Genetic analysis of a Japanese patient with butyrylcholinesterase deficiency

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SUMMARY

A patient (64-year-old, male) with familial cholinesterasemia caused by BChE deficiency was studied. DNA sequence analysis of all exons identified a point mutation, an A → G transition at codon 128, resulting in a Tyr → Cys substitution. The propositus showed extremely low BChE activity, but his other family members (three individuals) showed from intermediate to normal BChE activity. An immunological method revealed the absence of BChE protein in serum of the propositus. Both PCR primer introduced restriction analysis (PCR-PIRA) and sequence analysis revealed all three family members to be heterozygotes for this mutation.

INTRODUCTION

Human butyrylcholinesterase (BChE EC 3.1.1.18) is a glycoprotein enzyme that hydrolyses choline esters. Familial BChE deficiency is a rare autosomal recessive disorder characterized by resistance to hydrolysis of several cholinester drugs, particularly succinylcholine (SCC), a short-term muscle relaxant that is used in surgical operations. Prolonged apnea caused by SCC in individuals homozygous for this deficiency has been shown to be the result of decreased ability of mutant BChE to degrade this drug. A number of different types of missense mutation have been identified in the BChE gene in Japanese patients with BChE deficiencies; BCHE24M (Maekawa et al. 1994), BCHE199V (M. Sakamoto, personal communication), BCHE250P (Maekawa et al., 1995), BCHE330I (Maekawa et al. 1994), BCHE365R (Hidaka et al. 1992), BCHE418S (Maekawa et al. 1995) and BCHE515C (Maekawa et al. 1995). These mutations resulting in a nonfunctional BChE have been found in exon 2 and exon 3 of the BCHE gene. In this paper, we report a missense mutation (Tyr128→Cys) in exon 2 of the BCHE gene.

MATERIALS AND METHODS

1. Case report

The propositus was a 64-year-old Japanese male who visited Sakakibara Hospital for a surgical operation on his heart. When he visited this hospital for his heart disease several years earlier, laboratory data indicated unexpectedly diminished BChE activity for his condition. When he was hospitalized again for heart disease, a decrease in his BChE activity was still evident. BChE studies carried out on his family members indicated that his daughter, son and grandson might have the same BChE deficiency.

2. BChE activity and phenotyping

BChE activity in serum was measured by using butyrythiocholine iodide as a substrate. Inhibition numbers, dibucaine (DN) and fluoride
Table 1. Biochemical analysis of the propositus and other family members. I-1 is the propositus and other members are the members indicated in Figure 1. DN, Dibucaine number; FN, fluoride number

<table>
<thead>
<tr>
<th></th>
<th>BChE activity (U)</th>
<th>DN (%)</th>
<th>FN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>II-1</td>
<td>82</td>
<td>75</td>
<td>65</td>
</tr>
<tr>
<td>II-2</td>
<td>150</td>
<td>77</td>
<td>67</td>
</tr>
<tr>
<td>III-1</td>
<td>151</td>
<td>77</td>
<td>64</td>
</tr>
<tr>
<td>Control</td>
<td>a 177</td>
<td>78</td>
<td>67</td>
</tr>
</tbody>
</table>

*The normal range of BChE activity is 150–260 µmol ml⁻¹ h⁻¹.

1. BChE activity

The BChE activity and inhibition numbers of the family members are shown in Table 1. The propositus (I-1) showed no activity. The BChE activity of II-1 was the intermediate level. The BChE activity of II-2 was 30%, and the fluoride number (FN) of II-2 was 67%. The normal range of BChE activity is 150–260 µmol ml⁻¹ h⁻¹.

2. BChE isozyme analysis

The sera of the propositus and his three family members were subjected to electrophoresis on a 8% polyacrylamide slab gel and stained with 2-amino-5-chlorotoluene diazotate after incubation of the gel in α-naphthylacetate solution (Harris et al. 1962).

3. BChE protein analysis

Electrophoresis of the sera of the propositus and his family members was conducted 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, 1988. The membrane was incubated with antihuman BChE rabbit serum (DAKO, Glostrup, Denmark) as the first antibody, and then bands were visualized with horseradish peroxidase conjugated swine antirabbit IgG as the second antibody according to the method of Hangaard et al. (1991). The immunoreactive BChE protein was stained with Konica immunostain HRP-1000 (KONICA Co.) according to the manufacturer’s instructions.

4. Polymerase chain reaction–primer introduced restriction analysis (PCR–PIRA)

Genomic DNAs of the propositus and his three family members were amplified by polymerase chain reaction (PCR) with specific mismatched primer according to the method of McGuire et al. 1989. The oligonucleotides used as primers were 5’-GACCAAGTGCTGATATT-3’, which binds to codon 49–56 for the sense side and 5’-CTTTCAACCGAGCCAGAAACTTGCCGTCA-3’, which is a 30-mer, with a mismatch single base (A → G) at the fourth position (asterisk) from the 3’ end. This binds to codon 128–138 in exon 2 for the antisense side. The PCR products were digested with Tsp45I according to manufacturer’s instructions. The digest was separated by capillary gel electrophoresis (CGE) using a 270 A capillary electrophoresis system (Applied Biosystems, USA) with a polyacrylamide-coated capillary [efficient diameter × length, 100 µm × 30 cm], according to the method of Arakawa et al. (1994). The sample was loaded electrophoretically by application at 5 kV for 5–30 sec. The analysis was run with 100 mm Tris-borate buffer (pH 8.3) at 10 kV and at 30 °C. Peak detection was monitored at 260 nm.

5. Analysis of DNA

Genomic DNA of the four individuals including the propositus was amplified by polymerase chain reaction (PCR) as described above. The oligonucleotides used as primers for the PCR were 5’-CTTGGTAGACTTGCAAAA-3’, which binds to codon 38–44 in exon 2 for the sense side and 5’-GGGACAACAAATGCTTCATTCAGAAGAATTTCTTGGGA-3’, which binds to codon 269–281 in exon 2 for the antisense side. The PCR products were directly sequenced with a sequencing primer end-labelled with 32P by the dideoxy chain termination method and electrophoresed on 6% denaturing polyacrylamide gel.

RESULTS

1. BChE activity

The BChE activity and inhibition numbers of the family members are shown in Table 1. The propositus (I-1) showed no activity. The BChE activity of II-1 was the intermediate level. The

2. BChE isozyme analysis by electrophoresis

The C₄ band, which is a major component of the usual BChE isozyme, was not observed in the serum of propositus (I-1), but was clearly seen in the serum of the other family members. The intensity of the colouration of C₄ bands in the sera of the other family members was indistinguishable from normal (Fig. 2A).

3. BChE protein analysis by electrophoresis

An immunoreactive BChE protein band was absent in the propositus, but the presence of BChE protein was demonstrated in the other family members (Fig. 2B).
Fig. 3. Top: Scheme for detection of the Tyr<sup>128</sup>→Cys mutation of BChE variant. The specific primer is composed of 30 mer and contains a mismatched single base (A→G) at the fourth position from the 3′ end (asterisk). The PCR product of 267 bp derived from the mutant with A→G substitution has a new Tsp<sub>45I</sub> recognition site (square box) at codon 128 (under bar) and is digested into two fragments of 31 bp and 236 bp. Bottom: GC electrophoregram of DNA fragments resulting from Tsp<sub>45I</sub> digestion of PCR product obtained from the propositus (A), II-1 (B) and a control subject (C).

4. **PCR–PIRA**

The PCR product of 267 bp obtained from the propositus (I-1) created a new Tsp<sub>45I</sub> restriction site, which was completely digested with Tsp<sub>45I</sub>. It was cleaved into two fragment of 31 bp and 236 bp and the digests were analysed by CGE. However, only the peak of 236 bp (a) was present on the CGE chromatogram. The peak of 31 bp was not found because the absorbance for a nucleotide of this length is generally extremely low. Family member (II-1) showed two peaks of 236 bp (a) and 267 bp (b) and compared with the single peak of 267 bp (b) of the normal subject (Fig. 3). The Tsp<sub>45I</sub> digests in family members II-2 and III-1 were consistent with heterozygosity for the mutant allele (data not shown).

5. **Analysis of DNA**

Sequence analysis verified that the BCHE gene of the propositus (I-1) had a transition mutation of A to G in nucleotide 383, which changed codon 128 from TAT (Tyr) to TGT (Cys), and it further indicated the homozygous condition of this mutation (Fig. 4). The other three family members (II-1, II-2 and III-1) had two bases, A and G, at nucleotide 383, indicating they were heterozygotes (data not shown). The genotypic expression of this family is accordingly consistent with the result presented in Fig. 1. The other coding regions, namely, exon 1, exon 3, exon 4 and the remaining region of exon 2, were normal base sequences corresponding to those of normal BChE.
We have characterized the molecular deficiency in a propositus with familial butyrylcholinesterasemia and have determined that he is a homozygote for a missense mutation; an A→G transition at codon 128 and nucleotide 383, resulting in a Tyr128→Cys substitution. The propositus (I-1) was readily diagnosable as a homozygote for the silent BCHE gene because of a trace of BChE activity, whereas his daughter (II-1) among the three family members was presumed to be a heterozygote because of her intermediate BChE activity. However, detection of phenotyping of the remaining two individuals (II-2 and III-1) was difficult because their BChE activity was at the lower level of normal range. The final proof that they were heterozygotes for the same mutation was only obtained by PCR-PIRA and/or DNA sequence analysis.

The new mutation observed in this study is associated with total loss of BChE activity. Therefore, we used the ELISA method to measure BChE protein in plasma. The plasma sample of the propositus homozygous for this mutation showed no evidence of immunoreactive BChE protein, whereas those of the three family members did show evidence of the immunoreactive enzyme protein.

The importance of the amino acid Tyr at residue 128 in BChE is shown by its almost absolute conservation among the seven vertebrates shown in Table 2 (Arpagaus et al. 1991).

Therefore, the substitution of Cys for Tyr may have prevented stability or folding of the enzyme protein.

We have previously reported four genetic mutations associated with silent BCHE genes. The first case had a point mutation (GGA→CGA) at codon 365, resulting in substitution of Gly by Arg (Hidaka et al. 1992). The second case was a compound heterozygote for different two mutations; one type was a frameshift mutation, in which an extra A was inserted in codon 315 (ACC→AACC) to create a stop codon at position 322 and the other was the same mutation at codon 365 as the first mutant (Hidaka et al. 1992). The third case was a point mutation (GCA→GTA) at codon 199, resulting in replacement of Ala by Val (M. Sakamoto, personal communication). The last instance was a nonsense mu-
Table 2. Amino acid Tyr at residue 128 in BChE in seven vertebrates

<table>
<thead>
<tr>
<th>Species</th>
<th>BChE</th>
<th>L</th>
<th>H</th>
<th>V</th>
<th>Y</th>
<th>D</th>
<th>G</th>
<th>K</th>
<th>F</th>
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<tbody>
<tr>
<td>Human</td>
<td>BChE</td>
<td>L</td>
<td>H</td>
<td>V</td>
<td>Y</td>
<td>D</td>
<td>G</td>
<td>K</td>
<td>F</td>
</tr>
<tr>
<td>Monkey</td>
<td>BChE</td>
<td>L</td>
<td>H</td>
<td>V</td>
<td>Y</td>
<td>D</td>
<td>G</td>
<td>K</td>
<td>F</td>
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<tr>
<td>Bovine</td>
<td>BChE</td>
<td>L</td>
<td>H</td>
<td>V</td>
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<td>D</td>
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<td>F</td>
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<tr>
<td>Dog</td>
<td>BChE</td>
<td>L</td>
<td>H</td>
<td>V</td>
<td>Y</td>
<td>D</td>
<td>G</td>
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<tr>
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<td>H</td>
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<tr>
<td>Sheep</td>
<td>BChE</td>
<td>L</td>
<td>H</td>
<td>V</td>
<td>Y</td>
<td>D</td>
<td>G</td>
<td>K</td>
<td>F</td>
</tr>
</tbody>
</table>

Substitution (TGC→TGA) at codon 400, resulting in substitution of Cys by a stop codon (Hidaka et al. 1997).

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REFERENCES


