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SHORT COMMUNICATION

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α-1-Antichymotrypsin gene A1252G variant (ACT Isehara-1) is associated with a lacunar type of ischemic cerebrovascular disease

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Abstract α-1-Antichymotrypsin (ACT) is a plasma protease inhibitor belonging to the serpine superfamily; it has many functions, and thus qualitative change in ACT is likely to result in specific diseases. We previously reported a variant *A*ACT (ACT Isehara-1, Met389Val, A1252G) in patients with ischemic cerebrovascular disease (CVD). The present study was designed to examine the association of the variant with ischemic CVD, in 87 patients and 397 age-matched controls. We found that the frequency of the A1252G variant (ACT Isehara-1) was higher in the group with ischemic CVD than in the control group ($P = 0.0397$), which appeared to be independent of known risk factors. We subdivided the CVD group into lacunar and atherothrombotic subgroups. Further analysis by subtype of ischemic CVD showed an association of ACT Isehara-1 with lacunar infarction ($P = 0.0036$). These results suggest that ACT Isehara-1 is a new genetic risk factor for ischemic CVD, especially lacunar-type infarction, in Japan.

Key words α-1-Antichymotrypsin · Ischemic cerebrovascular disease · Lacunar infarction · Risk factor of cerebrovascular disease · Plasma protease inhibitor · Serpine

Introduction

α-1-Antichymotrypsin (ACT) is a plasma protease inhibitor belonging to the serpine superfamily, and has been found to have many functions in vitro (Travis and Salvesen 1983). Although the pathological and physiological roles of ACT are not established, it seems likely that qualitative change in ACT would result in specific diseases. We previously reported that a variant *A*ACT (ACT Isehara-1, Met389Val, A1252G) was found frequently in patients with cerebrovascular disease (CVD) (Tsuda et al. 1992a). The present study was designed to examine whether there is a direct association between ACT Isehara-1 and a particular subtype of symptomatic ischemic CVD.

Patients and methods

Informed consent was obtained from all subjects prior to their inclusion in the study. We studied 87 subjects with documented CVD from Tokai University Hospital, and 397 age-matched control subjects from the HIMEDIC Imaging Center at Lake Yamanaka. All subjects were Japanese, and were selected on the basis of clinical manifestations, history of CVD, and magnetic resonance imaging (MRI) findings. Subjects who were asymptomatic but had undifferentiated bright objects or any kind of hyperintensities on their MRI T2 images were excluded from the control group. We subdivided the patients with symptomatic ischemic CVD into atherothrombotic and lacunar subgroups according to the classification of CVD in the report of The National Institute of Neurological Disorders and Stroke (Whisnant et al. 1990). Atherothrombotic infarction occurs with atherosclerosis involving selected sites in extracranial and major intracranial arteries. The term "lacunar-type" infarction is commonly used as a clinical category for the small lesions that result from the involvement of deep, small, penetrating arteries. Subjects with cardiogenic cerebral embolism and cardiac diseases, including atrial fibrillation and rheumatic heart disease, were excluded from this study, thereby eliminating specific cardiac and systemic factors.

The data were analyzed by Fisher's exact test, and in part by the Mann-Whitney *U*-test.

Genomic leukocyte DNA was used for DNA analyses, including polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and PCR-restriction

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fragment length polymorphism (PCR-RFLP), and DNA sequencing, as described previously (Tsuda et al. 1992a; 1992b).

The PCR primers used were:
5′-TTACTGAGAGCCCCACTGCATGAT-3′ and
5′-CATAAGGCTGTGCTTGATGTA-3′

PCR conditions. The PCR conditions were 94°C for 2 min to denature the DNA, then 30 cycles of 94°C for 1 min, 72°C for 2 min, and finally, 72° for 5 min. PCR-SSCP and PCR-RFLP analyses were used to identify mutation.

PCR-SSCP analysis. Primers labeled at the 5′-end with ^{32}P were used for 30 PCR cycles. The PCR product labeled was diluted to 20 times its volume with 0.1% sodium dodecylsulfate and 10 mM ethylenediamine-tetraacetic acid (EDTA). Then this solution was diluted to twice its volume with 95% formamide, 20 mM EDTA, 0.1% bromophenol blue, and 0.1% xylene cyanol, and the resulting solution was heated in a boiling water bath for 3 min. The denatured PCR product was applied to 6% polyacrylamide gel containing 10% glycerol, and electrophoresed at room temperature.

PCR-RFLP analysis. The PCR product (234-bp) was digested with *BSPHI* (New England Biolabs, Inc., Beverly, MA, USA) at 37°C for 2 h. The digestion products were separated by 20% polyacrylamide gel electrophoresis and detected using ethidium bromide. The PCR digestion products from the native *ACT* gene showed fragments of 64 and 170 bp, while the A-to-G mutation (ACT Isehara-1) should result in no change of the molecular size by restriction enzyme treatment because of the loss of the cleavage site. To confirm the polymorphism, DNA sequencing was also used.

Results and discussion

Eleven of the 87 patients with ischemic CVD, and 24 of the 397 control subjects were heterozygotes with the ACT Isehara-1 gene (Table 1). No homozygote was observed in this study. The frequency of ACT Isehara-1 (heterozygote) in the ischemic CVD group (12.6%) was higher than that in the control group (6.0%), and the difference was significant ($P = 0.0397$; odds ratio, 2.25; 95% confidence interval [CI], 1.06–4.79). The etiology of CVD involves both environmen-

Table 1. Prevalence of risk factors for ischemic CVD

	CVD group ($n = 87$)	Control group ($n = 397$)	Odds ratio (95% CI)	P
Age (years; mean ± SD)	58 ± 13	56 ± 8		NS[a]
Sex (% male)	63.2	69.0		
ACT Isehara-1 and risk factors				
ACT Isehara-1 (heterozygote) (%)	12.6	6.0	2.25 (1.06–4.79)	0.0397
Hypertension (%)	70.1	33.5	4.66 (2.81–7.71)	<0.0001
Diabetes (%)	21.8	11.8	2.08 (1.15–3.76)	0.0235
Hyperlipidemia (%)	31.0	37.0	0.77 (0.46–5.08)	NS

Data were analyzed by Fisher's exact test, and, in part, by the Mann-Whitney *U*-test. Fisher's exact test was used to compare the values for the CVD group and the control group
CVD, Cerebrovascular disease; NS, not significant ($P > 0.05$); CI, confidence interval
[a] Mann-Whitney *U*-test

Table 2. Association of ACT Isehara-1 with other CVD risk factors in control subjects and CVD patients

	ACT Isehara-1 gene			
	With	Without	Odds ratio (95% CI)	P
Control subjects[c]	($n = 24$)	($n = 373$)		
Hypertension ($n = 133$)	4	129	0.38 (0.13–1.13)	NS[a]
Diabetes ($n = 47$)	3	44	1.07 (0.31–3.73)	NS[a]
Hyperlipidemia ($n = 147$)	13	134	2.11 (0.92–4.84)	NS[a]
CVD patients[c]	($n = 11$)	($n = 76$)		
Hypertension ($n = 61$)	8	53	1.15 (0.28–4.76)	NS[a]
Diabetes ($n = 19$)	0	19	[b]	[b]
Hyperlipidemia ($n = 28$)	1	27	[b]	[b]

Fisher's exact tests were used to compare each risk factor in controls with ACT Isehara-1 and native *A*ACT genes
NS, Not significant ($P > 0.05$)
[a] Fisher's exact test
[b] Number of patients was too small for statistical analysis
[c] Some subjects had more than one risk factor

Table 3. Frequency of ACT Isehara-1 compared in CVD subgroups and control group

	Subjects with ACT Isehara-1 (number of subjects)	Odds ratio (95% CI)	*P*
Control group ($n = 397$)	6.0% ($n = 24$)	1.0	
Subgroups of ischemic CVD ($n = 87$)			
Lacunar ($n = 38$)	21.1% ($n = 8$)	4.14 (1.71–10.02)	0.0036
Atherothrombotic ($n = 49$)	6.1% ($n = 3$)	1.01 (0.29–3.50)	NS

Fisher's exact test was used to compare the frequency of ACT Isehara-1 in lacunar infarction and atherothrombotic infarction patients with that in control subjects

tal and genetic factors, particularly hypertension, diabetes, and hyperlipidemia. In this study, hypertension and diabetes, but not hyperlipidemia, were associated with CVD. One must consider the possibility that ACT Isehara-1 is associated with these risk factors, and, hence, with lacunar infarction. However, no direct relation of ACT Isehara-1 with these risk factors was found by statistical analyses in either control or CVD subjects in this study (Table 2). In analysis by subtype of CVD, the frequency of ACT Isehara-1 in patients with lacunar infarction was 21.1%, while in patients with atherothrombotic cerebral infarction, it was only 6.1%, which was similar to that in the control group (Table 3). These results indicate an association of ACT Isehara-1 with lacunar infarction ($P = 0.0036$; odds ratio, 4.14; 95% CI, 1.71–10.02) and suggest that ACT Isehara-1 is a new genetic risk factor for ischemic CVD, especially lacunar infarction. The significance of ACT Isehara-1 (Met389Val) priority is not yet determined; however, it is known that ACT Isehara-1 has normal inhibitory activity for proteases such as chymotrypsin and cathepsin G. Determination of this ACT genotype may be useful in the assessments of risk for CVD, which could, in part, contribute to the prevention of this ailment.

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