Jpn J Human Genet **42**, 363-368, 1997

Mutation Report

*ITIH1*Q0$_{iwate}$*, A NULL ALLELE OF INTER-ALPHA-TRYPSIN INHIBITOR H1 CAUSED BY DELETION/FRAMESHIFT MUTATION

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Summary The molecular characterization of the first example of null allele in the inter-alpha-trypsin inhibitor H1 (ITIH1) system, *ITIH1*Q0$_{iwate}$*, encountered as apparent inverse homozygosity of ITIH1 phenotypes between mother and child in a paternity case, is described. Single-strand conformation polymorphism analysis and subsequent sequencing showed that deletion of a single nucleotide in the codon for Lys^{87} results in a frameshift causing a terminator codon downstream of the deletion. This leads to premature termination of ITIH1 protein translation at amino acid 128, resulting in a truncated protein.

Key Words inter-alpha-trypsin inhibitor H1, null allele, frameshift mutation, PCR, SSCP, sequencing

Inter-alpha-trypsin inhibitor (ITI) is a plasma serine protease inhibitor consisting of two heavy chains, ITIH1 and ITIH2, and one light chain, ITIL (Enghild *et al.*, 1989), for which the structural genes are located on chromosome 3p211-p212, 10p13 and 9q32-33, respectively (Diarra-Mehrpour *et al.*, 1989). The function of the heavy chains is not clear, but is likely to play a role of carrier protein as a regulator for hyaluronan (Huang *et al.*, 1993). The genetic polymorphism of plasma ITI has been described by isoelectric focusing (IEF) and immunostaining techniques (Vogt and Cleve, 1990; Vogt *et al.*, 1991a; Yuasa *et al.*, 1991; Harada

Received January 29, 1997; Revised version accepted March 11, 1997.
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et al., 1994) and has been revealed to arise from the variation for the ITIH1 chain by Southern hybridization analysis (Vogt *et al.*, 1994) and by an immunological assay with three monoclonal antibodies for each chain (Harada *et al.*, 1995). The ITIH1 chain is composed of 877 amino acid residues with a molecular mass of 92 kDa (Diarra-Mehrpour *et al.*, 1992). The ITIH1 gene spans about 14 kb and includes 22 exons with 15–281 bp in size (Bost *et al.*, 1993). Three common alleles, *ITIH1*1*, *ITIH1*2* and *ITIH1*3*, are characterized by amino acid substitutions at positions 551 and 561 in exon 14 (Ding *et al.*, 1995a).

We previously reported a paternity case showing an inverse homozygosity between a mother (ITIH1 2) and her child (ITIH1 1). However, no incompatible results were found in the other polymorphic markers including several DNA polymorphic systems. In addition, they have shared a rare alpha-2-HS-glycoprotein allele, *AHSG*15* (Nakayashiki *et al.*, 1994). We suspected that they shared an ITIH1 null allele, tentatively designated $ITIH1*Q0_{iwate}$, responsible for

Table 1. Oligonucleotide PCR primers for ITIH1 gene.

Exon	Primer sequence (5′ to 3′)		*T* (°C)	Amplified size (bp)
	Forward	Reverse		
1	AGGCAAAAAGCTCCACTGCC	GCTGCCTATTTCTCGCTGTG	58	330
2	CACCTGTTCCGACTCCCCTA	GGAGAGTACCCCATGCCTATC	64	217
3	TCCTTCATGTCTCACTTTAG	TTGGGCAGGCACGCACA	52	203
4	GTGTCCTTCCCTCCCTGCA	CAGCCCTAGAGATGAGACCA	58	162
5	TGTCTGTCTGCTGTTGCCTCTG	ATGGTGACGGAGAGAGGGG-CA	62	242
6	GTTGCAGATTGATGTGGAC	TGGGCTAAGGCTGGACATT	52	208
7	ACTCTCGACGGTTTTCCAG	ACTCTCACAGCACCCCTTG	54	203
8	CAAGGGGTGCTGTGAGAGT-GG	TGAGTGATGGGGCTGTGGA-AG	62	220
9	CTACTGACTGTTCTGCCTTGC	GTTACCCTTACCCTCATCCAG	60	203
10	GTCTGCTTTCTCTTCCTTGC-AG	CAGCTTGCATGTCTCGGGTGA	62	212
11	CATCCCGTCACTACCCAGG	TTGGAGGTACAGGGGGTTG	56	244
12	CCCATTAGGAGCCCAGG	CAGGATGGGGAACAGTG	52	318
13	CACCACCCCTCTCTGTACC	TGGATGGTGAGGTAGGCCC	58	152
14	AGGTGAAAGGATGAGGGCCG	GTCGATGGTGGGCTTCAGGC	64	203
15	TACAGAAGGGAGAGGCCATG	GTGGAGTCAGGGAAGAATGG	58	195
16	GTGGGGAACTGTGGGTGAC	ACGGAGGAAGAAAGCCAACG	58	150
17	AGGGAAGAGTCATCAGAGG-GA	AGCTAGGCAGGTCCCAGAGA	60	260
18	TTCCATGCTGTGCCCCC	TAGTCCTGTCCTGGCTGA	52	208
19	CGGATGCCCTCTTGCTCCAT-TG	CACACACCTCCCCCTGCATC-TT	64	268
20	CTGAATGGAGAGGGATGCAG-TG	CAGCACATAGAAGCCCAGGA-AG	62	191
21	CGCTGTCTCCAATGTGCCTT-TC	AGGAAGCTCACCCCACCCAT-GT	64	232
22	CTACTGGTCCGAAGGGTGA	TCCTCCTCCTCTGCCTTGG	58	227

T, annealing temperature; underline shows region overlapping to exon sequence.

their incompatibility. In this study the *ITIH1*Q0$_{iwate}$* was investigated by polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) analysis and direct sequencing.

Genomic DNAs were prepared from whole blood of the members of a family (parents and child) and healthy Japanese volunteers, whose plasma ITIH1 phenotypes were known. Oligonucleotide primer pairs for the amplification of 22 exons were designed from genomic sequence of ITIH1 gene (Bost *et al.*, 1993) as shown in Table 1. Each exon was amplified by PCR mostly according to Thiberville *et al.* (1994). The PCR products of each exon were subjected to SSCP analysis as described by Ding *et al.* (1995a). Some target fragments showing an altered migration pattern were excised from a gel, amplified by the second PCR and then purified for direct sequencing. Sequencing was carried out using the forward and reverse primers of the corresponding fragment in an autosequencer (373A DNA sequencer, Applied Biosystems).

Each exon was successfully amplified by PCR and the sizes of each fragment were consistent with ones expected from primer designs and the genomic sequence. SSCP analysis showed altered migration patterns in some exons including exons 4 and 14. As shown in Fig. 1, the mother and child were distinguishable in the SSCP pattern of exon 4 from other individuals with common ITI phenotypes. In the SSCP analysis of exon 14 which characterizing three common alleles (Ding *et al.*, 1995a), the mother (ITIH1 2-Q0$_{Iwate}$) and child (ITIH1 1-Q0$_{Iwate}$) were typed as ITIH1 2-1 and ITIH1 1, respectively (data not shown). Therefore, we hypothesize that the *ITIH1*Q0$_{iwate}$* is a derivative of *ITIH1*1*. Direct sequencing of amplified fragment of exon 4 from the mother and child revealed a deletion of the third nucleotide (guanine) of the codon for Lys^{87} (Fig. 2). This deletion results in a frameshift causing a terminator codon at position 128 in exon 5, leading to a truncated protein (Fig. 3). This protein is probably unstable, because it consists of only 127 amino acids, *i.e.*, about 15% of the mature protein, and is predicted to have an alternative carboxyl terminus (amino acids 87–127). Even if it were expressed in blood, the antigenic epitopes to commercially available polyclonal

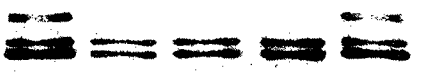

Fig. 1. Single-strand conformation polymorphism (SSCP) analysis for exon 4 of the ITIH1 gene.

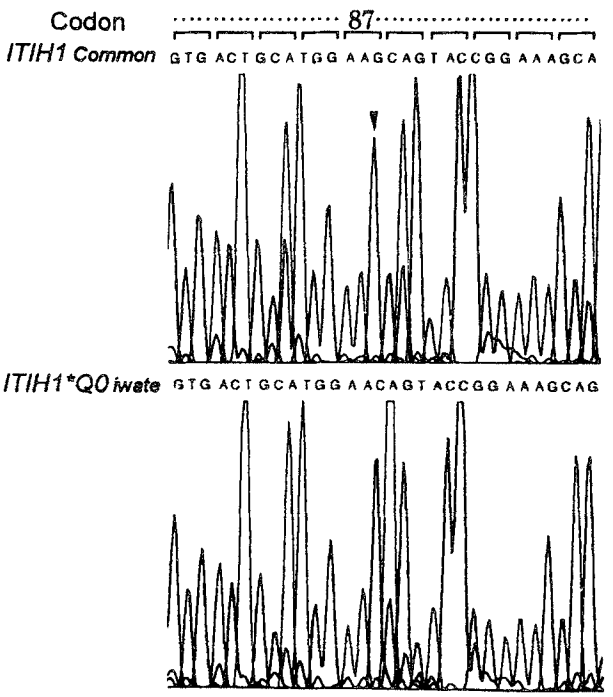

Fig. 2. Direct sequencing of amplified fragments for exon 4 of the *ITIH1 common* allele and *ITIH1*Q0$_{iwate}$* allele. A single base (arrowhead) in codon 87 was lacked in the *ITIH1*Q0$_{iwate}$* allele sequence.

Codon	87	88	89	···	127	128	129	···
ITIH1 Common alleles	AAG	CAG	TAC	···	CAG	CTG	ACT	···
Amino Acid	- Lys -	Gln -	Tyr -	···	- Gln -	Leu -	Thr -	···
	(G)							
*ITIH1*Q0 iwate*	AAC	AGT	ACC	···	AGC	TGA	CTT	···
Amino Acid	- Asn -	Ser -	Thr -	···	- Ser -	Ter		

Fig. 3. A schematic representation of regions around codon 87 in exon 4 and codon 128 in exon 5. (G): missing nucleotide in *ITIH1*Q0$_{iwate}$*, Ter: terminator codon.

anti-human ITI antibody would be highly or completely lost.

In this study, 22 pairs of primers were prepared to analyze every coding exons of ITIH1 gene. Some of primers contained nucleotide sequences of coding regions (Table 1), because of the limited genomic sequence data (Bost *et al.*, 1993). Only a few short regions of coding exons and a 5′-flanking region were not analyzed.

Though possible mutations in the unexamined regions were not denied, we supposed that the deletion of a guanine residue resulting in a terminator codon is a cause to form the *ITIH1*Q0$_{iwate}$*. The mother and child in this case were apparently healthy, suggesting that there are no clinical problems in a heterozygous state of *ITIH1*Q0$_{iwate}$*. It would be of much interest to know clinical influence and structural effect to ITI molecule in a homozygous state.

Vogt *et al.* (1991b) observed the significant increase of homozygotes in 81 tested children under 18 months and described that the incomplete expression of ITI heterozygous phenotypes in younger children might raise incorrect typing as homozygous ones. On the other hand, Ding *et al.* (1995b) investigated 79 newborns to compare the phenotypes by IEF of chondroitinase ABC-treated plasma ITIH1 with those by SSCP analysis of PCR products of exon 14, and concluded that there are no incompatible cases. In this study, we present the evidence for the existence of a null allele in the ITIH1 systems, so that attentions must be paid in conventional ITIH1 phenotyping of plasma samples. To dissolve such problems, PCR-SSCP typing of exon 4 and 14 should be useful for accurate ITIH1 genotyping.

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