as interventricular septal thickness (IVSTd), posterior wall thickness (PWTd), LV diameter at end-diastole (LVDd) and LV diameter at end-systole (LVDs). Fractional shortening was calculated as (LVDd–LVDs)/LVDd. Relative wall thickness (RWT) was calculated as (IVSTd + PWTd)/LVDd. LV mass was estimated using the formula validated by Devereux and Reichek:[13] LV mass (g) = $1.04 \times \{(\mathrm{IVSTd} + \mathrm{PWTd} + \mathrm{LVDd})^3 - \mathrm{LVDd}^3\} - 13.6$. LV mass was normalized for body surface area and expressed as LV mass index (LVMI). LV hypertrophy and LV concentric change were defined as a LVMI of $\geqslant$125 g m^{-2} and a RWT of $\geqslant$0.44, respectively.[2,3] The geometry of LV was stratified into four different patterns according to the values of LVMI ($<$ or $\geqslant$125 g m^{-2}) and RWT ($<$ or $\geqslant$0.44). Patients with increased LVMI and increased RWT were considered to have concentric hypertrophy, and those with increased LVMI and normal RWT were considered to have eccentric hypertrophy. Those with normal LVMI and increased or normal RWT were considered to have concentric remodelling or normal geometry, respectively.

To assess LV diastolic function, the diastolic filling of LV (LV inflow) was examined using Doppler echocardiography.[14] The peak velocity of early diastolic filling (*E*) and the peak velocity of atrial filling (*A*) were recorded and the *E* to *A* ratio (*E*/*A*) was calculated. The deceleration time was measured as the time between the top of the *E* wave and the point at which the descending part of the *E* wave or its asymptote crossed the zero line.

Detection and genotyping of single nucleotide polymorphisms (SNPs)

First, peripheral blood samples were obtained from 96 patients with essential hypertension, and all exons, part of the intron segments, and the promoter regions of the IGF-1 receptor gene were sequenced for the detection of SNPs. The method of direct sequencing has been described earlier.[15,16] The identified polymorphisms were numbered from the *A* of the initiator codon (ATG), according to the recommendations of the Nomenclature Working Group for human gene mutations.[17] Among all SNPs identified by sequencing, common SNPs with a minor allele frequency of $>$5% were chosen, and one SNP from several SNPs with tight linkage disequilibrium ($r^2 \geqslant 0.5$) was selected for genotyping. As a result, 12 SNPs were genotyped using the TaqMan-polymerase chain reaction system for all patients, as described earlier.[18,19]

Statistical analysis

The values are expressed as mean ± s.d. To assess the association of IGF-1 receptor SNPs with LV structural change, differences in frequency among the groups were tested by χ^2 analysis. An unpaired Student's *t*-test was used for comparison of clinical and echocardiographic parameters between the two groups with different SNP genotypes. A multiple

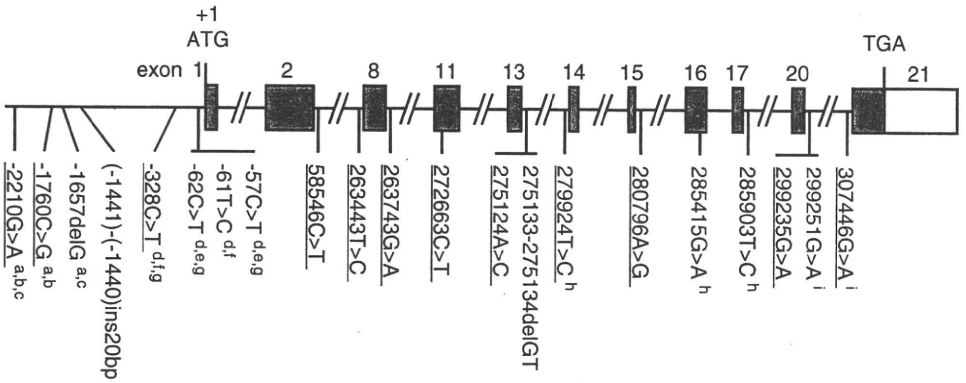

Figure 1 The locations of identified and genotyped single nucleotide polymorphisms (SNPs) in the insulin-like growth factor (IGF)-1 receptor gene. Only SNPs of which allele frequency is >5% are shown. ATG represents the initiation codon, and TGA represents the stop codon. a, b, c, d, e, f, g, h and i indicate haplotype blocks with tight linkage disequilibrium ($r^2>0.5$), and the underlined 12 SNPs were genotyped in this study. In all, 11 of 12 genotyped SNPs had been recorded in public databases (dbSNPs); that is, −2210G>A was identical to dbSNP ID rs8034564, −328C>T to rs13379905, 58546C>T to rs7174918, 263443T>C to rs2272037, 263743G>A to rs951715, 272663C>T to rs3743262, 275124A>C to rs1464430, 279924T>C to rs4486868, 280796A>G to rs2229765, 299235G>A to rs2684789 and 307446G>A to rs2593053.

logistic regression analysis was performed to identify the independent relation of some SNPs to LV hypertrophy and geometric change. Hardy–Weinberg equilibrium was calculated using a χ^2 test. A value of $P<0.05$ was accepted as statistically significant. All analyses were performed using StatView Version 5 Software (Abacus Concepts Inc., Berkeley, CA, USA). Linkage disequilibrium was evaluated by obtaining an r^2 value between polymorphisms using the SNP Alyze ver. 2.0 software (DYNACOM Co, Ltd, Shigehara, Japan).

Results

Locations of identified and genotyped SNPs in the IGF-1 receptor gene were shown in Figure 1. In total, 12 SNPs (three SNPs in promoter, one in exon and eight in intron regions) in the IGF-1 receptor gene were genotyped in the present study. In all, 11 of 12 SNPs had been recorded in public databases (dbSNPs, http://www.ncbi.nlm.nih.gov/SNP/), and the remaining one SNP (−1760C>G) was novel. The genotype distribution of all analysed SNPs did not significantly deviate from the Hardy–Weinberg expectation.

The association of SNP genotypes in the IGF-1 receptor gene with LV structural change was assessed by χ^2 analysis (Table 1). Among 12 SNPs, genotype frequencies of promoter −328C>T and intron-13 275124A>C polymorphisms were significantly associated with LV hypertrophy ($\chi^2=7.513$ and $P=0.023$) and LV concentric change ($\chi^2=7.949$ and $P=0.019$), respectively. Similarly, in allele frequencies, the C allele of −328C>T had a significant relation to LV hypertrophy (odds ratio 1.78, $\chi^2=6.828$ and $P=0.009$), and the A allele of 275124A>C was related to LV concentric change (odds ratio 1.38, $\chi^2=7.259$ and $P=0.007$).

Next, we compared clinical characteristics and echocardiographic parameters between different genotype groups of IGF-1 receptor −328C>T and 275124A>C polymorphisms. There were no significant differences in clinical parameters, such as age, sex, body mass index, hypertension duration, blood pressure and the use of antihypertensive agents between the two subject groups with CC ($n=702$) and CT+TT ($n=92$) of −328C>T (Table 2). However, LV wall thickness, LVMI, and the prevalence of LV hypertrophy were significantly increased in the group with CC genotype. LV systolic and diastolic function (fractional shortening, *E*/*A* ratio and deceleration time) did not differ between the two groups. As for 275124A>C polymorphism, similarly, no significant differences were found in clinical characteristics between the two-genotype groups with AA ($n=470$) and AC+CC ($n=321$) (Table 3).

Table 1 Association of SNP genotypes in the IGF-1 receptor gene with LV structural change

SNP	*Region*	*LV hypertrophy (LVMI≥ 125 g m^{-2})*		*LV concentric change (RWT≥0.44)*	
		χ^2	P	χ^2	P
−2210G>A	Promoter	1.561	0.458	1.550	0.461
−1760C>G	Promoter	0.480	0.787	0.403	0.818
−328C>T	Promoter	7.513	0.023	2.186	0.335
58546C>T	Intron 2	1.691	0.429	1.921	0.383
263443T>C	Intron 7	2.160	0.340	1.494	0.474
263743G>A	Intron 8	1.313	0.519	2.475	0.290
272663C>T	Exon 11	0.582	0.747	3.879	0.144
275124A>C	Intron 13	4.897	0.086	7.949	0.019
279924T>C	Intron 13	2.032	0.362	1.946	0.378
280796A>G	Intron 15	1.597	0.450	3.205	0.201
299235G>A	Intron 20	4.565	0.102	0.164	0.922
307446G>A	Intron 20	3.274	0.195	0.062	0.969

Abbreviations: IGF, insulin-like growth factor; LV, left ventricular; LVMI, left ventricular mass index; RWT, relative wall thickness; SNP, single nucleotide polymorphism.

Table 2 Comparison of clinical characteristics and echocardiographic parameters between the two groups with CC and CT+TT of IGF-1 receptor −328C>T polymorphism

	CC (n = *702*)	*CT+TT* (n = *92*)	P
Age, years	65 ± 10	63 ± 11	0.070
Sex (male), %	55	54	0.867
Body mass index, kg m^{-2}	24.4 ± 3.4	24.0 ± 3.3	0.278
Duration of hypertension, years	18 ± 11	17 ± 11	0.154
Diabetes mellitus, %	23	26	0.522
Fasting plasma glucose, mg per 100 ml	104 ± 20	102 ± 24	0.393
Haemoglobin A1c, %	5.7 ± 0.8	5.7 ± 0.8	0.908
Total cholesterol, mg per 100 ml	202 ± 32	207 ± 37	0.164
Triglycerides, mg per 100 ml	136 ± 118	149 ± 110	0.318
Serum creatinine, mg per 100 ml	1.0 ± 1.1	1.0 ± 1.1	0.999
Systolic blood pressure, mmHg	146 ± 19	143 ± 19	0.185
Diastolic blood pressure, mmHg	85 ± 13	83 ± 14	0.264
Heart rate, b.p.m.	70 ± 11	69 ± 8	0.702
Antihypertensive treatment			
Ca channel blockers, %	72	72	0.900
RAS inhibitors, %	52	48	0.500
β-blockers, %	36	36	0.996
Diuretics, %	24	16	0.121
Others, %	15	12	0.507
Total number of classes	2.0 ± 1.1	1.8 ± 1.1	0.264
Echocardiographic parameters			
IVSTd, mm	10.9 ± 2.0	10.4 ± 1.8	0.020
PWTd, mm	10.7 ± 1.8	10.3 ± 1.5	0.046
LVDd, mm	46.6 ± 5.0	45.8 ± 4.8	0.125
LVDs, mm	28.4 ± 5.4	28.1 ± 5.3	0.598
Fractional shortening	0.39 ± 0.08	0.39 ± 0.07	0.566
LVMI, g m^{-2}	130 ± 39	119 ± 28	0.007
RWT	0.47 ± 0.09	0.46 ± 0.08	0.323
Prevalence of LV hypertrophy, %	48	35	0.019
Prevalence of LV concentric change, %	58	52	0.256
E/A ratio	0.87 ± 0.32	0.88 ± 0.30	0.812
Deceleration time, ms	215 ± 48	217 ± 48	0.673

Abbreviations: IGF, insulin-like growth factor; IVSTd, interventricular septal thickness at end-diastole; LV, left ventricular; LVDd, left ventricular diameter at end-diastole; LVDs, left ventricular diameter at end-systole; LVMI, left ventricular mass index; PWTd, posterior wall thickness at end-diastole; RAS, renin–angiotensin system; RWT, relative wall thickness.
RAS inhibitors represent angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors. Values are mean ± s.d. or percentage.

Table 3 Comparison of clinical characteristics and echocardiographic parameters between the two groups with AA and AC+CC of IGF-1 receptor 275124A>C polymorphism

	AA (n = *470*)	*AC+CC* (n = *321*)	P
Age, years	66 ± 10	65 ± 11	0.209
Sex (male), %	55	56	0.831
Body mass index, kg m^{-2}	24.4 ± 3.3	24.4 ± 3.5	0.859
Duration of hypertension, years	18 ± 11	18 ± 11	0.758
Diabetes mellitus, %	24	23	0.672
Fasting plasma glucose, mg per 100 ml	104 ± 21	104 ± 21	0.820
Haemoglobin A1c, %	5.7 ± 0.8	5.7 ± 0.9	0.926
Total cholesterol, mg per 100 ml	202 ± 32	202 ± 34	0.887
Triglycerides, mg per 100 ml	138 ± 128	137 ± 100	0.902
Serum creatinine, mg per 100 ml	1.0 ± 1.0	1.0 ± 1.3	0.542
Systolic blood pressure, mmHg	145 ± 18	146 ± 20	0.428
Diastolic blood pressure, mmHg	84 ± 13	86 ± 14	0.113
Heart rate, b.p.m.	70 ± 10	70 ± 10	0.554
Antihypertensive treatment			
Ca channel blockers, %	73	71	0.548
RAS inhibitors, %	52	50	0.628
β-blockers, %	37	34	0.346
Diuretics, %	25	20	0.072
Others, %	14	15	0.516
Total number of classes	2.0 ± 1.1	1.9 ± 1.2	0.191
Echocardiographic parameters			
IVSTd, mm	10.9 ± 1.9	10.6 ± 2.0	0.036
PWTd, mm	10.7 ± 1.6	10.5 ± 1.9	0.334
LVDd, mm	46.5 ± 4.9	46.7 ± 5.1	0.500
LVDs, mm	28.3 ± 5.2	28.5 ± 5.7	0.587
Fractional shortening	0.39 ± 0.07	0.39 ± 0.08	0.981
LVMI, g m^{-2}	130 ± 37	127 ± 40	0.276
RWT	0.47 ± 0.08	0.45 ± 0.09	0.047
Prevalence of LV hypertrophy, %	49	42	0.069
Prevalence of LV concentric change, %	61	53	0.027
E/A ratio	0.86 ± 0.32	0.89 ± 0.31	0.389
Deceleration time, msec	214 ± 45	216 ± 51	0.652

Abbreviations: IGF, insulin-like growth factor; IVSTd, interventricular septal thickness at end-diastole; LV, left ventricular; LVDd, left ventricular diameter at end-diastole; LVDs, left ventricular diameter at end-systole; LVMI, left ventricular mass index; PWTd, posterior wall thickness at end-diastole; RAS, renin–angiotensin system; RWT, relative wall thickness.
RAS inhibitors represent angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors. Values are mean ± s.d. or percentage.

Among echocardiographic parameters, interventricular septal thickness, RWT and the prevalence of LV concentric change were significantly increased in the group with AA genotype, although LV dimension, LVMI, and LV systolic and diastolic function did not differ between the two groups.

To confirm whether the influence of these specific SNP genotypes in the IGF-1 receptor gene on LV structural changes was independent of various clinical parameters, we analysed possible predictive factors using a multiple logistic regression analysis in all subjects. As shown in Table 4, the presence of CC genotype of −328C>T was a significant predictor of LV hypertrophy, independent of age, sex, body mass index, hypertension duration, complication of diabetes mellitus, and systolic and diastolic blood pressure (odds ratio 1.67 vs CT + TT and $P = 0.033$). In addition, AA genotype of 275124A>C was found to be an independent determinant for LV concentric change (odds ratio 1.37 vs AC + CC and $P = 0.039$).

Finally, the combined effect of CC of −328C>T and AA of 275124A>C genotypes on LV geometric patterns was analysed. The patient group with both CC genotype of −328C>T and AA genotype of 275124A>C (group 2, $n = 424$) had a significantly higher rate of LV concentric hypertrophy compared with the other subjects (group 1, $n = 366$) (Figure 2). In contrast, the rate of patients with normal geometry was significantly lower in group 2 than in group 1. There were no differences in basal

Table 4 Independent relation of two IGF-1 receptor SNPs to LV hypertrophy and concentric change by multiple logistic regression analysis

	LV hypertrophy (LVMI ≥ 125 g m^{-2})		*LV concentric change (RWT ≥ 0.44)*	
	OR (95% CI)	P	*OR (95% CI)*	P
Age, 10 years	1.14 (0.97–1.34)	0.101	1.13 (0.97–1.33)	0.121
Sex, male	1.97 (1.44–2.68)	<0.001	1.38 (1.01–1.87)	0.040
Body mass index, 1 kg m^{-2}	1.06 (1.01–1.10)	0.020	1.09 (1.04–1.14)	<0.001
Hypertension duration, 1 year	1.01 (0.99–1.03)	0.168	1.01 (1.00–1.03)	0.052
Diabetes mellitus, yes	1.21 (0.84–1.73)	0.304	1.25 (0.86–1.82)	0.235
Systolic blood pressure, 10 mm Hg	1.14 (1.03–1.27)	0.015	1.06 (0.95–1.18)	0.328
Diastolic blood pressure, 10 mm Hg	0.84 (0.72–0.97)	0.019	0.86 (0.74–1.00)	0.051
IGF-1 receptor SNP genotype				
CC of −328C>T	1.67 (1.03–2.69)	0.033	1.19 (0.76–1.89)	0.449
AA of 275124A>C	1.22 (0.91–1.65)	0.187	1.37 (1.01–1.85)	0.039

Abbreviations: CI, confidence interval; IGF, insulin-like growth factor; LV, left ventricular; LVMI, left ventricular mass index; OR, odds ratio; RWT, relative wall thickness; SNP, single nucleotide polymorphism.

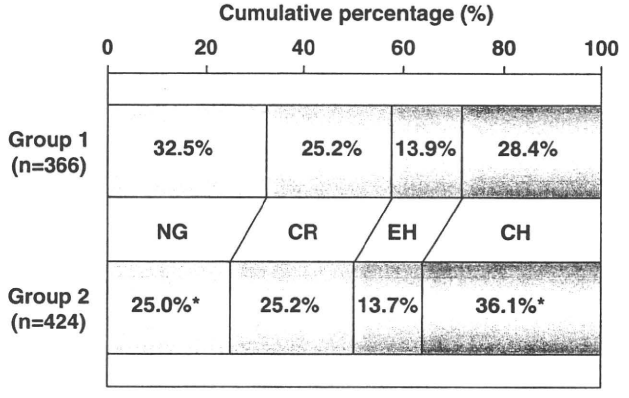

Figure 2 The combined effect of CC of −328C>T and AA of 275124A>C genotypes in the insulin-like growth factor (IGF)-1 receptor gene on left ventricular (LV) geometric patterns. Group 2 indicates the subjects with both CC genotype of −328C>T and AA genotype of 275124A>C, and the other subjects (that is, with CT+TT of −328C>T and/or AC+CC of 275124A>C) belong to group 1. CR, concentric remodelling (normal LVMI and increased RWT); CH, concentric hypertrophy (increased LVMI and RWT); EH, eccentric hypertrophy (increased LVMI and normal RWT); NG, normal geometry (normal LVMI and RWT). *$P<0.05$ compared with group 1.

clinical characteristics and antihypertensive treatment between the two groups (data not shown).

Discussion

Earlier studies have revealed that a promoter polymorphism in the IGF-1 gene is related to several cardiovascular complications, such as myocardial infarction, heart failure and cardiac hypertrophy.[20–23] On the other hand, the genetic variations in the IGF-1 receptor have been shown to be associated with non-cardiovascular human disorders, such as growth retardation, cancer, dementia and osteoporosis.[24–27] However, because IGF-1 receptor expression is also observed in the cardiovascular system, especially abundantly in the heart, the possible association of IGF-1 receptor gene polymorphisms with cardiac structure and function should be elucidated. In this study, we showed that two SNPs of the IGF-1 receptor gene, promoter −328C>T and intron-13 275124A>C, were significantly associated with LV hypertrophy in hypertensive patients. Thus, this is the first study that reported the significant influence of the IGF-1 receptor gene variation on cardiac hypertrophic change in human hypertension.

This study also showed that the combination of IGF-1 receptor −328C>T and 275124A>C polymorphisms was related to specific patterns of LV geometry. The patients with both CC genotype of −328C>T and AA genotype of 275124A>C had a significantly higher rate of LV concentric hypertrophy compared with the other subjects. These findings suggest the possibility that blood pressure level-independent diversity of LV structure in hypertensives is partially attributable to the genetic variation of the IGF-1 receptor. In addition, as for the association between LV geometry and cardiovascular prognosis, hypertensive patients with concentric hypertrophy among four LV geometric patterns have the highest incidence of cardiovascular events and death.[2,28] Therefore, having both CC genotype of −328C>T and AA genotype of 275124A>C may be a risk marker for poor cardiovascular prognosis in hypertensive subjects.

Our earlier *in vitro* study showed that IGF-1 promotes not only hypertrophy of cardiomyocytes but also collagen production by cardiac fibroblasts.[29] As cardiac fibrosis induces the deterioration of LV function, we examined whether −328C>T and 275124A>C polymorphisms were associated with LV systolic and diastolic dysfunction as well as LV hypertrophy. However, neither systolic function (fractional shortening) nor diastolic function (*E*/*A* ratio and deceleration time) had a significant association with these SNPs. Therefore, it is unlikely that LV function, apart from LV hypertrophy, may be influenced by IGF-receptor gene polymorphisms.

This study has not provided specific information regarding the mechanism by which the observed SNPs of the IGF-1 receptor gene influence LV mass and geometry. That is, it remains unclear whether these SNPs are functional or just risk markers. As −328C>T polymorphism is present in the promoter region, this SNP may affect the expression level of the IGF-1 receptor gene. On the other hand, 275124A>C polymorphism in intron-13 does not functionally affect the expression of the IGF-1 receptor protein. Further studies will be necessary to clarify the function of these polymorphisms or to identify the causative polymorphisms that are in linkage disequilibrium with these polymorphisms.

In addition, it remains to be elucidated whether the significant association of IGF-1 receptor gene polymorphisms with LV hypertrophy observed in hypertensive patients is also seen in other cardiac diseases, such as hypertrophic cardiomyopathy, valvular heart disease and old myocardial infarction.

In conclusion, this study showed that two SNPs of the IGF-1 receptor gene, promoter −328C>T and intron-13 275124A>C, were significantly associated with LV hypertrophy, and that the combination of these two polymorphisms was related to specific patterns of LV geometry in patients with essential hypertension. Thus, the genetic variation of the IGF-1 receptor may be involved in the diversity of LV structure in hypertensives, particularly in the progression of LV concentric hypertrophy.

What is known about this topic

- Blood pressure explains only 10–25% of the variation in left ventricular mass, suggesting that non-haemodynamic factors are involved in the cardiac growth in human hypertension.
- Insulin-like growth factor (IGF)-1 is a strong promoter of cardiomyocyte growth through its receptors abundantly expressed in myocardium.
- Circulating levels of IGF-1 are associated with left ventricular hypertrophy and geometric change in hypertensive subjects.

What this study adds

- Two single nucleotide polymorphisms of the IGF-1 receptor gene, promoter −328C>T and intron-13 275124A>C, were associated with left ventricular hypertrophy in patients with essential hypertension.
- The genetic variation of the IGF-1 receptor may be involved in the diversity of left ventricular structure in hypertensives.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO) of Japan, and research grant from the Japanese Ministry of Health, Labor, and Welfare.

References

1 Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990; **322**: 1561–1566.
2 Koren MJ, Devereux RB, Casale PN, Savage DD, Laragh JH. Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. *Ann Intern Med* 1991; **114**: 345–352.
3 Ganau A, Devereux RB, Roman MJ, de Simone G, Pickering TG, Saba PS *et al.* Patterns of left ventricular hypertrophy and geometric remodeling in essential hypertension. *J Am Coll Cardiol* 1992; **19**: 1550–1558.
4 Morgan HE, Baker KM. Cardiac hypertrophy: mechanical, neural, and endocrine dependence. *Circulation* 1991; **83**: 13–25.
5 Schunkert H, Hense HW, Holmer SR, Stender M, Perz S, Keil U *et al.* Association between a deletion polymorphism of the angiotensin-converting-enzyme gene and left ventricular hypertrophy. *N Engl J Med* 1994; **330**: 1634–1638.
6 de Simone G, Palmieri V, Bella JN, Celentano A, Hong Y, Oberman A *et al.* Association of left ventricular hypertrophy with metabolic risk factors: the HyperGEN study. *J Hypertens* 2002; **20**: 323–331.
7 Verdecchia P, Reboldi G, Schillaci G, Borgioni C, Ciucci A, Telera MP *et al.* Circulating insulin and insulin growth factor-1 are independent determinants of left ventricular mass and geometry in essential hypertension. *Circulation* 1999; **100**: 1802–1807.
8 Ito H, Hiroe M, Hirata Y, Tsujino M, Adachi S, Shichiri M *et al.* Insulin-like growth factor-I induces hypertrophy with enhanced expression of muscle specific genes in cultured rat cardiomyocytes. *Circulation* 1993; **87**: 1715–1721.
9 Tokudome T, Horio T, Yoshihara F, Suga S, Kawano Y, Kohno M *et al.* Different effects of high glucose and insulin on cultured cardiac myocyte hypertrophy and fibroblast proliferation. *Metabolism* 2004; **53**: 710–715.
10 Straus DS. Growth-stimulatory actions of insulin *in vitro* and *in vivo*. *Endocr Rev* 1984; **5**: 356–369.
11 Iwashima Y, Horio T, Kamide K, Rakugi H, Ogihara T, Kawano Y. Uric acid, left ventricular mass index, and risk of cardiovascular disease in essential hypertension. *Hypertension* 2006; **47**: 195–202.
12 Tomiyama M, Horio T, Kamide K, Nakamura S, Yoshihara F, Nakata H *et al.* Reverse white-coat effect as an independent risk for left ventricular concentric hypertrophy in patients with treated essential hypertension. *J Hum Hypertens* 2007; **21**: 212–219.
13 Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man: anatomic validation of the method. *Circulation* 1977; **55**: 613–618.
14 Iwashima Y, Horio T, Kamide K, Rakugi H, Ogihara T, Kawano Y. Pulmonary venous flow and risk of cardiovascular disease in essential hypertension. *J Hypertens* 2008; **26**: 798–805.
15 Okuda T, Fujioka Y, Kamide K, Kawano Y, Goto Y, Yoshimasa Y *et al.* Verification of 525 coding SNPs in 179 hypertension candidate genes in the Japanese population: identification of 159 SNPs in 93 genes. *J Hum Genet* 2002; **47**: 387–394.

16 Yang J, Kamide K, Kokubo Y, Takiuchi S, Tanaka C, Banno M *et al.* Genetic variations of regulator of G-protein signaling 2 in hypertensive patients and in the general population. *J Hypertens* 2005; **23**: 1497–1505.
17 Antonarakis SE. Recommendations for a nomenclature system for human gene mutations. Nomenclature Working Group. *Hum Mutat* 1998; **11**: 1–3.
18 Tanaka C, Kamide K, Takiuchi S, Miwa Y, Yoshii M, Kawano Y *et al.* An alternative fast and convenient genotyping method for the screening of angiotensin converting enzyme gene polymorphisms. *Hypertens Res* 2003; **26**: 301–306.
19 Yasuda H, Kamide K, Takiuchi S, Matayoshi T, Hanada H, Kada A *et al.* Association of single nucleotide polymorphisms in endothelin family genes with the progression of atherosclerosis in patients with essential hypertension. *J Hum Hypertens* 2007; **21**: 883–892.
20 Vaessen N, Heutink P, Janssen JA, Witteman JC, Testers L, Hofman A *et al.* A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. *Diabetes* 2001; **50**: 637–642.
21 Bleumink GS, Rietveld I, Janssen JA, van Rossum EF, Deckers JW, Hofman A *et al.* Insulin-like growth factor-I gene polymorphism and risk of heart failure (the Rotterdam Study). *Am J Cardiol* 2004; **94**: 384–386.
22 Bleumink GS, Schut AF, Sturkenboom MC, Janssen JA, Witteman JC, van Duijn CM *et al.* A promoter polymorphism of the insulin-like growth factor-I gene is associated with left ventricular hypertrophy. *Heart* 2005; **91**: 239–240.
23 Schut AF, Janssen JA, Deinum J, Vergeer JM, Hofman A, Lamberts SW *et al.* Polymorphism in the promoter region of the insulin-like growth factor I gene is related to carotid intima-media thickness and aortic pulse wave velocity in subjects with hypertension. *Stroke* 2003; **34**: 1623–1627.
24 Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E *et al.* IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med* 2003; **349**: 2211–2222.
25 Chen C, Freeman R, Voigt LF, Fitzpatrick A, Plymate SR, Weiss NS. Prostate cancer risk in relation to selected genetic polymorphisms in insulin-like growth factor-I, insulin-like growth factor binding protein-3, and insulin-like growth factor-I receptor. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 2461–2466.
26 Garcia J, Ahmadi A, Wonnacott A, Sutcliffe W, Nagga K, Soderkvist P *et al.* Association of insulin-like growth factor-1 receptor polymorphism in dementia. *Dement Geriatr Cogn Disord* 2006; **22**: 439–444.
27 Lee DO, Jee BC, Ku SY, Suh CS, Kim SH, Choi YM *et al.* Relationships between the insulin-like growth factor I (IGF-I) receptor gene G3174A polymorphism, serum IGF-I levels, and bone mineral density in postmenopausal Korean women. *J Bone Miner Metab* 2008; **26**: 42–46.
28 Krumholz HM, Larson M, Levy D. Prognosis of left ventricular geometric patterns in the Framingham Heart Study. *J Am Coll Cardiol* 1995; **25**: 879–884.
29 Horio T, Maki T, Kishimoto I, Tokudome T, Okumura H, Yoshihara F *et al.* Production and autocrine/paracrine effects of endogenous insulin-like growth factor-1 in rat cardiac fibroblasts. *Regul Pept* 2005; **124**: 65–72.

2. ProGEAR研究

国立循環器病センター研究所病因部部長 宮田 敏行 (Miyata, Toshiyuki)
国立循環器病センター研究所病因部室員 嘉田 晃子 (Kada, Akiko)
国立循環器病センター輸血管理室医長 宮田 茂樹 (Miyata, Shigeki)
名古屋市立大学大学院薬学研究科助教 岡田 浩美 (Okada, Hiromi)
国立循環器病センター脳内科医長 長束 一行 (Nagatsuka, Kazuyuki)

THROMBOSIS and Circulation

§論文のポイント

[1] アスピリン投与にもかかわらず血小板機能が効果的に抑制されない状態をアスピリンレジスタンスと呼ぶ.

[2] アスピリンレジスタンスを示す患者は血栓塞栓症を発症するリスクが高いと報告されているが, その統一された定義ならびに原因について, 明確にされていない.

[3] ProGEAR研究は, 2次予防としてアスピリン投与を受けている患者群を対象に, アスピリンレジスタンスに関する各種の因子に検討を加えることにより, アスピリンレジスタンスの発症要因, 発症頻度, 予後ならびに遺伝子背景を明らかにするための多施設共同前向き観察研究である.

[4] ProGEAR研究の患者登録は終了した. 今後, 2次イベント, 検査学的パラメーター, 遺伝子多型の間の関連解析を行う.

§キーワード

脳梗塞・急性冠症候群／アスピリンレジスタンス／血小板凝集能／血清トロンボキサン B_2／ゲノム網羅的遺伝子解析

はじめに

近年，アスピリン投与にもかかわらず血小板機能が効果的に抑制されない患者群がアスピリン投与群の数％から20％以上の割合で存在するとの報告がある[1)2)]．これらの患者群は，アスピリンに感受性を示す患者群と比較して，血栓塞栓症を発症するリスクが高いことが報告されている．しかしながら，アスピリンレジスタンスの統一された定義ならびにその原因について，いまだに明確にされていない[3)-5)]．

私達は，脳梗塞ならびに急性冠症候群に対する2次予防としてアスピリン投与を受けている患者群を対象に，アスピリンレジスタンスの発症要因，発症頻度，予後ならびに遺伝子背景を明らかにするため，多施設共同前向き観察研究 The Study on Profile and Genetic factors of Aspirin Resistance (ProGEAR study)「アスピリンレジスタンスの実態ならびにその遺伝子背景に関する研究」を計画し，2005年より研究を開始した．本研究は，アスピリンレジスタンスの発症要因などを明らかにすることで，個別医療の実現を目指すものである．本研究は，安価なアスピリンの安全・安心な服薬につながり，本研究結果から得られる社会的・経済的効果は著しいと考えられる．

ProGEAR研究参加23医療機関は次の通りである．中村記念病院，広南病院，東宝塚さとう病院，大西脳神経外科病院，奈良県立医科大学，自治医科大学，東京女子医科大学，日本医科大学，東京都保健医療公社荏原病院，大阪大学，香川大学，北海道脳神経外科記念病院，熊本大学，国立病院機構福岡東医療センター，三重大学，佐渡総合病院，川崎医科大学，帝京大学，名古屋市立大学，国立循環器病センター，東海大学，国立病院機構嬉野医療センター，秋田県立脳血管センター．

ProGEAR研究の対象者

ProGEAR研究対象者は，以下の①～③の基準を満たす症例とした．①脳梗塞(心原性脳塞栓症を除く)／一過性脳虚血発作および急性冠症候群の2次予防としてアスピリンの投与を受けている長期服薬患者(服薬開始後1ヵ月以上，最終イベント後1ヵ月以上で，最終イベントから2年以内，可能な限り最終イベントから6ヵ月以内にエントリーする，他の抗血小板薬やワルファリンは服用していない)，②登録時年齢20歳以上，③患者自身から文書により同意を得られた患者．症例の除外基準は，以下の基準に1つでも該当する場合とした．①悪性腫瘍のある患者，あるいはその疑いのある症例，②血小板数が10万/μL以下もしくは45万/μL以上，③先天性出血性素因のある患者，④他の抗血小板薬やワルファリンの服用者，⑤心房細動のある患者，⑥登録2週間前から登録までに手術やカテーテルインターベンションなどの処置をした患者，⑦登録2週間前から登録までにヘパリン(低分子ヘパリン)，ダナパロイド等の抗凝固薬の投与を受けた患者，⑧Modified Rankin scale 4以上(中等度から重度の障害：援助なしでは歩行できず，身の回りのこともできない)の患者．

症例数の検討は次のように行った．2006年に発表された自治医科大学のアスピリンレジスタンスに関する報告では，2次イベントに関するハザード比が8と報告された[6)]．そこで，アスピリンレジスタンスの頻度を20％，両群合わせたイベント発症率を15％とし，ハザード比を2，3，4とした場合，登録数600例を想定すると，検出力は68％，95％，99％と計算された．これをもとに，600例を収集目標とした．

ProGEAR研究で収集する項目

脳梗塞ならびに急性冠症候群に対する2次予防としてアスピリン投与を受けている患者群を対象に，アラキドン酸(2濃度)とコラーゲン(2濃度)を用いた血小板凝集能，血清トロンボキサンB_2量，尿11-デヒドロトロンボキサンB_2量，ずり応力下での血小板凝集能(国立循環器病センターのみ)を測定する．これら各種因子により，検査学的アスピリンレジスタンスのリスクを評価する．登録開始後2年間を観察期間とし，この間にイベント発症(脳梗塞，一過性脳虚血発作，心筋梗塞，その他血栓塞栓症の発症，心血管疾患による死亡)の有無を追跡する．また，以下の評価項目の情報を収集する．患者背景，アスピリンの種類(腸溶錠かどうか)，服薬量，服薬期間，compliance (問診ならびに，空腹時でない場合として外来に来院されたときに，抜き打ちでサリチル酸濃度を1回測定する)，併用薬として

nonsteroidal anti-inflammatory drugs (NSAIDs), プロトンポンプインヒビター, スタチン, アンジオテンシンⅡ受容体ブロッカーの服薬を調査する. アスピリンレジスタンスの候補遺伝子として, プロスタグランジン受容体関連遺伝子6個およびプロスタグランジン合成酵素関連遺伝子9個, 計15個を選び, 収集した遺伝子試料を対象に遺伝子解析を行う. ゲノム網羅的解析は, 蛋白質コード遺伝子領域を中心に約50万ヵ所の一塩基多型(SNPs)を搭載した遺伝子多型チップ Illumina Human Exon 510S-Duo BeadChip を用いて行う. 試料の施設外への搬出には, Material transfer agreement を取り交わし, 試料管理の責任の所在を明らかにして行う.

これらのデータを用いて, アスピリンレジスタンスの定義を, ①イベント発症の有無(臨床的アスピリンレジスタンス), および, ②生体内でアスピリンの効き目を追跡できる残存血小板凝集能やCOXの代謝産物の量(検査学的アスピリンレジスタンス)とし, アスピリンレジスタンスに影響を与える因子を解析する.

ProGEAR研究の成果(途中経過)

研究全体は終了していないが, これまでに次の3点が終了した. ①全国23施設の前向き観察研究として, 患者592名の登録を終了した. ②患者の心血管イベント発症や併用薬等の各種情報に関して追跡調査を進めた. ③遺伝子試料のゲノム網羅的解析およびアスピリンの作用機序を考慮した候補遺伝子の塩基配列解析を終了した.

1. 患者登録と追跡調査

患者592名の登録を終了した. 登録数は目標である600例にわずかながら届かなかったが, 99%の登録目標達成率であった. これまでのイベント発症の頻度は, 欧米でのメタアナリシスで報告された値に近い値を得ている. このことは, 日本人においてもアスピリンの服薬にもかかわらず, 心血管イベントの再発が少なからず発生していることを意味している.

2. 血小板凝集能など

コラーゲン惹起血小板凝集能は広い分布を示し, 50%以上残存している患者はコラーゲン濃度により大きく異なった. 血清トロンボキサン B_2 と尿11-デヒドロトロンボキサン B_2 は上位20%でアスピリンレジスタンスを評価する. 尿11-デヒドロトロンボキサン B_2 量は比較的広い分布を示した. アスピリンはCOX-1の阻害薬なので, アスピリン服薬患者は全例においてアラキドン酸惹起血小板凝集能が強く抑制されるべきであるが, 本研究では数%の患者に残存した凝集能を検出した. このことは, アスピリンの服薬が遵守されていない可能性によっても説明できる. 本研究では, 抜き打ちで血中サリチル酸量を測定し, 服薬コンプライアンスの評価を行っている. これは, 他の研究がいまだなしえていない本研究における重要な特徴の1つであり, 今後, 服薬コンプライアンスとアラキドン酸惹起血小板凝集能残存との関係について, 注意深く評価を行っていく予定である.

3. 遺伝子解析

ゲノム網羅的解析は遺伝子多型チップを用いて行った. また, アスピリンレジスタンスの候補遺伝子として, 15遺伝子を選び収集した遺伝子試料を対象に塩基配列再解析およびタイピングを行った. アスピリンで活性が阻害される *PTGS1 (COX-1)* 遺伝子の全蛋白質コード領域を, シークエンスした結果, 約8.4%の患者に12個のミスセンス変異を同定した. *PTGS2 (COX-2)* 遺伝子の全蛋白質コード領域のシークエンスでは, 約6.6%の患者に6個のミスセンス変異を同定した. その他の受容体関連遺伝子6個(*PTGER1*, *PTGER2*, *PTGER3*, *PTGER4*, *PTGIR*, *TBXA2R*)およびプロスタグランジン合成酵素関連遺伝子7個(*PTGIS*, *TBXAS1*, *PGDS*, *PTGDS*, *PTGES*, *PTGES2*, *ALOX5AP*)の塩基配列再解析を行い, 詳細な遺伝子変異・多型の同定を行った. 同定された遺伝子変異のうち, 蛋白質に影響を与える可能性が高く, 頻度がまれではない35個の変異のタイピングを行った. また, 公開されているデータベースから, 血小板凝集に関係する遺伝子の98個の多型(nonsynonymous変異もしくはsynonymous変異ではアレル頻度は0.05以上)を選択し, 収集した遺伝子試料を対象に, それらのタイピングを行った.

ProGEAR研究の今後の方針

ProGEAR研究は, 4つの関連解

析を行うことによりアスピリンレジスタンスの発症要因ならびに遺伝子背景を明らかにする予定である(図1). すなわち, 第1の解析は, 登録時の血小板凝集能やCOX機能などの検査学的パラメーター間の関連解析, 第2の解析は, 登録時の検査学的パラメーターと遺伝子多型(ゲノム網羅的および候補遺伝子)との間の関連解析, 第3の解析は, 登録時の検査学的パラメーターを含む臨床情報と2次イベントとの間の関連解析, 第4の解析は, 2次イベントと遺伝子多型との間の関連解析である. 患者の2次イベント情報の収集は2010年3月末で終了する. 第3と第4の関連解析は, 2次イベントの収集後に行う.

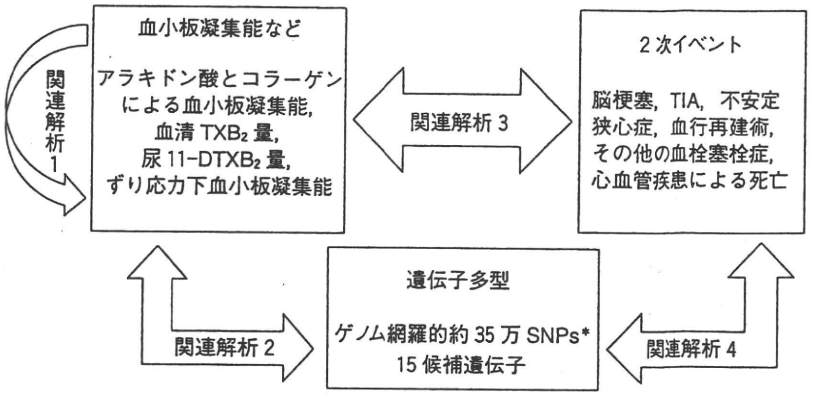

図1 アスピリンレジスタンスのリスク因子の同定のための関連解析
4つの関連解析を行うことにより, リスク因子の同定とその評価を行う予定である.
TXB_2 : トロンボキサン B_2, $DTXB_2$: デヒドロトロンボキサン B_2
*約50万SNPsをタイピングしたが, 約15万SNPsは多型を示さなかった.

本稿を終えるに当たり, 患者登録と追跡調査を行っていただいている全国の施設の担当医の先生方に深く御礼申し上げます. 本研究は独立行政法人医薬基盤研究所保健医療分野における基礎研究推進事業の支援を受けて行ったものである.

References

1) Collaborative meta-analysis of randomized trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 324 : 71-86, 2002
2) Baigent C, Blackwell L, Collins R, et al : Aspirin in the primary and secondary prevention of vascular disease : collaborative meta-analysis of individual participant data from randomised trials. Lancet 373 : 1849-1860, 2009
3) Patrono C, Rocca B : Drug insight : aspirin resistance-fact or fashion? Nat Clin Pract Cardiovasc Med 4 : 42-50, 2007
4) Sweeny JM, Gorog DA, Fuster V : Antiplatelet drug 'resistance'. part 1 : mechanisms and clinical measurements. Nat Rev Cardiol 6 : 273-282, 2009
5) Gorog DA, Sweeny JM, Fuster V : Antiplatelet drug 'resistance'. part 2 : laboratory resistance to antiplatelet drugs-fact or artifact? Nat Rev Cardiol 6 : 365-373, 2009
6) Ohmori T, Yatomi Y, Nonaka T, et al : Aspirin resistance detected with aggregometry cannot be explained by cyclooxygenase activity : involvement of other signaling pathway(s) in cardiovascular events of aspirin-treated patients. J Thromb Haemost 4 : 1271-1278, 2006

連載

知って得するワンポイントアドバイス

抗血栓薬の不応症（レジスタンス）

国立循環器病研究センター脳血管内科 医長

長束 一行

Kazuyuki NAGATSUKA

抗血栓薬の不応症（レジスタンス）として，早くから注目されたのはアスピリン抵抗性である．アスピリンは多くの血栓症発症予防のために用いられてきたが，全体でみると血栓症を約23%予防するとされているが[1]，逆の方向から考えるとアスピリンを服用していても年間約6%の血栓症が1年間に生じるということになる．血小板機能に対するアスピリンの作用に個人差がみられるという研究が数多く発表されるようになり，血小板機能からみたアスピリン抵抗性をもつ群に血栓症の再発が多いとのデータも発表されたことにより，一気にアスピリン抵抗性が注目されることとなった．

血小板機能からみたアスピリン抵抗性については，検査法によりその頻度が大きく異なり，数%から45%とかなりのばらつきがある．どの検査が最も臨床的なアスピリン抵抗性，すなわちアスピリンによる再発予防効果が弱い群をみつけるのに適しているかは，まだ明らかではない．血小板機能検査には血小板凝集能，血小板トロンボキサン産生能，尿中トロンボキサン代謝産物排泄量，ずり応力下での血小板凝集能，VerifyNowという抗血小板薬の抵抗性を評価する専門機器などさまざまである．血小板凝集能は古くからある血小板機能検査であるが，凝集を起こさせるための惹起物質に何を選ぶのかが問題となる．アスピリンはアラキドン酸凝集，コラーゲン凝集を主に抑制するが，ADP凝集は一次凝集しか抑制しない．アラキドン酸凝集はアスピリンが主に抗血小板剤作用を発揮する，アラキドン酸カスケードそのものによる凝集をみるために，高感度にアスピリンの作用を反映するが，その凝集反応は通常凝集するかしないかに大きく分かれるために，微妙な差はわかりにくい．一方，コラーゲン凝集はアスピリン投与による抑制効果はばらつきが多くなるし，試薬の濃度にも依存する．

血小板のトロンボキサン産生能はアラキドン酸カスケードそのものを評価可能であるが，逆にそこだけしか評価できない．尿中トロンボキサン代謝産物はサイクロオキシゲナーゼ2の機能も反映するといわれている．

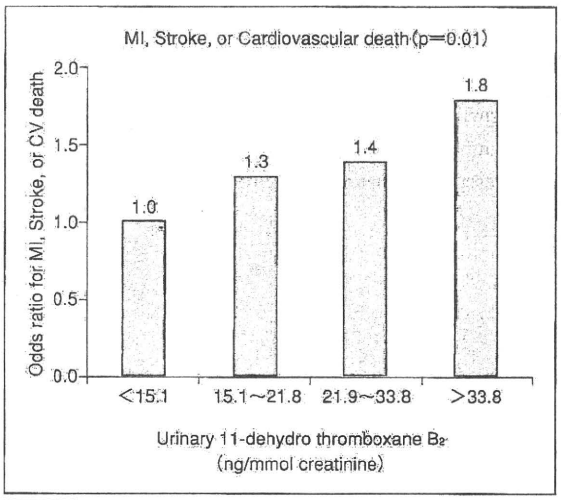

図1 尿中トロンボキサン代謝産物量と血栓症発症リスク

尿中トロンボキサン代謝産物量が多い群では血栓症のリスクが高くなっている．

（文献2より引用改変）

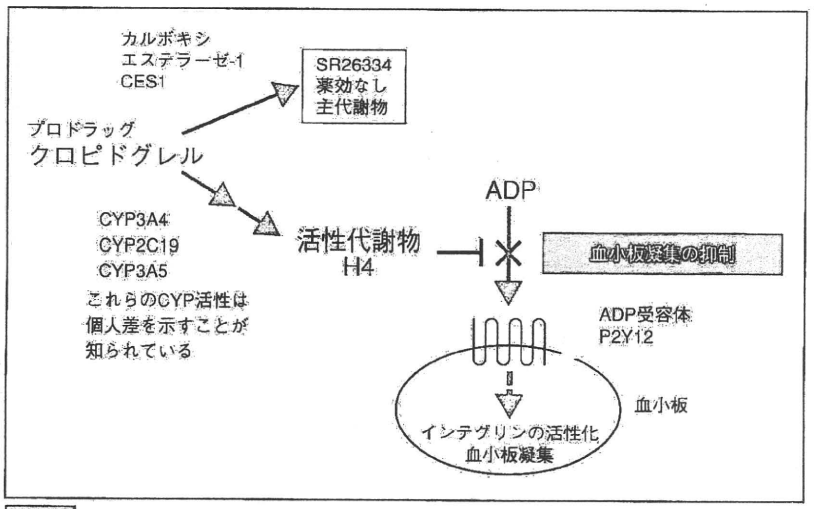

図2 クロピドグレルの代謝と作用機序

クロピドグレルはプロドラッグで，CYP2C19などの代謝酵素で代謝され，活性化物質へと変化し，抗血小板機能を発揮する．

アスピリン抵抗性に関する代表的な論文を紹介すると，Eikelboomら[2]は，尿中トロンボキサン代謝産物量を測定し，4分位に分けて5年間血栓症をイベントとしてフォローしたところ，尿中トロンボキサン代謝産物量が多いほど血栓症の発生が多かったと報告している（図1）．

アスピリン抵抗性の原因については，さまざまな説がある．投与量，代謝産物の影響，併用薬の影響，遺伝子多型，服薬コンプライアンスなどさまざまなものが推測されているが，いまだ明らかにされていない．Ohmoriら[3]は，コラーゲン凝集をマーカーとして4分位に分け，81mgのアスピリンを服用している脳梗塞，心筋梗塞の既往のある136例をフォローしたところ，特に1年以内の血栓症の発症率は最もコラーゲン凝集が抑制されていない群で明らかに高かったと報告している．現在，国立循環器病研究センター研究所病因部の宮田敏行が主任研究者となり，「アスピリンレジスタンスの実態ならびにその遺伝子背景に関する研究（ProGEAR study）」が進行中であるが，この研究ではアラキドン酸およびコラーゲン凝集，血小板トロンボキサン産生能，尿中トロンボキサン代謝産物量，遺伝子解析に加え，血中アセチルサリチル酸濃度血中濃度を抜き打ちで計測することにより，服薬コンプライアンスも評価している．592例が登録され，2010年2月末に2年間の追跡がすべての症例で終了し，間もなく解析結果が報告できる．

アスピリン以外の抗血小板剤ではクロピドグレル抵抗性が最近注目されているが，こちらはメカニズムがかなり解明されている．クロピドグレルはプロドラッグで，代謝産物が抗血小板機能を有している．その代謝に関わるCYP2C19には遺伝子多型が存在し，遺伝子多型をもつ症例では代謝産物の産制量が減り，同じ投与量では抗血小板作用が弱くなる[4][5]（図2，3）．遺伝子多型をもつ頻度は欧米では30%前後とされているが，日本人では60～70%とむしろ遺伝子多型をもつ頻度が非常に高いことがわかっている．また，プロトンポンプ阻害薬を併用すると抗血小板作用が弱まることも最近欧米で報告され，話題となっている[6]．

本当にクロピドグレルは日本人では効いていないことが多いのであろうか．クロピドグレルと同じ系統のチエノピリジン系抗血小板薬のチクロピジンは，欧米では500mgの投与量であったが，日本では合併症が多いということで，いつの間にか200mgの投与量が標準となっているが，日本人でのエビデンスを集めたわけではない．このことからクロピドグレルは，当初は欧米と同じ75mgという投与量は多いのではないかと危惧されていたが，製薬会社が行ったチクロピジン200mgとクロピドグレル75mg投与の二重盲検試験で

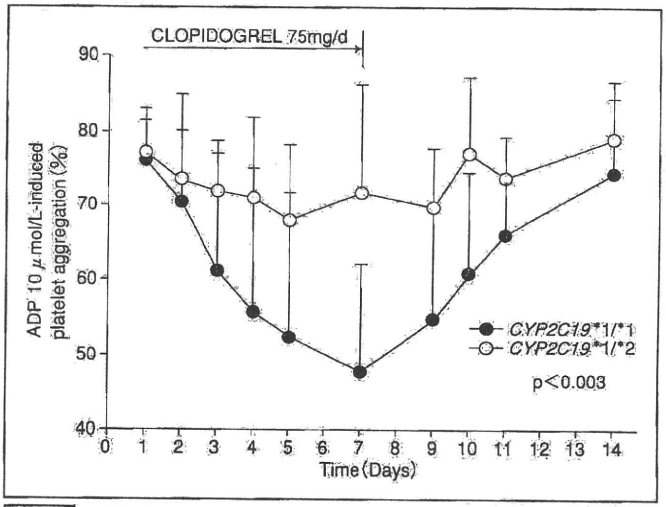

図3 健常者にクロピドグレルを投与した場合のADP凝集抑制効果

CYP2C19の遺伝子多型をもたない群（○）は大きく凝集能が抑制されるが，遺伝子多型をもつ群（●）では凝集能の抑制効果が弱い．

（文献5より引用改変）

は出血合併症に差がなかったと報告されている．今後日本人におけるクロピドグレルと遺伝子多型，抗血小板作用，イベント抑制率については，独自にしかも公的資金で調査する必要があり，現在，循環器病研究委託費「複雑化する脳・心血管疾患病態における適切な抗血栓治療の開拓」による研究の一環として，多施設前向き共同試験（Cognac study）を開始している．

文献

1）Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ **324**: 71-86, 2002

2）Eikelboom JW, Hirsh J, Weitz JI, et al: Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. Circulation **105**: 1650-1655, 2002

3）Ohmori T, Yatomi Y, Nonaka T, et al: Aspirin resistance detected with aggregometry cannot be explained by cyclooxygenase activity: Involvement of other signaling pathway(s) in cardiovascular events of aspirin treated patients. J Thromb Haemost **4**: 1271-1278, 2006

4）Simon T, Verstuyft C, Mary-Krause M, et al: Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med **360**: 363-375, 2009

5）Hulot JS, Bura A, Villard E, et al: Cytochrome p450 2c19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. Blood **108**: 2244-2247, 2006

6）Ho PM, Maddox TM, Wang L, et al: Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. JAMA **301**: 937-944, 2009

Int J Hematol (2010) 92:468–473
DOI 10.1007/s12185-010-0687-5

ORIGINAL ARTICLE

Usefulness of antithrombin deficiency phenotypes for risk assessment of venous thromboembolism: type I deficiency as a strong risk factor for venous thromboembolism

Mana Mitsuguro · Toshiyuki Sakata · Akira Okamoto · Sachika Kameda · Yoshihiro Kokubo · Yoshiaki Tsutsumi · Michitaka Sano · Toshiyuki Miyata

Received: 25 June 2010 / Revised: 26 August 2010 / Accepted: 31 August 2010 / Published online: 23 September 2010

Abstract Inherited antithrombin deficiency, an established risk factor for venous thromboembolism (VTE), can be classified into type I (quantitative deficiency) or type II (qualitative deficiency). In the present study, we assessed the VTE risk associated with the phenotypes of antithrombin deficiency in patients admitted to our hospital. We found that patients with type I deficiency ($n = 21$) had more VTE events and earlier onset of VTE than those with type II deficiency ($n = 10$). The VTE-free survival analysis showed that the risk for VTE in patients with type I deficiency was sevenfold greater than that in patients with type II deficiency (hazard ratio: 7.3; 95% confidence interval: 1.9–12.2; $P = 0.0009$). The prevalence of type I deficiency in the VTE group (5.6%, 6/108) was higher than that in the general population (0.04%, 2/4,517) (odds ratio: 132.8; 95% confidence interval: 26.5–666.1; $P < 0.0001$). However, the prevalence of type II deficiency was not different between the VTE group and the general population. Our study indicated that the risk for VTE in patients with type I deficiency was much higher than that in patients with type II deficiency. Thus, simple phenotypic classification of antithrombin deficiency is useful for assessment of VTE risk in Japanese.

Keywords Antithrombin deficiency · Deep vein thrombosis · Phenotype · Risk assessment · Venous thromboembolism

1 Introduction

Antithrombin is a serine protease inhibitor and functions as a potent natural anticoagulant by inactivating proteases in the coagulation cascade [1–3]. Since Egeberg's [4] first report of antithrombin deficiency in a Scandinavian family in 1965, numerous additional families with antithrombin deficiency have been reported [5, 6]. The majority of individuals with antithrombin deficiency are heterozygotes, and homozygotes are extremely rare.

Antithrombin deficiency is phenotypically classified into two types. In type I deficiency, both antithrombin activity and antigen levels are low in the plasma, indicating that the protein is not produced by the mutant allele. In type II deficiency, low antithrombin activity within normal antigen limits is observed, indicating a functional impairment of the molecule. Type II deficiency can be further divided into three subtypes: a heparin-binding site subtype, in which heparin binding is abnormal; a reactive site subtype, in which the reactive center loop is abnormal; and a pleiotropic effect subtype, in which the influence is pleiotropic [3, 5].

M. Mitsuguro · T. Sakata (✉) · A. Okamoto · S. Kameda · M. Sano
Laboratory of Clinical Chemistry,
National Cerebral and Cardiovascular Center,
5-7-1, Fujishirodai, Suita, Osaka 565-8565, Japan
e-mail: tsakata@hsp.ncvc.go.jp

Y. Kokubo
Department of Preventive Cardiology,
National Cerebral and Cardiovascular Center,
5-7-1, Fujishirodai, Suita, Osaka 565-8565, Japan

Y. Tsutsumi
Department of Medicine, National Cerebral and Cardiovascular Center, 5-7-1, Fujishirodai, Suita, Osaka 565-8565, Japan

T. Miyata
Research Institute, National Cerebral and Cardiovascular Center,
5-7-1, Fujishirodai, Suita, Osaka 565-8565, Japan

Antithrombin deficiency or low antithrombin levels are associated with increased incidence of VTE and are a risk for first and recurrent VTE [1–3, 7–12]. Asymptomatic antithrombin deficiency is present as frequently as 1:600 [9, 13, 14], and type II deficiency is more prevalent than type I deficiency in the general population [13]. In a cumulative analysis of the literature, more than half of the patients with antithrombin type I deficiency or the reactive site or pleiotropic effect subtypes of type II deficiency manifested VTE events [15–17]. However, the incidence of VTE events in patients with the heparin-binding site subtype of type II deficiency was very low (only 6%), although patients who were homozygous or compound heterozygous for the heparin-binding site subtype mostly manifested VTE events [15–18]. These observations were obtained from cumulative evidence collected worldwide from patients with antithrombin deficiency. However, the methods used for identifying antithrombin deficiency in each institution were different and not standardized. To date, there has been no direct comparison of the differential thrombotic risk associated with type I and type II antithrombin deficiency at a single institution.

We have measured hemostatic factors including antithrombin, protein C, and plasminogen in patients admitted to the National Cerebral and Cardiovascular Center Hospital [9, 19, 20]. We have thus far identified 31 patients with antithrombin deficiency based on their heparin-dependent activity [9]. We also measured antithrombin activity in a Japanese general population consisting of more than 4,500 individuals, who were selected randomly from the municipal population registry stratified by sex and 10-year age group. Comparing the prevalence of antithrombin deficiency in the VTE patient group with that in the general population, we concluded that antithrombin deficiency is a strong risk factor for VTE with an odds ratio of 38 [9].

Here, we extend our previous study to clarify the VTE risk associated with the antithrombin deficiency phenotypes (type I and type II) using two studies. In the first study, 31 patients with antithrombin deficiency admitted to our hospital were classified as type I or type II and their VTE events were retrospectively collected and compared between the two types. In the second study, we compared the prevalence of type I and type II antithrombin deficiency in the VTE patient group and that in the general population and assessed whether the phenotypes of antithrombin deficiency affected VTE events. Both studies showed that type I antithrombin deficiency was a stronger risk factor for VTE than type II deficiency. Thus, the phenotypic classification of antithrombin deficiency is important for assessing VTE risk, and a simple phenotypic classification for type I and type II is useful for assessment of VTE risk in Japanese.

2 Materials and methods

2.1 Study population: retrospectively followed patients with antithrombin deficiency classified by phenotypes

We measured antithrombin and protein C activities, in response to a doctor's request, in addition to routine hemostatic parameters including prothrombin time and activated partial thromboplastin time in patients admitted to the National Cerebral and Cardiovascular Center Hospital from January 1986 to May 2006. We ultimately identified 31 patients with antithrombin deficiency from 30 families on the basis of antithrombin and protein C activities and the family study. We classified them into type I or type II antithrombin deficiency groups based on the antithrombin activity/antigen ratio. These patients were admitted to our hospital due to cardiovascular problems and did not always have VTE. We, therefore, retrospectively followed them for VTE events and VTE onset age by their medical records for assessment of thrombotic risk. Information on hypertension, hyperlipidemia, diabetes mellitus, and current smoking status was also collected. The diagnosis of deep vein thrombosis was based on radioisotope venography, contrast venography, magnetic resonance imaging, and/or continuous-wave Doppler ultrasonography. The diagnosis of pulmonary embolism was confirmed by pulmonary angiography, computed tomography, and/or magnetic resonance imaging.

2.2 Study populations: the VTE group and the general population

We previously enrolled 108 outpatients with VTE (54 men and 54 women), who were admitted to the National Cerebral and Cardiovascular Center Hospital between 1994 and 1998, and identified 6 patients with antithrombin deficiency [9]. These 6 patients were included in the 31 patients with antithrombin deficiency described above. We also previously enrolled 4,517 individuals (2,090 men and 2,427 women), who were randomly selected from the residents of Suita city in Japan and stratified by sex and 10-year age group as a general population, and identified 7 individuals with antithrombin deficiency [9, 21, 22]. Here, we measured the antigen levels of antithrombin in these 13 patients (6 with antithrombin deficiency described above and 7 from Suita city), classified them as having type I or type II deficiency, and compared the prevalence of the phenotypes of antithrombin deficiency in the VTE patient group with that in the general population.

2.3 Assay methods

Blood samples were collected in siliconized plastic vacuum tubes containing a 1/10 volume of 3.13% trisodium citrate.

The tubes were centrifuged at 4,000 rpm for 10 min at room temperature and the plasma samples were obtained. Antithrombin activity was determined as heparin-cofactor activity based on the antithrombin assay by the use of chromogenic substrate S-2238 (CHROMOGENIX AB, Stockholm, Sweden) [9, 19]. Protein C activity was measured using the chromogenic substrate S-2236 (CHROMOGENIX AB) after activation by Protac (Pentapharm, Basel, Switzerland) [9, 19]. Antithrombin antigen levels were determined using a latex photometric immunoassay kit (Mitsubishi Chemical Medience Co., Tokyo, Japan) and LPIA-A700, a fully automated quantitative latex photometric immunoassay instrument (Mitsubishi Chemical Medience Co.). The activity and antigen levels were expressed as percentages of the levels obtained from commercially available standard human plasma (Siemens AG, Bayern, Germany). As measured in our laboratory, the inter-assay coefficients of variation were 2.2% for the antithrombin activity assay, 1.9% for the antithrombin antigen assay, and 2.4% for the protein C activity assay. Antithrombin deficiency was defined by the antithrombin and protein C activities and the family study. In this study, patients having an antithrombin activity <3 standard deviations below the mean (70%) and a protein C/antithrombin activity ratio >3 standard deviations above the mean (1.65) [9] and at least one relative with antithrombin deficiency were considered to have antithrombin deficiency.

2.4 Statistical analysis

Statistical analysis was performed using Prism (GraphPad Software, La Jolla, CA, USA). Quantitative data were assessed using the Mann–Whitney test. Associations between qualitative variables were analyzed with the Chi-square test. Odds ratios with 95% confidence intervals were used to assess the differences between prevalence rates in the different groups. VTE-free curves in patients with type I or type II antithrombin deficiency were created using the method of Kaplan–Meier and compared using the log-rank test.

3 Results

3.1 Classification of type I and type II antithrombin deficiency

To classify antithrombin deficiency into type I and type II, the antithrombin activity/antigen ratio (AT-act/AT-ag ratio) was calculated. Figure 1 shows the distribution and box plots of the AT-act/AT-ag ratio in the 31 patients with antithrombin deficiency. We arbitrarily divided the patients into two groups based on this ratio; the median (range) of the ratio in the two groups was 0.98 (0.84–1.29) and 0.52 (0.42–0.62), respectively. We considered 0.75 as the cut-off value for discrimination between the two groups. This cut-off value was calculated as the mean of the median in the two groups. There was no overlap of the AT-act/AT-ag ratio between the two groups. Based on these criteria, 21 patients with type I (circles) and 10 patients with type II (triangles) were identified.

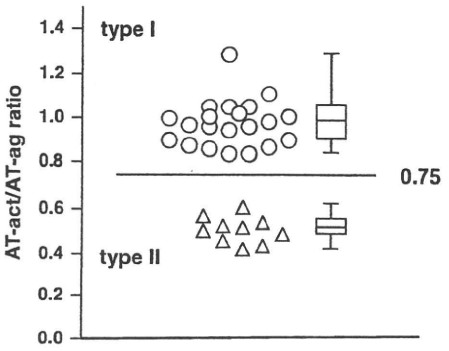

Fig. 1 Discrimination of antithrombin deficiency phenotypes using the antithrombin activity/antigen ratio. Thirty-one patients with antithrombin deficiency admitted to our hospital were classified into type I (*circles*, $n = 21$) or type II (*triangles*, $n = 10$) deficiency groups using the antithrombin activity/antigen ratio (AT-act/AT-ag) of 0.75. In the box plots, the *error bars* represent minimum and maximum values, the *horizontal rules* indicate median values, and the *ends of the boxes* indicate interquartile ranges

3.2 Retrospective follow-up study to compare VTE-free curves in patients with type I and type II antithrombin deficiency

We retrospectively collected the VTE events of the 21 patients with type I antithrombin deficiency and the 10 patients with type II antithrombin deficiency (Table 1). In the type I group, 33 VTE events occurred in 18 of the 21 patients (85.7%), and 16 patients (76.2%) experienced a VTE event before 50 years of age. Recurrent VTE events were observed in 6 of the 18 patients with VTE. The median age for VTE events was 37 years with a range of 15–89 years of age. In contrast, in the type II group, only 3 VTE events occurred in 2 of the 10 deficient patients (20.0%), and the first VTE events occurred at the ages of 57 and 70. There were no significant differences in the risk factors for coronary artery disease, including hypertension, hyperlipidemia, diabetes mellitus, and smoking, between the type I and type II groups.

To evaluate the VTE risk for patients with type I and type II antithrombin deficiency, we compared the VTE-free curves between the groups (Fig. 2). The VTE risk was increased by approximately sevenfold in patients with type I

Table 1 Characteristics of patients with type I or type II antithrombin deficiency

	Phenotypes		*P* value
	Type I	Type II	
Number of patients	21	10	
Sex (male/female)	11/10	4/6	0.52
Antithrombin levels			
Activity, median (range) (%)	54 (29–69)	62 (40–68)	0.27
Antigen, median (range) (%)	55 (30–71)	116 (87–127)	<0.0001
Activity/antigen ratio, median (range)	0.97 (0.84–1.29)	0.51 (0.42–0.61)	<0.0001
Onset of VTE events			
Number of total events	33	3	
Number of patients (%)	18/21 (85.7)	2/10 (20.0)	<0.0001
Number of patients, age < 50 years (%)	16/21 (76.2)	0/10 (0.0)	<0.0001
Recurrence of VTE onset			
Number of patients (%)	6/18 (33.3)	1/2 (50.0)	0.64
Age at VTE onset (years)			
Median (range)	37 (15–89)	64 (57–70)	0.09
Risk for coronary artery disease			
Hypertension (%)	2/21 (10)	3/10 (30)	0.30
Hyperlipidemia (%)	2/21 (10)	3/10 (30)	0.30
Diabetes (%)	0/21 (0)	2/10 (20)	0.10
Current smoking (%)	8/21 (38)	5/10 (50)	0.70

VTE venous thromboembolism, *Hypertension* 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 non-fasting plasma glucose ≥ 11.1 mmol/L (200 mg/dL) or HbA1c ≥ 6.5% or antidiabetic medication

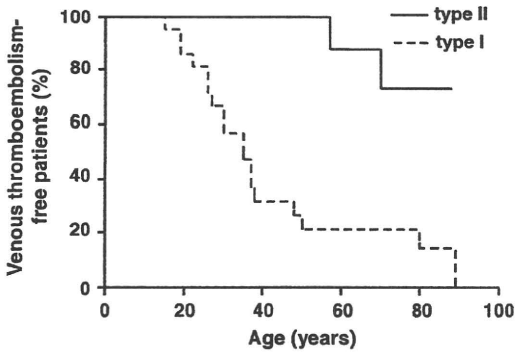

Fig. 2 VTE-free curves of patients with type I or type II antithrombin deficiency. In type I antithrombin deficiency, 18 out of 21 patients developed VTE. In type II antithrombin deficiency, only 2 out of 10 patients developed VTE. For the Kaplan–Meier analysis, the remaining three or eight patients were treated as the censored patients

deficiency relative to those with type II deficiency (hazard ratio: 7.3; 95% confidence intervals: 1.9–12.2; $P = 0.0009$).

3.3 Comparison of the prevalence of type I and type II antithrombin deficiency in the VTE patient group and the general population

We previously identified 6 patients with antithrombin deficiency among 108 consecutive outpatients with VTE [9]. We also identified 7 individuals with antithrombin deficiency in a general population consisting of 4,517 individuals. Comparing these frequencies, we reported that antithrombin deficiency was a strong risk factor for VTE with an odds ratio of 38 [9]. To extend this previous work, we measured the antithrombin antigen levels in these patients in the present study. We found that all 6 VTE patients with antithrombin deficiency were type I (Table 2). In the general population, two individuals were type I and the remaining 5 were type II (Table 2). The prevalences of type I antithrombin deficiency in the VTE group (6/108, 5.56%) and the general population (2/4,517, 0.04%) were significantly different, and the odds ratio between the two groups was 132.8 (95% confidence intervals: 26.5–666.1; $P < 0.0001$). In contrast, the prevalences of type II antithrombin deficiency in the VTE group (0/108, 0.00%) and the general population (5/4,517, 0.11%) were not significantly different (odds ratio: 3.8; 95% confidence interval: 0.2–68.9; $P = 0.7294$).

4 Discussion

In the present study, we identified 21 patients with type I antithrombin deficiency and 10 patients with type II antithrombin deficiency. Among these patients, more than 75% with type I deficiency had VTE events before the age of 50; in contrast, only 2 out of 10 patients with type II deficiency had VTE events, and these occurred at the ages of 57 and 70. The VTE-free curves showed that type I antithrombin

Table 2 Prevalence of type I and type II antithrombin deficiency in the VTE patient group and the general population

Phenotype of antithrombin deficiency	Number of individuals (%)		Odds ratio (95% CI vs. general population)	*P* value
	VTE patient group (n = 108)	General population (n = 4,517)		
Type I	6 (5.56%)	2 (0.04%)	132.8 (26.5–666.1)	<0.0001
Type II	0 (0.00%)	5 (0.11%)	3.8 (0.2–68.9)	0.73
Type I + II[a]	6 (5.56%)	7 (0.15%)	37.9 (12.5–114.8)	<0.0001

CI confidence interval, *VTE* venous thromboembolism

[a] Data taken from Ref. [9]

deficiency was a strong risk factor for VTE, with a hazard ratio of 7.3 compared to type II deficiency. These findings were reinforced by a comparison of the prevalence of type I and type II deficiency in the VTE group and the general population, which showed very high odds ratio for VTE for type I, but not for type II.

Based on a cumulative analysis of the literature, type I antithrombin deficiency as well as both the reactive site and the pleiotropic subtypes of type II deficiency have an increased risk for VTE [15–17]. However, the heparin-binding site subtype of type II deficiency showed a relatively low risk for VTE [15–17]. Thus, the VTE risk conferred by type I antithrombin deficiency was consistent between our study and the cumulative analysis. However, the risk assessment of type II antithrombin deficiency for VTE is in part debatable. In our analysis, we could not classify our patients with type II deficiency into three subtypes, a heparin-binding site subtype, a reactive site subtype, and a pleiotropic effect subtype, due to the sample limitations. Therefore, we could not evaluate the risk assessment for VTE by subtypes. Despite this limitation, we demonstrated that type I antithrombin deficiency was a greater risk for VTE than type II.

The plausible explanation for type II deficiency as a low VTE risk would be that mutations responsible for a low VTE risk, the heparin-binding defects, would be more common than those for a high VTE risk, the reactive site defects and pleiotropic effect defects, in a Japanese population. This could explain the clearly differentiated risk between type I and type II deficiency in our study. In the public database, the cases of at least three Japanese VTE patients homozygous for the R47C mutation, that causes a heparin-binding defect, have been deposited (Japanese Thrombophilia Mutation Database, http://hes.met.nagoya-u.ac.jp/KENSA WEB/labo/blood/bunshi_hp/mutation.html, Antithrombin Mutation Database, http://www1.imperial.ac.uk/medicine/divisions/olddivisions/is/haematology/coag/antithrombin/) [18, 23, 24], suggesting the prevalence of this mutation in the Japanese population. As for the reactive site defects in the Japanese population, two heterozygous patients, one with the R425C mutation [25] and another with the R425H mutation [26] have been deposited in the public database. Thus, the frequency of patients with the reactive site defects seemed lower than those with the heparin-binding defects.

We identified 2 individuals with type I antithrombin deficiency and 5 individuals with type II deficiency out of 4,517 individuals, who were selected randomly from the municipal population registry stratified by sex and 10-year age group. Thus, the prevalences of type I and type II deficiency in the Japanese general population were 0.04% (2/4,517) and 0.11% (5/4,517), respectively, which were similar to those obtained in western Scotland (type I: 0.02%, type II: 0.15%) [13], although the selection criteria for antithrombin deficiency were different. The Japanese population is now about 128 million. Extrapolating from the prevalence, we estimate that as many as 57,000 Japanese have type I antithrombin deficiency and are at risk of developing VTE.

In conclusion, considerable differences in VTE risk were observed between Japanese individuals with type I and type II antithrombin deficiency and a simple phenotypic classification for type I and type II antithrombin deficiency was shown to be useful for assessment of VTE risk in Japanese.

Acknowledgments This study was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO), a Grant-in-Aid from the Ministry of Health, Labor and Welfare of Japan, and the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1. De Stefano V, Finazzi G, Mannucci PM. Inherited thrombophilia: pathogenesis, clinical syndromes, and management. Blood. 1996; 87:3531–44.
2. Perry DJ, Carrell RW. Molecular genetics of human antithrombin deficiency. Hum Mutat. 1996;7:7–22.
3. Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V, et al. Inherited thrombophilia: Part 1. Thromb Haemost. 1996;76:651–62.
4. Egeberg O. Inherited antithrombin deficiency causing thrombophilia. Thromb Diath Haemorrh. 1965;13:516–30.

5. Lane DA, Bayston T, Olds RJ, Fitches AC, Cooper DN, Millar DS, et al. Antithrombin mutation database: 2nd (1997) update For the Plasma Coagulation Inhibitors Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Thromb Haemost. 1997;77: 197–211.
6. Miyata T, Sato Y, Ishikawa J, Okada H, Takeshita S, Sakata T, et al. Prevalence of genetic mutations in protein S, protein C and antithrombin genes in Japanese patients with deep vein thrombosis. Thromb Res. 2009;124:14–8.
7. Koster T, Rosendaal FR, Briet E, van der Meer FJ, Colly LP, Trienekens PH, et al. Protein C deficiency in a controlled series of unselected outpatients: an infrequent but clear risk factor for venous thrombosis (Leiden Thrombophilia Study). Blood. 1995; 85:2756–61.
8. Sanson BJ, Simioni P, Tormene D, Moia M, Friederich PW, Huisman MV, et al. The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. Blood. 1999;94: 3702–6.
9. Sakata T, Okamoto A, Mannami T, Matsuo H, Miyata T. Protein C and antithrombin deficiency are important risk factors for deep vein thrombosis in Japanese. J Thromb Haemost. 2004;2:528–30.
10. Vossen CY, Walker ID, Svensson P, Souto JC, Scharrer I, Preston FE, et al. Recurrence rate after a first venous thrombosis in patients with familial thrombophilia. Arterioscler Thromb Vasc Biol. 2005;25:1992–7.
11. Vossen CY, Conard J, Fontcuberta J, Makris M, VDM FJ, Pabinger I, et al. Risk of a first venous thrombotic event in carriers of a familial thrombophilic defect. The European Prospective Cohort on Thrombophilia (EPCOT). J Thromb Haemost. 2005;3:459–64.
12. Lijfering WM, Brouwer JL, Veeger NJ, Bank I, Coppens M, Middeldorp S, et al. Selective testing for thrombophilia in patients with first venous thrombosis: results from a retrospective family cohort study on absolute thrombotic risk for currently known thrombophilic defects in 2479 relatives. Blood. 2009;113: 5314–22.
13. Tait RC, Walker ID, Perry DJ, Islam SI, Daly ME, McCall F, et al. Prevalence of antithrombin deficiency in the healthy population. Br J Haematol. 1994;87:106–12.
14. Wells PS, Blajchman MA, Henderson P, Wells MJ, Demers C, Bourque R, et al. Prevalence of antithrombin deficiency in healthy blood donors: a cross-sectional study. Am J Hematol. 1994;45:321–4.
15. Finazzi G, Caccia R, Barbui T. Different prevalence of thromboembolism in the subtypes of congenital antithrombin III deficiency: review of 404 cases. Thromb Haemost. 1987;58:1094.
16. Hirsh J, Piovella F, Pini M. Congenital antithrombin III deficiency. Incidence and clinical features. Am J Med. 1989;87:34S–8S.
17. Demers C, Ginsberg JS, Hirsh J, Henderson P, Blajchman MA. Thrombosis in antithrombin-III-deficient persons. Report of a large kindred and literature review. Ann Intern Med. 1992;116: 754–61.
18. Koide T, Odani S, Takahashi K, Ono T, Sakuragawa N. Antithrombin III Toyama: replacement of arginine-47 by cysteine in hereditary abnormal antithrombin III that lacks heparin-binding ability. Proc Natl Acad Sci USA. 1984;81:289–93.
19. Okamoto A, Sakata T, Mannami T, Baba S, Katayama Y, Matsuo H, et al. Population-based distribution of plasminogen activity and estimated prevalence and relevance to thrombotic diseases of plasminogen deficiency in the Japanese: the Suita study. J Thromb Haemost. 2003;1:2397–403.
20. Miyata T, Kimura R, Kokubo Y, Sakata T. Genetic risk factors for deep vein thrombosis among Japanese: importance of protein S K196E mutation. Int J Hematol. 2006;83:217–23.
21. Kokubo Y, Nakamura S, Okamura T, Yoshimasa Y, Makino H, Watanabe M, et al. Relationship between blood pressure category and incidence of stroke and myocardial infarction in an urban Japanese population with and without chronic kidney disease: the Suita study. Stroke. 2009;40:2674–9.
22. Kokubo Y, Kamide K, Okamura T, Watanabe M, Higashiyama A, Kawanishi K, et al. Impact of high-normal blood pressure on the risk of cardiovascular disease in a Japanese urban cohort: the Suita study. Hypertension. 2008;52:652–9.
23. Ueyama H, Murakami T, Nishiguchi S, Maeda S, Hashimoto Y, Okajima K, et al. Antithrombin III Kumamoto: identification of a point mutation and genotype analysis of the family. Thromb Haemost. 1990;63:231–4.
24. Shimizu K, Toriyama F, Ogawa F, Katayama I, Okajima K. Recurrent leg ulcers and arterial thrombosis in a 33-year-old homozygous variant of antithrombin. Am J Hematol. 2001;66:285–91.
25. Okajima K, Abe H, Wagatsuma M, Okabe H, Takatsuki K. Antithrombin III Kumamoto II; a single mutation at Arg393-His increased the affinity of antithrombin III for heparin. Am J Hematol. 1995;48:12–8.
26. Nagaizumi K, Inaba H, Amano K, Suzuki M, Arai M, Fukutake K. Five novel and four recurrent point mutations in the antithrombin gene causing venous thrombosis. Int J Hematol. 2003;78:79–83.