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*PI*S*$_{iiyama}$, A DEFICIENCY GENE OF ALPHA$_1$-ANTITRYPSIN: EVIDENCE FOR THE OCCURRENCE IN WESTERN JAPAN

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Summary An alpha$_1$-antitrypsin deficiency associated with pulmonary emphysema was investigated in a 32-year-old Japanese male. Polymerase chain reaction (PCR)-amplified fragments and dot blot hybridization with allele-specific oligonucleotide probes revealed that the patient was homozygous for a C to T transition at codon 53, resulting in the substitution of Phe^{53} for Ser^{53} (*PI*S*$_{iiyama}$). Crossedimmunoelectrophoresis after isoelectric focusing and agarose gel electrophoresis showed atypical banding patterns. *PI*S*$_{iiyama}$ is a rare deficiency gene, but it can occur sporadically all over the Japan.

Key Words alpha$_1$-antitrypsin, deficiency gene, point mutation, pulmonary emphysema

INTRODUCTION

Alpha$_1$-antitrypsin (AAT) is a plasma glycoprotein, synthesized mainly in the liver and released into the circulation. It is present in normal plasma at concentrations of 150–350 mg/dl. AAT is an acute-phase protein and is markedly elevated during inflammation and infection. It is an about 52,000 Da constituent, composed of a single polypeptide chain of 394 amino acids and three *N*-linked oligosaccharide chains. Its most important function is the inhibition of neutrophil elastase, capable of destroying most components of the extracellular matrices. A deficiency of AAT is associated with pulmonary emphysema and childhood liver diseases (Arnaud and Chapuis-Cellier, 1988; Cox, 1989; Crystal, 1990).

In the Japanese, deficiency and null genes are extremely rare and *PI*Z*, a deficiency gene occurring at polymorphic frequencies in the Caucasians, has not been

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reported (Yuasa *et al.*, 1984). In 1990 we reported the first Japanese case of AAT deficiency, caused by a deficiency gene, $PI^*M_{nichinan}$ (Matsunaga *et al.*). In this paper another case with a deficiency of AAT in a Japanese patient with pulmonary emphysema is investigated.

MATERIALS AND METHODS

Patient. The proband is a 32-year-old male Japanese living in Yonago, western Japan. He had asthma in the childhood. Since the age of 28, he sometimes suffered from cough, phlegm, and dyspnea. After hospitalization his disease was diagnosed as emphysema by various clinical tests. The development of emphysema seemed to be accelerated by his smoking 20–30 pieces of cigarette per day for more than 10 years. Laboratory data showed no apparent liver dysfunction. His parents were cousins. The serum levels of AAT were 12 mg/dl in the patient, 173 mg/dl in his father and 133 mg/dl in his mother by laser nephelometry. The AAT level in the patient's serum collected later for this study was estimated to be 5.5 mg/dl by Laurell's rocket immunodiffusion. He had no sibling.

Electrophoretic analyses. Isoelectric focusing (IEF) was carried out according to an instruction of supplier of carrier ampholytes. The pH gradients were established with Pharmalytes (Pharmacia). Crossed immunoelectrophoresis after IEF and agarose gel electrophoresis (AGE) was carried out with anti-AAT antibodies (Dako) by the lay-on method as described previously (Laurell, 1965; Söderholm *et al.*, 1975).

Polymerase chain reaction (PCR) and related techniques. Amplification of the AAT gene of the proband and direct sequencing of the PCR-amplified products were performed under the condition as described (Fucharoen *et al.*, 1989; Matsunaga *et al.*, 1990). The sequence of primers used for amplification of the coding exons of the AAT gene was as follows:

(BP3) 5′TAAGCTGACTGCAGGAGCAT3′, and
(BP2) 5′GTTCTGCAGAGCGTCAGTAG3′ for exon II;
(BP4) 5′AAGCTGAGCCTCGAGGGATG3′, and
(BP6) 5′TCACAAAAGCTTGAAATGCCAC3′ for exon III;
(BP5) 5′GGTGGCATTTCAAGCTTTTG3′, and
(BP8) 5′ACCAGCTCAACCCTTCTTTA2′ for exons IV and V.

Primers used in direct sequencing were described previously (Matsunaga *et al.*, 1990). Amplified products of exon II were dot-blotted onto nylon membrane and hybridized with allele-specific oligonucleotide probes at 42°C for 1 hr and then washed at 55°C for 10 min (Fucharoen *et al.*, 1989). The sequence of the two probes was as follows:

(SA7) 5′TCACTGGGGAGAAGAAGAT3′ for the normal allele and
(SA10) 5′TGCTCACTGGGAAGAAGAA3′ for the mutant allele.

RESULTS

A serum sample from the proband was analyzed by IEF in a pH gradient of 4.2–4.9 followed by protein staining (Fig. 1). No AAT bands were observed in the patient. Neither immunofixation nor immunoblotting could identify any unambiguous AAT bands. In contrast, the products of deficiency genes, *PI*Z* and *PI*Z$_{augsburg}$* (Faber *et al.*, 1990) were detected at the cathodic side.

Crossed immunoelectrophoresis was performed to detect AAT bands after IEF and AGE (Fig. 2). Atypical patterns of precipitation peaks were observed as compared with the control. IEF showed that AAT of the patient consisted of three major peaks. Two of them were located at similar positions to m6 and m8 isoproteins of the normal PI M phenotype, and the other peak was between them. A broad shoulder was formed at m4 zone. In AGE a major broad precipitation peak was observed at a similar position to that in the control. A shoulder peak was also accompanied at an anodal position.

DNA sequencing showed that a gene responsible for the deficiency of AAT

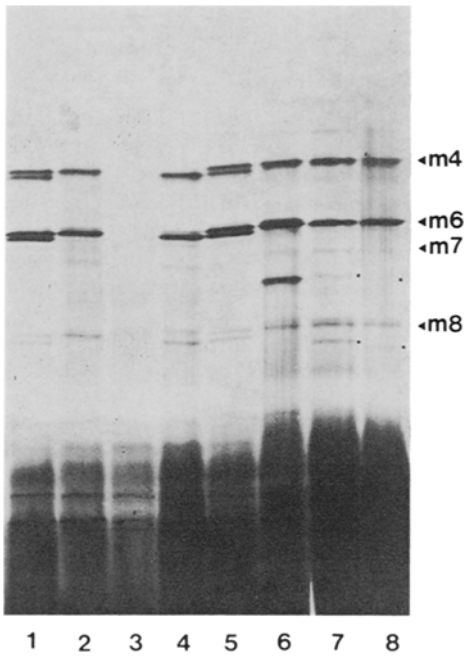

Fig. 1. Banding patterns of AAT as revealed by IEF at pH 4.2–4.9 followed by staining with Coomassie Brilliant Blue R-250. Anode at top. The isoproteins of PI M1 are indicated by arrowheads. The major 4 and 6 bands of PI Z and PI Z$_{augsburg}$ are indicated by dots. Lanes 1 and 5, M1M2; lane 2, M1S$_{iiyama}$-mother; lane 3, S$_{iiyama}$-proband; lane 4, M2S$_{iiyama}$-father; lane 6, M1S; lane 7, M1Z; lane 8, M1Z$_{augsburg}$.

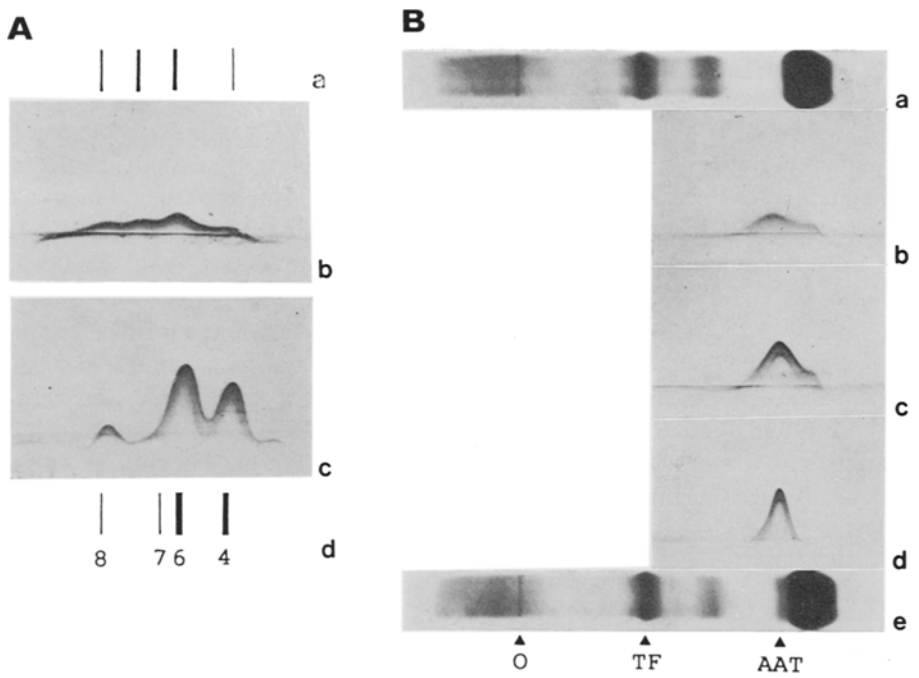

Fig. 2. Crossed immunoelectrophoresis after IEF (A) and AGE (B). The second dimensional gel (FMC corporation, agarose LE) contained 1/200 diluted anti-AAT antibodies (Dako). Control serum with a phenotype of PI M1M3 was diluted (about 1/50) to give the same peak height as native serum from the patient. (A) The first dimensional IEF was performed in a pH gradient established with a 1 : 1 mixture of Pharmalytes pH 4.2–4.9 and 4–6.5. Anode at right. Panel a, diagram of panel b; panel b, precipitation peaks in the patient's serum; panel c, precipitation peaks in the control serum; panel d, diagram of panel c. Note that the isoproteins m6 and m7 separated by IEF were fused in crossed immunoelectrophoresis due to a low resolution capacity. The numbers indicate the locations of the isoproteins m4, m6, m7, and m8. (B) The first dimensional AGE was performed using conventional barbiturate buffer, pH 8.6. Anode at right. Panel a, pattern after AGE of the patient's serum; panel b, native serum from the patient; panel c, a 50: 1 mixture of patient and control sera; panel d, 1/50-diluted control serum; panel e, pattern after AGE of the control serum. O and TF indicate the locations of origin and transferrin, respectively.

in the patient differed from a normal PI M gene by a single base transition in exon II in the codon for residue 53, resulting in the amino acid change Ser^{53} TCC to Phe^{53} TTC (Fig. 3). As the polymorphic sites of the codons 101, 213, and 376 were CGT (Arg), GTG (Val), and GAA (Glu), respectively, AAT of the proband had the PI M1 (Val^{213}) background.

Dot blot hybridization of the amplified DNA of the proband and his parents with the allele-specific oligonucleotide probes confirmed that he was homozygous and his parents were heterozygous for this mutation (Fig. 4).

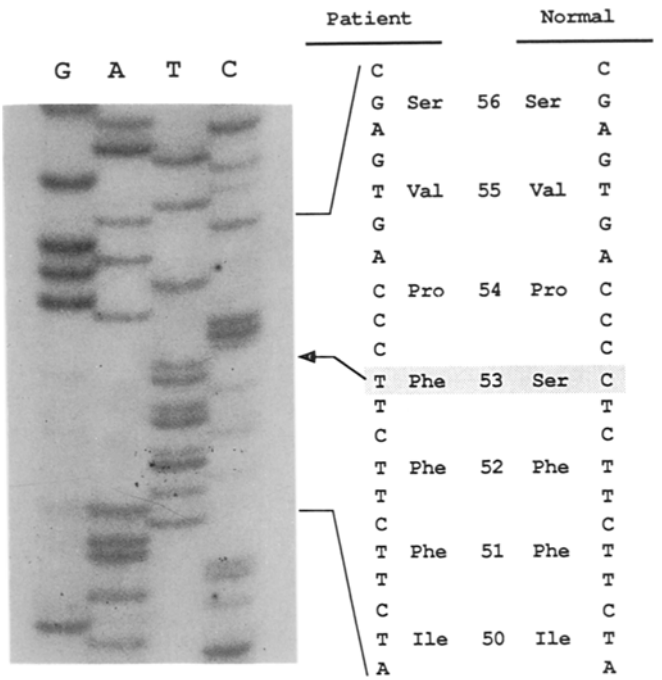

Fig. 3. Sequence of the region around a mutation in exon II found in the proband. It is identical to that of *PI*Siiyama*, having the same sequence as PI M1(Val^{213}) except for the C to T alteration indicated by an arrow, resulting in the substitution of Phe^{53} for Ser^{53}. Codon numbers and corresponding amino acids in the mature protein are indicated.

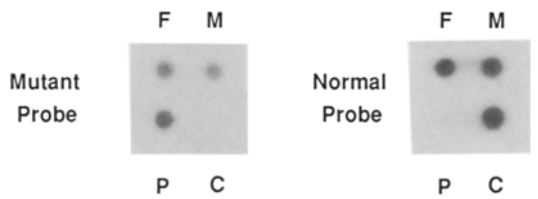

Fig. 4. Dot blot hybridization with specific oligonucleotide probes for the detection of the C-T substitution at the second position of codon 53. P, F, M, and C are the proband, father and mother of the proband, and a normal individual, respectively.

DISCUSSION

The deficiency genes of AAT are extremely rare in the Japanese population and only two genes have been reported thus far. One gene is *PI*M$_{nichinan}$*, found in the southern Japan. It was characterized by a deletion of Phe (TTC) at codon 51 or 52 and a mutation from Gly (GGG) to Arg (AGG) at codon 148 (Matsunaga

et al., 1990). The other gene is PI^*S_{iiyama}, observed in the central Japan. It had a substitution of Phe (TTC) for Ser (TCC) at codon 53 (Seyama *et al.*, 1991). The mutation revealed by PCR-related techniques in this study was identical to that of PI^*S_{iiyama}. This deficiency gene is rare but it can occur sporadically all over the Japan, because both families are unrelated. This gene is easily overlooked in heterozygous states. By IEF the banding patterns in the parents were similar to those of PI M2 and PI M1 phenotypes, respectively. These findings also suggest the existence of a deficiency gene showing an inverse homozygosity between alleged father and child in paternity testing.

In spite of a neutral amino acid mutation from Ser to Phe, the product of PI^*S_{iiyama} was shifted cathodally by about one positive charge to the normal PI M product after IEF. This mutation affects the three-dimensional structure of AAT. The Ser^{53} plays important roles in forming of the helix B and in stabilizing of the sheet 5B (Loebermann *et al.*, 1984; Huber and Carrell, 1989). Electrophoretic analyses have shown that the homozygous products of deficiency genes such as PI^*Z (Jeppsson *et al.*, 1978), $PI^*M_{heerlen}$ (Kramps *et al.*, 1981), and PI^*M_{rouen} (Martin *et al.*, 1975) also have the same microheterogeneity as the normal AAT. In contrast, the product of PI^*S_{iiyama} in serum was characterized by atypical banding patterns, differing in number, location and relative concentration from normal PI products.

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REFERENCES

Arnaud P, Chapuis-Cellier C (1988): α_1-Antitrypsin. Methods Enzymol **163**: 400–418

Cox DW (1989): α1-Antitrypsin deficiency. In: Scriver CR, Beaudet AL, Sly WL, Valle D (eds). The metabolic basis of inherited disease. 6th ed. McGraw-Hill, New York, pp 2409–2437

Crystal RG (1990): α1-Antitrypsin deficiency, emphysema, and liver disease. Genetic basis and strategies for therapy. J Clin Invest **85**: 1343–1352

Faber J-P, Weidinger S, Olek K (1990): Sequence data of the rare deficient $alpha_1$-antitrypsin variant PI Zaugsburg. Am J Hum Genet **46**: 1158–1162

Fucharoen SP, Fucharoen G, Sriroongrueng W, Laosombat V, Jetsrisuparb A, Prasatkaew S, Tanphaichitr VS, Suvatte V, Tuchinda S, Fukumaki Y (1989): Molecular basis of β-thalassemia in Thailand: analysis of β-thalassemia mutations using the polymerase chain reaction. Hum Genet **84**: 41–46

Huber R, Carrell RW (1989): Implications of the three-dimensional structure of α_1-antitrypsin for structure and function of serpins. Biochemistry **28**: 8951–8966

Jeppsson J-O, Laurell C-B, Fagerhol M (1978): Properties of isolated human α_1-antitrypsins of Pi types M, S and Z. Eur J Biochem **83**: 143–153

Kramps JA, Brouwers JW, Maesen F, Dijkman JH (1981): $Pi^{Mheerlen}$, a Pi^M allele resulting in very low α_1-antitrypsin serum levels. Hum Genet **59**: 104–107

Laurell CB (1965): Antigen-antibody crossed electrophoresis. Anal Biochem **10**: 358–361

Loebermann H, Tokuoka R, Deisenhofer J, Huber R (1984): Human α_1-proteinase inhibitor. Crystal structure analysis of two crystal modifications, molecular model and preliminary analysis of the implications for function. J Mol Biol **177**: 531–556

Martin J-P, Sesboue R, Charlionet R, Ropartz C (1975): Does alpha-1-antitrypsin PI null phenotype exist? Humangenetik **30**: 121–125

Matsunaga E, Shiokawa S, Nakamura H, Maruyama T, Tsuda K, Fukumaki Y (1990): Molecular analysis of the gene of the α_1-antitrypsin deficiency variant, Mnichinan. Am J Hum Genet **46**: 602–612

Seyama K, Nukiwa T, Takabe K, Takahashi H, Miyake K, Kira S (1991): *Siiyama* (serine 53 (TCC) to phenylalanine 53 (TTC)). A new α1-antitrypsin-deficient variant with mutation on a predicted conserved residue of the serpin backbone. J Biol Chem **266**: 12627–12632

Söderholm J, Smyth CJ, Wadström T (1975): A simple and reproducible method for crossed immunoelectrofocusing. Scand J Immunol **4** (suppl 2): 107–113

Yuasa I, Suenaga K, Gotoh Y, Ito K, Yokoyama N, Okada K (1984): PI (α_1-antitrypsin) polymorphism in the Japanese: Confirmation of *PI*M4* and description of new PI variants. Hum Genet **67**: 209–212