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The first instance of nonsense mutation(BChE, 400 stop) in exon 2 of the butyrylcholinesterase gene detected in Yamaguchi Prefecture

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ABSTRACT

A point mutation that causes a silent phenotype for human serum butyrylcholinesterase(BChE) was proved by DNA analyses of a 64-year old Japanese female who visited the hospital because of a common cold. The propositus and her two siblings showed extremely low BChE activity, but other family members (six individuals) manifested from intermediate to normal values of BChE activity. An immunological method revealed that the propositus and her two siblings showed absence of the BChE protein in serum. DNA sequence analysis of the propositus identified a point mutation at codon 400(TGC → TGA), resulting in the production of a stop codon. This alteration exists upstream of the Cys 571 of the subunit, which forms a disulfide bridge with the Cys 571 of another partner subunit.

Key words: Butyrylcholinesterase activity deficiency; succinylcholine; gene sequence analysis; point mutation

Introduction

Human butyrylcholinesterase(BChE) is a homotetramer of about 430,000 daltons. A subunit is glycoprotein made of 574 amino acids in sequence[1]. Hereditary serum BChE deficiency is a rare autosomal recessive disease characterized by resistance to hydrolysis of sevral cholinester drugs, particulary succinylcholine(SCC), a short-acting muscle relaxant. Individuals homozygous for this deficiency experience prolonged apnea after intravenous administration of SCC in usual doses, but it is harmless to the carrier in daily life[2].

Seven types of mutations responsible for BChE deficiency have been reported to date with DNA variation in the Japanese population. The first case was characterized by an insertion of an Alu-like element in exon 2, namely, BCHEALU355[3], the second case by an insertion of a single nucleotide, resulting in the making of a frameshift at position 315, BCHEFS315[4], the third case by a nucleotide substitution, resulting in a termination codon at codon 465, BCHE465STOP[5], and the fourth to seventh instances were BCHE365R[4,6],

BCHE250P, BCHE418S and BCHE515C[5].

This paper aims to report another form of point mutation creating a stop codon at position 400 which might produce immature BChE.

MATERIALS AND METHODS

The propositus was a 64-year old Japanese female who visited Yamaguchi Prefectural Central Hospital because of a common cold. Laboratory data indicated unexpectedly diminished BChE activity for a cold. Physical examination showed no particularly abnormal findings. She had no previous history of organophosphorus compound poisoning. BChE studies carried out on her family members led to the suspicion that her elder sister and elder brother had the same BChE deficiency.

BChE activity in serum and the inhibition numbers, namely, dibucaine(DN) and fluoride(FN) were measured using butyrylthiocholine iodide as a substrate by the method of Iuchi et al[7].

The sera of the propositus and her eight family members were subjected to electrophoresis on 8% polyacrylamide slab gel and stained with 2-amino-5 chlorotoluene diazotate after incubation of the gel in α-naphthylacetate solution.

Electrophoresis of the sera of propositus and her family members was carried out on 8% polyacrylamide slab gel, after which they were transferred onto a nylon membrane with the help of electric semidry equipment according to the method of Hirano[8]. The membrane was incubated with antihuman BChE rabbit serum(DAKO, Glostrup, Denmark) as the first antibody, and then with horseradish peroxidase conjugated swine antirabbit IgG as the second antibody to visualize bands of BChE protein according to the method of Hangaard et al[9].

The immunoreactive BChE protein was stained with Konica immunostain HRP-1000(KONICA Co.) according to the manufacturer's instruction.

Genomic DNA of 10 individuals including the propositus was amplified by the polymerase chain reaction(PCR) according to the method of McGuire, et al[10]. The oligonucleotides used as the primer for the PCR were 5'-ACATCTCTTTATGAAGCTACGAACAGA-3', which binds to codon 234-242 in exon 2 for the sense side and 5'-AAGCCAGAGAACAATGACAAAAA-TCAGCACTTAC-3', which binds to the 35 nucleotide downstream from the exon 2 / intron 2 junction for the antisense side. A nucleoitde of C to A transversion change at codon 400 created a new BspMI restriction site ($ACCTGC(N)_4$). Therefore, the PCR products were digested with BspMI and the digests were separeted electrophretically on 1.0% agarose gel.

The PCR products amplified with primers described above were directly sequenced with a sequencing primer end-labeled with ^{32}P by the dideoxy chain termination method. Since a sufficient amount of fresh blood was not obtainable in one individual(III-2), DNA was amplified directly from her minute whole blood volume (10μl) and then a nested PCR was performed to obtain an adequate amount of purified PCR product for sequencing. The pair of primers for the nested PCR were 5'-ACGTTGAACTTAGCTAAATTG-3', which binds to codon 243-249 and 5'-CCATATTTTGCAAAATTTGTCCA-3', which binds to

codon 478-466 as a oligonucleotide of the sense and antisense primers, respectively.

RESULTS

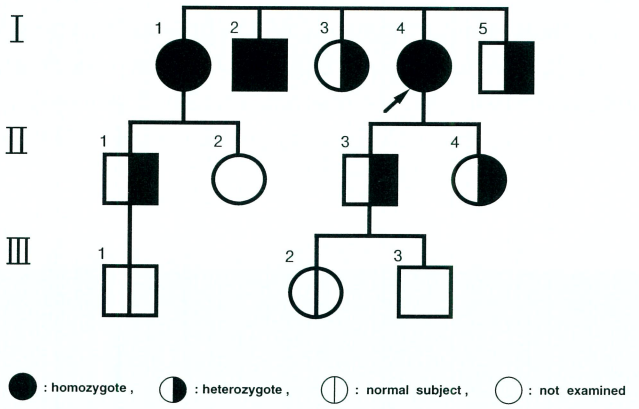

Figure 1. Family tree. An arrow revealed the propositus.

The propositus(I -4) as well as individuals of I -1, I -2 in figure 1 showed no BChE activity. The activity of I -3, I -5, II-1 and II-4 was intermediate level. The inhibition numbers of these members were within the usual limit. Individuals III-1 and II-3 showed normal values in both activity and in hibition numbers (data are not shown).

The C_4 band, which is a major component of the usual BChE isozyme, was not demonstrated in serum of the propositus(I -4) and her two siblings(I -1 and I -2), but was clearly seen in the sera of other family members. The intensity of the coloration of the C_4 bands in the sera of her other family members seemed normal (Fig. 2A).

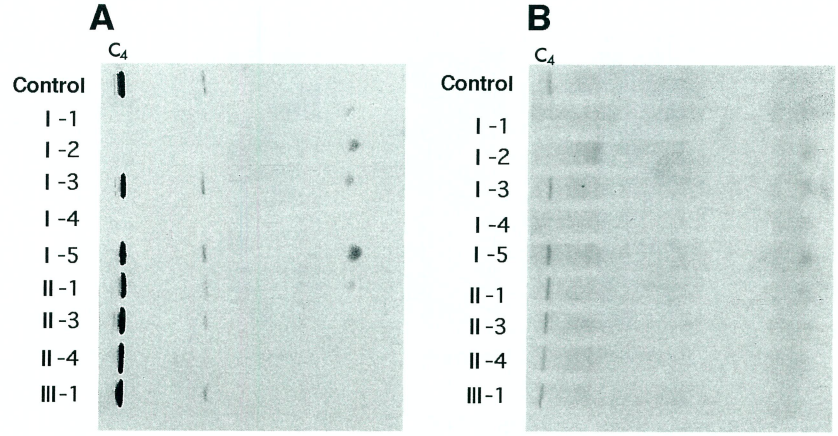

Figure 2. Butyrylcholinesterase zymogram.
A : BChE activity staining of the propositus and family members.
B : Peroxidase staining of immunoreactive BChE protein on nylon. Note that I -1, I -2 and I -4 show absence of the C_4 band.

The immunoreactive BChE protein band was not seen in the propositus and two family members (I -1 and I -2), but the presence of BChE as protein was clearly demonstrated in other family members (Fig. 2B).

The PCR product (395 bp) derived from the propositus and her two siblings(I -1 and I -2) was completely digested into fragments of 135 bp and 260 bp by BspMI and that of five other members(I -3, I -5, II-1, II-3 and II-4) showed the three fragments bands of 135, 260 and 395 bp, and that of two other members(III-1 and III-2) showed only one band of 395 bp. These results were consistent with the genotypes detected by DNA sequencing (Fig. 3).

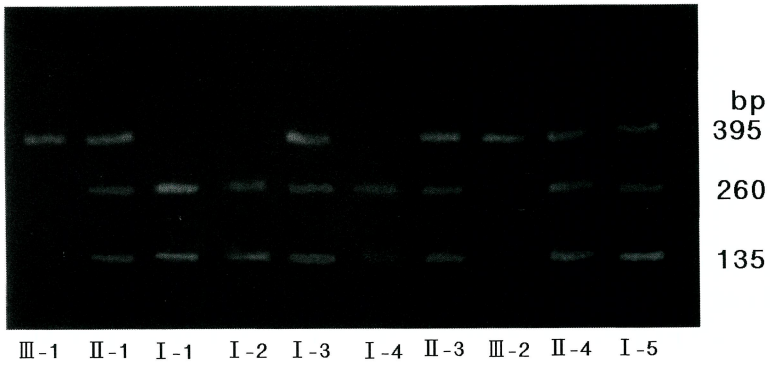

Figure 3. Electrophoresis of BspMI digests of amplified DNA.
PCR product (395bp) was purified, digested with BspMI, electrophoresed on 4% agarose gel and stained with ethidium bromide. The mutant allele showed bands of 135 bp and 260 bp, while normal allele remained undigest.

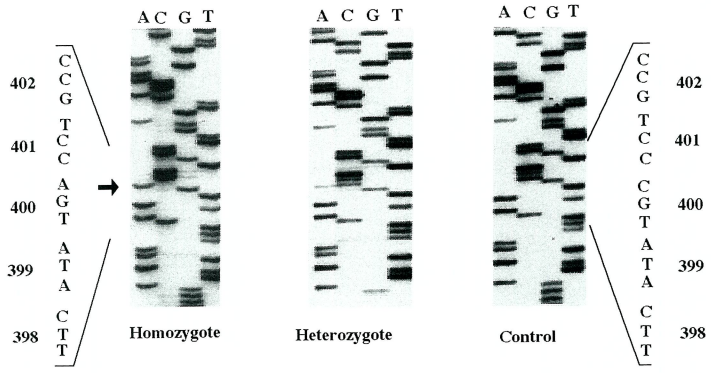

Figure 4. Sequence analysis of amplified DNA.
The propositus (I -4) is homozygous for the C → A transversion at codon 400. An individual(I -3) who is heterozygous for this mutation shows the mutant A and the usual C. A normal control is shown to the right. The C → A transversion at codon 400 is indicated by an arrow.

Sequence analysis verified that the BChE gene of the propositus has a transversion mutation of C to A in nucleotide 1200, which changes codon 400 from TGC(Cys) to TGA(termination codon) and, further more, it indicates the homozygous condition of this mutation (Fig. 4). Her two siblings(I -1 and I -2) were also homozygotes of the same mutation. Five other members (I -3, I -5, II-1, II-3 and II-4) had two bases, C and A, at nucleotide 1200, indicating they are heterozygous. Individuals III-1 and III-2 showed one base, C, only at nucleotide 1200, which is usually seen in the normal BChE DNA sequence (Fig. 4).

The genotypic expression of this family is accordingly consistent with the results in figure 1. The other coding regions ; namely, exons 1 through 4, were normal base sequences corresponding to those of normal BChE.

DISCUSSION

There are four allelic genes recognized on the locus E_1 that participate in directing BChE 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 combination of three abnormal genes(E_1^a, E_1^f and E_1^s) and E_1^u may give rise to 10 genotypes. The homozygote and compound heterozygotes among E_1^a, E_1^f and E_1^s invariably exhibit from moderate to severe hypersensitivity for SCC. The genotypes determined by E_1^a, E_1^f, and E_1^s genes in both homozygote and compound heterozygote conditions except homozygous E_1^s can be differentiated by use of representative inhibitors ; i.e., dibucaine and sodium fluoride. The E_1^s gene in homozygous condition is readily detected by measuring activity where there is a trace or no enzyme activity. However, the detection of an individual heterozygous for the E_1^s gene is very difficult because the BChE activity of an individual with E_1^u/E_1^s overlaps with that of the usual E_1^u/E_1^u. Therefore, to establish such a heterozygosity, gene analyses are essentially necessary. Because the BChE activity of two members, II-3, and III-1, seemed to be at the lower limit of the normal range. It was difficult to decide their heterozygosity only from BChE activity. A final conclusion was made after REA and/or DNA sequence analysis (figure 3, figure 4).

Several cases of nonsense mutations of the BChE molecule leading to silent type BChE have been reported. They include BCHEFS6(10%)[12], BCHEFS117(22%)[13], BCHE271STOP(47%)[11], BCHEFS315(56%)[4], BCHE400STOP(71%)(the present case) and BCHE500STOP(87%)[11]. The values in the parentheses show the percentage of the peptide length as an amino acid sequence to the total peptide length (574 amino acid). The first four cases may be expected to have no activity of the BChE enzyme because the length of aberrant BChE peptide is too short when it is considered that the peptide in usual BChE consists of 574 amino acids. Our present case, BCHE400STOP, and the last case, BCHE500STOP, may be questionablely explainable only by peptide length. According to Lockridge et al.,[14] the quarternary structure of tetramic serum BChE consists of four identical subunits arranged as a dimer of dimers ; that is, the Cys^{571} of the subunit forms a disulfide bridge with the Cys^{571} of another identical subunit to stabilize the dimers, and two such dimers are hydrophobically linked into a tetramer. Both the interchain disulfide

bonds and the hydrophobic linkage region are believed to be located close near the carboxyl terminus of the subunit.

The last two cases, therefore, may not have the interchain disulfide and hydrophobic bonds and would obviously be expected to produce a sterically imcomplete BChE molecule.

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