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A New Type of Silent Butyrylcholinesterase Genotype found in Hyogo Prefecture

— a case of familial cholinesterasemia —

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ABSTRACT

A point mutation that causes a silent phenotype for human serum butyrylcholinesterase(BChE) was identified in the DNA of a 47-year old Japanese woman visited the hospital for complaining hypertension.

The propositus had extremely low BChE activity, but her younger sister and her dauther had intermediate value of BChE activity and her elder sister showed normal level.

An immunological method revealed that the propositus showed the presence of usual amounts of BChE protein in serum. DNA sequence analysis of the propositus identified a point mutation at codon 199(GCA → GTA), resulting in a Ala → Val substitution. This alteration site is one amino acid downstream of the esteratic site(Ser, 198). A new Pst1 restriction site was introduced into the PCR product from the normal subject by PCR-primer introduced restriction analysis(PCR-PIRA) using a specific mismatched primer and the family analysis by digestion with Pst1 disclosed that her younger sister and her daughter had the same mutation.

Key Words: silent type hereditary serum cholinesterase deficiency; succinylcholine; gene analysis; point mutation

Introduction

Hereditary serum butyrylcholinesterase(BChE) deficiency is a rare autosomal recessive disease characterized by resistance to hydrolysis of sevral esteratic drug, particularly succinylcholine(SCC), a short acting muscle relaxant. When this drug is injected intravenously into individuals homozygous for this disease, a dangerous prolonged apnea occur as the result of muscle paralysis, but the carrier with this disease has no harmful disabilities in daily life. Recently, several genetic variants of BChE deficiency have been reported in Japanese population, such as BCHE ALU355[1], BCHE365R[2,3], BCHE FS315[3], BCHE418S, BCHE515C, BCHE210P and BCHE 465P[4]. All these variants were characterized by silent type of BChE. In this paper, we show a new case of a point mutation of the silent type of BChE.

MATERIALS AND METHODS

The propositus was a 47-year old Japanese woman who visited the hospital for complaining hypertension. Laboratory data revealed that the level of BChE was markedly decreased. Physical examination showed no abnormal findings except that her blood pressure was 160/92. She had no previous history of organophosphate compound poisoning. BChE activity mesured on her family members indicated that her younger sister and her daughter were suspected of having the BChE deficiency.

BChE activity in serum was measured using butyrylthiocholine iodide as a substrate by the method of Iuchi et al[5] and phenotype was determined by measuring the inhibition numbers, dibucaine number(DN) and fluoride number(FN).

Sera of the propositus and her three family members were electrophoresed on 8% polyacrylamide slab gel and stained with 2-amino-5-chlorotoluene azotate and α-naphthylacetate as substrates.

Antihuman BChE rabbit serum purchased from Dako (Glostrup, Denmark) was used as the first antibody and then with horseradish peroxidase conjugated swine antirabbit IgG as the second antibody for Western Blotting analysis(Hangaard et al[6] and Hirano[7]).

Genomic DNAs were prepared from peripheral white blood cells according to the method of Maniatis et al [8]with slight modification and PCR was carried out according to the method of McGuire et al [9]with specific primers: sense 5'-CCTAAAAGTGTAA-CTCTCTTTGGAGAAA*CTG-3', which binds from codon 189 to the first base of codon 199 and contains a single base mismatch at the third position from the 3' end(asterisk), and antisense 5'-GGGACAACAAATGCTTCATTCAGAAGAATTTCTTGGGGA-3', which binds to codon 281-268. The amplified DNA fragments(278bp) were digested with Pst I restriction enzyme according to the manufacturer's instructions. The digests were separated by electrophoresis on 1.0% agarose gel.

The DNA of the four individuals was amplified by PCR with one pair of primers : sense 5'-CAAGCATCATATTTTAGGTAATTATCATCAATAAAG-3', which binds at 91 nucleotides upstream from the intron1/exon2 junction, antisense 5'-GGGACAACAAATGC-TTCATTCAGAAGAATTTCTTGGGGA-3',which binds to codon 268-281 in exon2. The PCR products were directly sequenced by means of a cycle sequencing kit with an automated DNA sequencer(Applied Biosystems, 373 DNA Sequencer).

RESULTS AND DISCUSSION

The family tree of the propositus is showh in figure 1.

The propositus(I -2) had unusually low SChE activity. Her younger sister (I -3) and her daughter(II-1) had intermediate values for BChE activity, but her elder sister (I -1) showed normal level. Inhibition numbers of the propositus could not be measured, but other three members of this family had dibucaine and fluoride numbers characteristic of the usual phenotype(data not shown).

Electrophoretically seen C_4 band was not identified for the serum of propositus, but was identified for the serum of other family members. But intensity of the bands for the serum of her younger sister and her daughter were much weaker than that of her elder

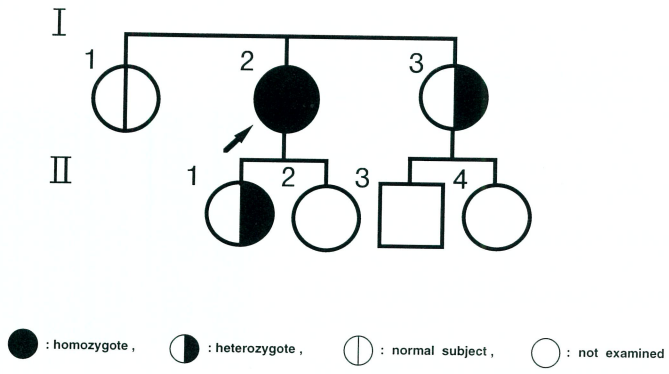


Fig. 1. Family tree. An arrow indicates the propositus.

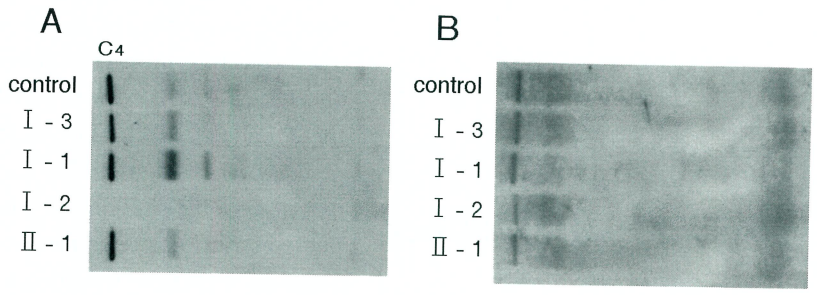
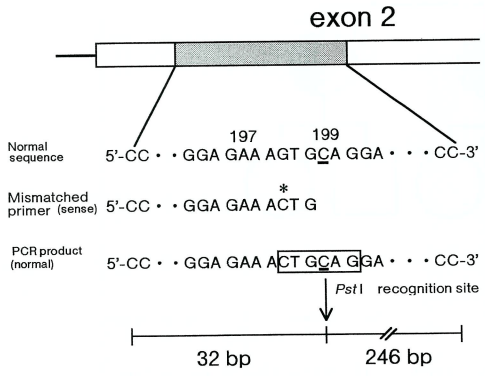


Fig. 2. A : BChE activity staining of the propositus and her three family members. B : Peroxidase staining of immunoreactive BChE protein on nylon membrane. Note that the propositus and other family members demonstrated immunoreactive BChE protein in their serum.

sister and normal subject(Fig. 2a). Immunoreactive BChE protein in serum of both the propositus and three family members was detected by treatment of anti-BChE antibody after Western Blotting analysis (Fig. 2b).

The PCR product of 278 bp resulted from the elder sister(I -1) of propositus created a new Pstl restriction site and was completely digested into two fragments of 32 and 246 bp by Pst I. The PCR product from the propositus was found to be resistant to Pst I digestion because of the absence of Pstl site. Two other family members(I -3 and II -1) showed the two restriction fragments bands of 246 and 278 bp (Fig. 3). Sequence analysis revealed that BChE gene of the propositus had a transition mutation of C to T in nucleotide 596, which convert codon 199 from GCA(Ala) to GTA(Val), who was homozygote of this mutation. Her younger sister and her daughter had two bases C and T on the same position in the nucleotide 596, suggesting hetrozygous for this mutation and her elder sister showed only one base, C, in the nucleotide 596 (Fig. 4).

There are four genes recognized on the locus E_1 that participate in directing ChE



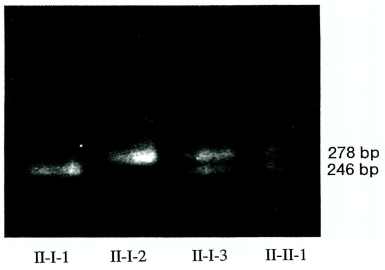


Fig. 3. Scheme for detection of Ala199Val mutation of BChE variant. Specific primer is composed of 31 mer and contains a mismatched single base(G → C) at the third position from the 3' end(asterisk). The pCR product of 278bp derived from the normal subject at codon 199(under bar) has a new Pst I restriction site(square box) and is digested into two fragments of 32bp and 246bp.
Right: separation of Pst I restriction fragments of four members(I -1, I -2, I -3 and II -1) on 4% agarose gel.

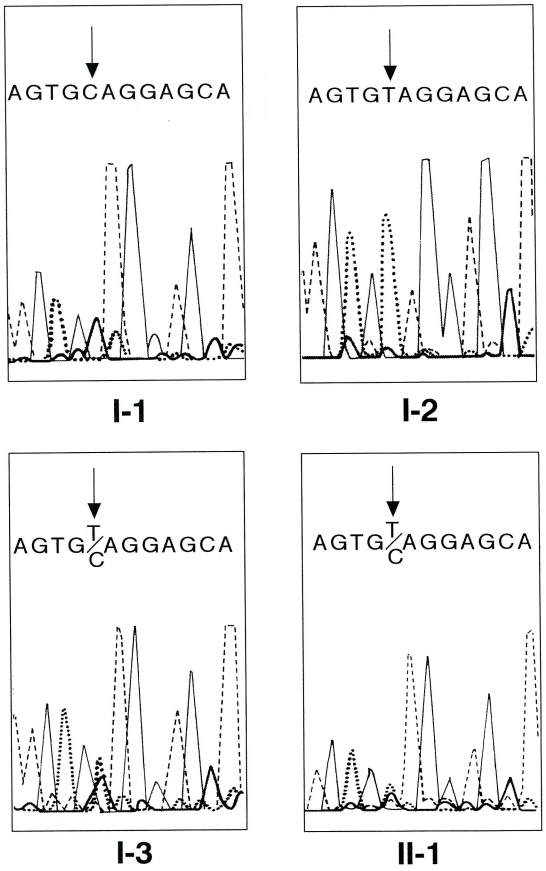


Fig. 4. Sequence analysis of the automated DNA sequencer for the region of the mutation. a) the propositus(I -2) is homozygous for the substitution GCA → GTA at codon 199(Ala → Val), b) the elder sister (I -1) and the younger sister(I -3) and daughter(II -1) had a normal and a heterozygous condition for the same mutation, respectively.

biosynthsis. They are E_1^u(usual : normal enzyme), E_1^a(atypical : dibucaine-resistant type), E_1^f(fluoride-resistant type) and E_1^s(silent type).

The E_1^s gene includes at least two BChE variants. One has zero activity and does not cross-react with antibody to normal human BChE(type I). The other has about 2 % of normal activity and is recoganized as the presence of cross-reactive protein(type II). Our case is supported to be the latter type.. Recently, three BChE variants of silent type, which produce normal amounts of immunoreactive materials but enzymatically inactive BChE protein in the serum, have been reported by Primo-Parmo et al[10], but this kinds of BChE variant have not been found in Japan. Therefore, the present case is the first one detected in Japan. In this investigation, we found a point mutation (GCA → GTA) at codon 199, resulting in a missense mutation from alanine to valine.. This alternation site is one amino acid downstream from esteratic region (Ser 198).

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REFERENCES

1) Muratani K, Hada T, Yamamoto Y. et al : Inactivation of the cholinesterase gene by Alu insertion : possible mechanism for human gene transposition. Proc Natl Acad Sci USA 88 : 11315-11319, 1991
2) Hada T, Muratani K, Ohue T, Imanishi H, Moriwaki Y, Itoh M, Amuro Y, Higashino K. : A variant serum cholinesterase and a confirmed point mutation at Gly-365 to Arg found in a patient with liver chirrhosis. Internal Medicine 31 : 357-362, 1992
3) Hidaka K, Iuchi I, Yamasaki T, Ohhara M, Shoda T, Primo-Parmo SL, LaDu BN : Identification of two different genetic mutations associated with phenotypes for human serum cholinesterase in Japanese. J Clin Path 40 : 535-540, 1992
4) Maekawa M, Sudo K, Kanno T, Kotani K, Dey DC, Ishikawa J, Izumi M, Etoh K : Genetic basis of the silent phenotype of serum bytyrylcholinesterase in three compound heterozygotes. Clin Chim Acta 235, : 41-57, 1995
5) Iuchi I, Ameno S, Shibata S : Survey of atypical pseudocholinesterasemia in Okayama district and a new method for its detection. Bull Yamaguchi Med Sch 16 : 35-44, 1969
6) Hangaard J, Whittaker M, Loft AGR, Norgaard-Pedersen B : Quantification and phenotyping of serum cholinesterase by enzyme antigen immunoassay : methodological aspects and clinical applicability. Scand J Clin Lab Invest 51 : 349-358, 1991
7) Hirano H : Microsequence analysis of protein electroblotted from polyacrylamide gels. Protein Nucleic Acid and Enzyme 33 : 2388-2396, 1988
8) Maniatis T, Fritsch EF, Sambrook J : Molecular Cloning : A Laboratory Manual,1st ed. New York : Cold Spring Harbor, 1982, p122, 280-2811982
9) McGuire MC, Nogueira CP, Bartels CF, Lightstone H, Hajra A, Van Der Spek AFL. Lockridge O, LaDu BN : Identification of the structural mutation responsible for the dibucaine-resistance(atypical) variant form of human serum cholinesterase. Proc Natl Acad Sci USA 86 : 953-957, 1989
10) Primo-Parmo SL, Bartels C, Wiersema B, van der Spek AFL, Innis JW, La Du BN : Charac-

terization of 12 silent alleles of the human butyrylcholinesterase(BChE) gene. Am J Hum Genet 58 : 52-64, 1996