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Detection of a new mutant α-1-antichymotrypsin in patients with occlusive-cerebrovascular disease

Michio Tsuda[a], Yukari Sei[a], Masaichi Yamamura[a], Masahiro Yamamoto[b] and Yukito Shinohara[b]

[a]*Department of Biochemistry and* [b]*Department of Neurology, Tokai University, School of Medicine, Isehara, Kanagawa, 259-11, Japan*

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A new mutant α-1-antichymotrypsin (variant ACT) was found by direct sequencing and PCR-single strand conformation polymorphism (PCR-SSCP). This variant ACT was a point mutation of exon V of ACT, with the substitution of Met by Val. Four out of six individuals with this variant ACT had occlusive-cerebrovascular disease, leading to one hypothesis that there might be an association between this mutation and occlusive-cerebrovascular disease.

α-1-Antichymotrypsin; Variant α-1-antichymotrypsin; PCR-SSCP; Occlusive-cerebrovascular disease

1. INTRODUCTION

The glycoprotein α-1-antichymotrypsin (ACT) is an acute phase protein and present in normal human serum at high concentration (294 ± 44 μg/ml). However the physiological activity of ACT is not yet known, although it has been reported that ACT inhibits the activity of pancreatic chymotrypsin and elastase, human neutrophil cathepsin G and mast cell chymase [1]. ACT has also been shown to increase the number of anti-sheep erythrocyte antibody producing cells in mice [2] and to inhibit cellular lysis mediated by cytotoxic T lymphocytes and cell-medited cytotoxicity (ADCC) in vitro [3]. We also reported that ACT has the ability to bind DNA [4] and inhibits purified DNA polymerase α [5] and DNA primase [6]. Recently it has been shown that ACT is present in senile plaque in the brain, particularly in the hypocampus of Alzheimer's disease patients [7], in which the concentration of ACT in cerebro-spinal fluid increased [8], suggesting that ACT plays a role in the formation of senile plaque in Alzheimer's disease. From these properties of ACT, it is possible to consider that ACT participates in the regulation of the biological response including inflammatory reactions, connective tissue damage, anaphylaxic reactions and the regulation of immune response, cell growth and so on. Therefore qualitative and quantitative change of ACT is considered to cause some dysfunction of the physiological response, resulting in specific diseases. We report here a variant ACT frequently found in patients with occlusive-cerebrovascular disease.

Correspondence address: M. Tsuda, Department of Biochemistry, Tokai University, School of Medicine, Isehara, Kanagawa, Japan, 259-11. Fax: (81) 463964828.

2. MATERIALS AND METHODS

2.1. *PCR conditions and sequencing*

PCR was carried out using the following primers; 5'-TTACTGAGAGCCCCACTGCATGAT-3' and 5'-CATAAGCCTGTGCTTGATGTA-3'.

PCR conditions were 94°C for 2 min to denature, then 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min, and a single cycle of 72°C for 5 min. The reaction volume of 100 μl containing 100 pmol primers, dNTPs at 200 μM, $MgCl_2$ at 1.5 mM and 0.5 μg of leucocyte genomic DNA. After 2% agarose electrophoresis, the PCR product was extracted and a second PCR was performed as the first one. The second PCR product was purified on a ULTRAFREE (C3TK, Millipore) and used for sequencing with a Sequenase kit (version 2.0) (USB) following the manufacturer's protocol.

2.2. *PCR-SSCP analysis of point mutation in exon V of ACT gene*

Primers described above were labelled at the 5' end with ^{32}P and used in PCR-SSCP analysis as described by Orita et al. [9]. Thirty-five cycles of the reaction at 94°C, 55°C and 72°C for 1 min, 1 min and 2 min, respectively, were performed. The PCR product (2 μl) was diluted with 40 μl of 0.1% sodium dodecyl sulphate and 10 mM EDTA. Then 4 μl of this solution was mixed with 4 μl of 95% formamide, 20 mM EDTA, 0.1% Bromophenol blue, and 0.1% xylene cyanol, and heated at 90°C for 3 min. The denatured PCR product was applied to a 6% polyacrylamide gel containing 10% glycerol, and electrophoresed at room temperature using a fan.

2.3. Bsp*HI digestion of the exon V PCR product*

PCR product (10 μl) of exon V was digested with 2 units of *Bsp*HI (Biolabs) at 37°C for 1 to 2 h, then electrophoresed in 3% agarose containing ethidium bromide.

3. RESULTS AND DISCUSSION

We sequenced the exon V of the ACT gene, contain-

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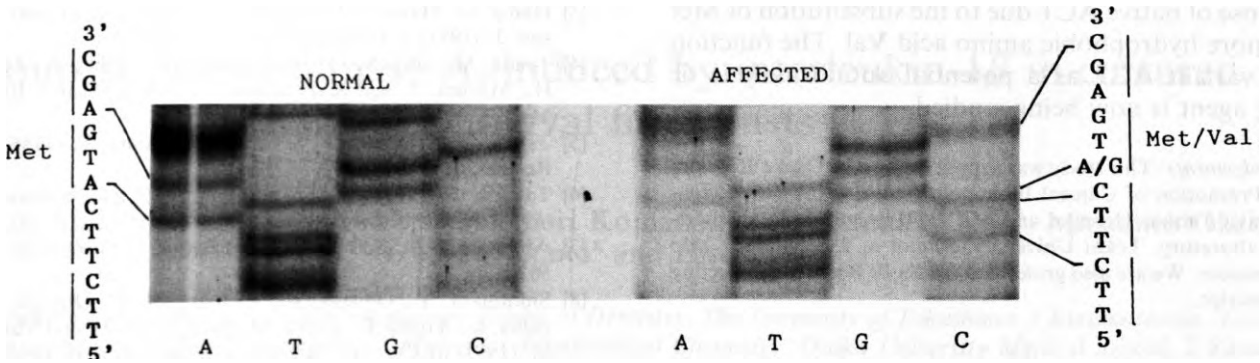

Fig. 1. Autoradiograph of a sequencing gel from the PCR product of exon V of the ACT gene in a normal and an affected individual, showing a single base pair change in the affected individual (heterozygote). This A to G transition leads to an amino acid substitution of Met by Val at codon 389.

ing the reactive site of this protease inhibitor, in a patient with multi-infarct dementia (MID). Direct sequencing revealed adenine to guanine transition (heterozygote) at base pair 1252 (Fig. 1). This mutation causes an amino acid substitution (Met to Val) at codon 389 close to the reactive site and the carboxy terminal of the protease inhibitor. This mutation was detected by PCR-single strand conformation polymorphism (PCR-SSCP) shown in Fig. 2, so that screening of 95 unrelated individuals including healthy subjects (n=32; age, 24–51 years old), Alzheimer's disease (n=6, age, 62–66 years old), occlusive-cerebrovascular disease (n=32; age, 38–80 years old), headache (n=8; age, 42–54 years old) and others (multiple sclerosis, epilepsy, Parkinson's disease and so on; n=17; age, 28–65 years old) was performed by PCR-SSCP [9]. Only 6 patients revealed the mutation. The PCR product from the normal ACT gene has a *Bsp*HI restriction site and was completely digested by *Bsp*HI (Fig. 3). The PCR products of the genomic DNA from individuals with abnormal PCR-SSCP patterns were digested with *Bsp*HI. It was found that the digestion was incomplete showing that these PCR products have both cutting and non-cutting sites. The sequencing of the PCR products with abnormal PCR-SSCP patterns confirmed that they were heterozygotes in the ACT gene and that the variant ACT gene was the same as described above, with valine substituting methionine. Four out of six patients with the variant ACT had occlusive-cerebrovascular disease (age, 38, 43, 69 and 80 years old), one suffered from tension-type headache (age, 54 years old) and the other one was a healthy subject (age, 24 years old).

This result leads us to one hypothesis that there might be an association between this mutation and occlusive-cerebrovascular disease. It is likely that this variant is pathogenic, because the variants of the serpin family of protease inhibitors have diminished activity or different protease specificity. The mutation of the reactive site of α-1-antitrypsin (AT) from Met to Arg at the P1 site (Pittsburgh), for example, converts α-1-antitrypsin into a potent inhibitor of thrombin and factor XI, causing a severe bleeding disorder [10].

The properties of this variant ACT may be different

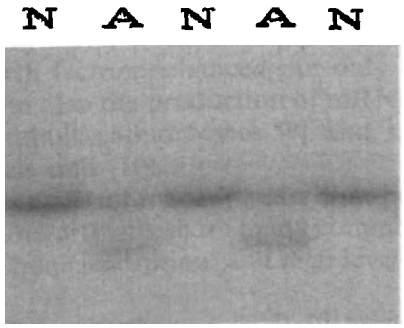

Fig. 2. PCR-SSCP analysis of the point mutation in exon V of the ACT gene in normal (N) and affected individuals (A). Thirty-five cycles of the reaction at 94°C, 55°C and 72°C for 1 min, 1 min and 2 min, respectively, were performed as described in Materials and Methods. PCR products were denatured, applied to a 6% polyacrylamide gel containing 10% glycerol, and electrophoresed at 20°C.

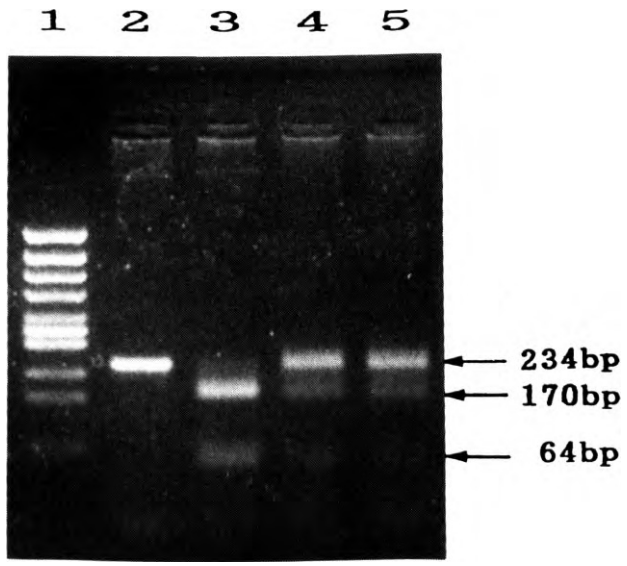

Fig. 3. *Bsp*HI digests of the Exon V of the PCR product from normal (lane 3) and affected (lanes 4 and 5) individuals. Lane 1, size markers; lane 2, not digested.

from those of native ACT due to the substitution of Met to the more hydrophobic amino acid Val. The function of this variant ACT as a potential antifibrinolytic or clotting agent is now being studied.

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