

Identification of Missense Mutation (G365R) of the Butyrylcholinesterase (BCHE) Gene in a Japanese Patient with Familial Cholinesterasemia

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A point mutation which caused a silent phenotype of human serum butyrylcholinesterase (BChE) was identified in the genomic DNA of a 57-year-old Japanese woman who visited our hospital because of pneumonia. The proband exhibited an unusually low level of BChE activity, whereas her son and daughter had an intermediate level. Immunologically, there was an absence of BChE protein in the proband's serum. DNA sequence analysis of the proband demonstrated a point mutation at codon 365 (GGA CGA), resulting in a Gly Arg substitution. A family study showed her son and daughter to have the same mutation.

Hereditary serum butyrylcholinesterase (BChE) deficiency is a rare autosomal recessive abnormality characterized by a resistance to the hydrolysis of several drugs, particularly succinylcholine (SCC), a short-acting muscle relaxant. When this drug is injected intravenously into individuals homozygous for this anomaly, a dangerous prolonged apnea occurs as a result of muscle paralysis. However, the carrier of this gene has no harmful disabilities in daily life(8). Recently, several genetic variants of BChE deficiency have been reported in the Japanese population. Some patients were found to have point mutations in the BCHE gene, while others were found to have frame shift mutations. All these variants were characterized as the silent type of BChE, which had no BChE activity and very low BChE activity. In this paper, we describe a case of a point mutation on the silent gene of BChE.

MATERIAL AND METHODS

Patient

A 57-year-old Japanese female was admitted to our hospital because of pneumonia. Laboratory data incidentally revealed that the patient's level of BChE activity was extremely low (Table). On physical examination, the general condition of the patient appeared to be

Table . Laboratory data on admission.

Hematology		
WBC	7800 /mm ³	(3400~8500)
RBC	477 × 10 ⁴ /mm ³	(345 × 10 ⁴ ~460 × 10 ⁴)
Hb	14.9 g/dl	(10.1~14.6)
Ht	45 %	(33~43)
PLT	14 × 10 ⁴ /mm ³	(15 × 10 ⁴ ~35 × 10 ⁴)

Blood Chemistry		
TP	8.5 g/dl	(6.5~8.3)
GOT	28 IU/I	(8~40)
GPT	17 IU/I	(5~35)
ChE	<u>25</u> IU/I	(3600~7600)
T-Bil	1.00 mg/dl	(0.30~1.10)
LDH	361 IU/I	(230~510)
ALP	164 IU/I	(60~260)
-GTP	24 IU/I	(5~60)
CPK	43 IU/I	(38~180)
T-Chol	230 mg/dl	(130~230)

Butyrylthiocholine were used as the substrate in determination of BChE activity. Normal activity is 3600(IU/l) to 7600(IU/l).

Table . Activity of serum butyrylcholinesterase (BChE) in the members of the the patient's family.

	ACTIVITY	DN (%) ^b	FN (%) ^b
II-3	-	-	-
III-1	120	86	68
III-3	58	81	65
CONTROL ^a	165	82	69

a:The normal range of BChE activity is 150~260μmol/ml/h.

b:The dibucaine number(DN) and fluoride number(FN) are the percentage inhibition of hydrolysis of substrate by dibucaine and sodium fluoride, respectively.

fair. Her blood pressure was 132/94 mmHg. The pulse rate was 83/min and regular. Her body temperature was 37.5 °C. Examination of the chest revealed a slightly fine crackle. But examination of the abdomen and nervous system revealed no abnormal findings. She had no previous history of organophosphate compound poisoning and showed extremely low BChE

A CASE OF FAMILIAL CHOLINESTERASEMIA

activity in her serum. She seemed to be homozygous for a silent phenotype of the BCHE gene. BChE studies carried out on her family members indicated that her son and daughter had an intermediate level of BChE activity (Fig. 1) (Table).

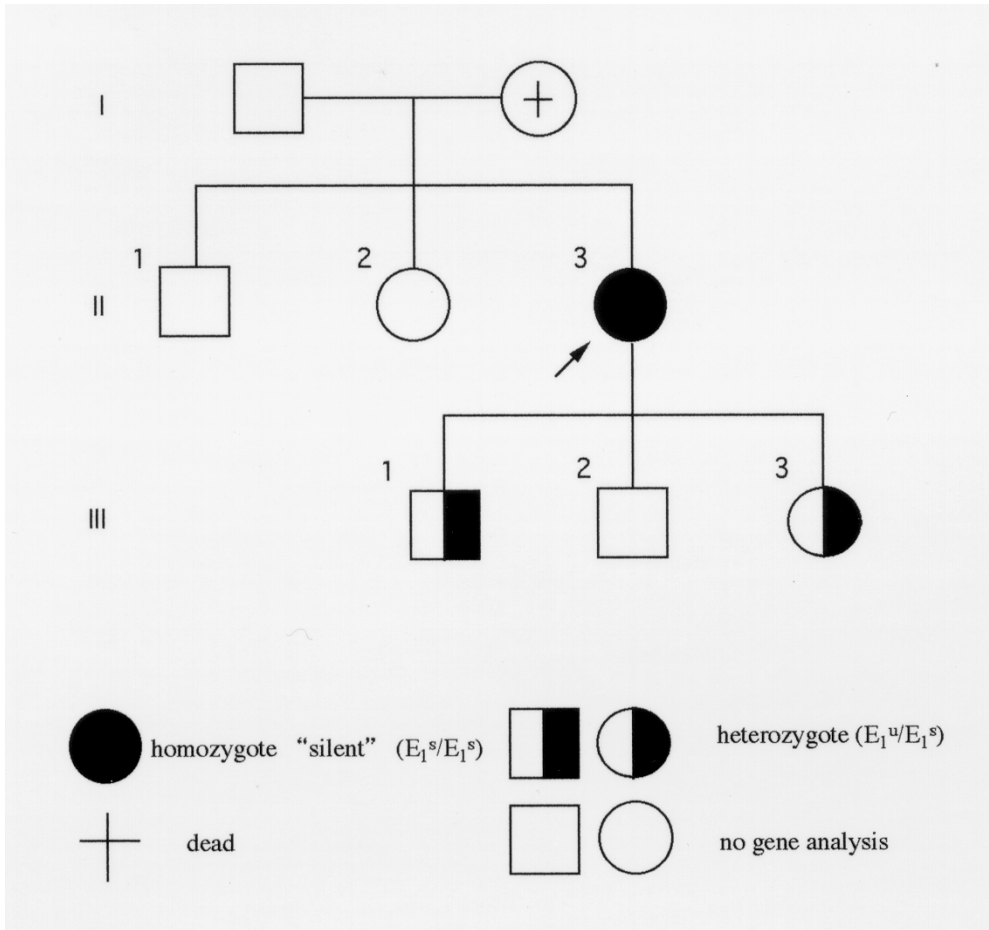


Fig. 1. Family tree of the propositus and genetic inheritance of abnormal butyrylcholinesterase. Black symbols indicate a homozygous mutation, half black symbols indicate a heterozygous point mutation in the BChE gene. The arrow indicates the propositus.

BChE activity and phenotyping

BChE activity in serum was measured with butyrylthiocholine iodide as a substrate by the method of Iuchi et al (7). Dibucaine number (DN) and fluoride number (FN) were defined as the percentage inhibition by dibucaine and sodium fluoride of the rate of hydrolysis of butyrylthiocholine iodide with butyrylcholinesterase.

BChE protein analysis and BChE isozyme analysis

Electrophoresis of the sera of the propositus and her two family members (son and daughter) were carried out on 8.0% polyacrylamide slab gel, after which they were transferred onto nylon membranes with the help of electric semi-dry equipment according to the method of Hirano(6). These membranes were used for two staining procedures. For

analysis of the BChE isozyme, the membrane was stained with 2-amino-5-chlorotoluene diazotate and *a*-naphthylacetate in phosphate buffer (pH 7.1). For analysis of BChE protein, the other 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* (3). The immunoreactive BChE protein was stained with Konica immunostain HRP-1000 (Konica Co.) according to the manufacturer's instructions.

Analysis of DNA

Informed consent was obtained from the three individuals (including the propositus, her son and daughter). The analysis of their genomic DNA was permitted by the Committee of the Executives of Harima Hospital of Ishikawajima-Harima Heavy Industries, Health Insurance Society. Their genomic DNA was extracted from peripheral white blood cells and amplified by the method of McGuire *et al* (10) with one pair of primers: 5'-GCTCCAGGGAACATGGGTTTATTTGATCAACAG-3', which binds to codon 162-172 in exon 2 for the sense side and 5'-TCAAACCAAGCCAGAGAACAATGACAAAAAATCAG-3', which binds to the 42 nucleotide downstream from the exon2/intron2 junction for the antisense. The polymerase chain reaction (PCR) product was ligated in the pT7Blue T-cloning vector and cloned into Nova Blue competent cells (TA Cloning Kit, Novagen Co. USA). Individual positive clones were identified and plasmid DNA was isolated and sequenced with a dye terminator cycle sequencing kit (Applied Biosystems, (ABI), USA) and DNA Sequencer (Model 373:ABI).

Restriction endonuclease analysis

The PCR products obtained as described above were digested with the *TaqI* restriction enzyme according to the manufacturer's instructions. The digests were electrophoresed on 1.0% agarose gel.

RESULTS

Determination of BChE activity

The propositus (II-3) exhibited an unusually low level of BChE activity. Her son (III-1) and daughter (III-3) had intermediate level of BChE activity. The inhibition numbers of the propositus could not be measured, but those of the other two members had dibucaine and fluoride numbers characteristic of the usual phenotype (Table).

BChE isozyme analysis

The C₄ band was not identified in the serum of the propositus, but was found in that of the two children (son and daughter). The intensity of the bands for her son was a little weaker than that of a normal subject while that for her daughter was about half that of a normal subject (Fig. 2A).

BChE protein analysis

An immunoreactive BChE protein band was absent in the propositus, but the presence of BChE protein was detected in the two children (Fig. 2B).

A CASE OF FAMILIAL CHOLINESTERASEMIA

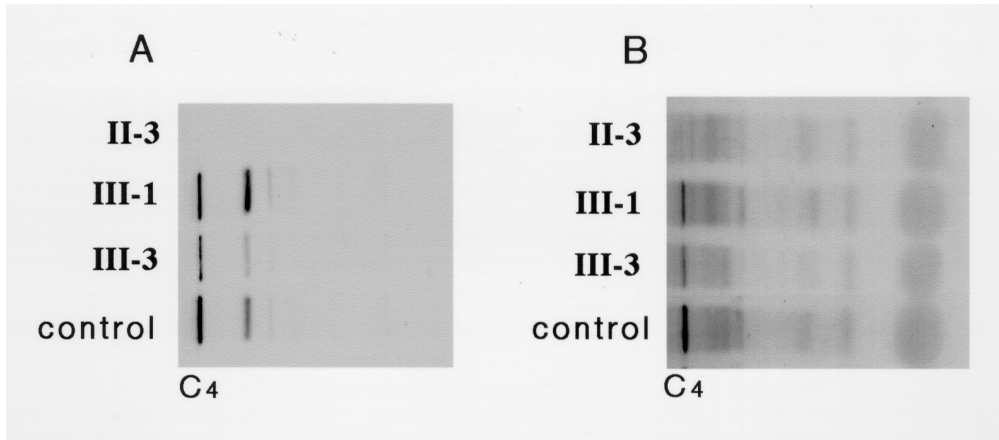


Fig. 2. (A) Polyacrylamide gel electrophoresis of BChE isozyme fractions of the proband and other family members. Note that the proband (II-3) shows an absence of the C₄ band. (B) BChE protein analysis by immunological examination. Note that the proband (II-3) shows an absence of immunoreactive BChE protein in her serum.

Analysis of DNA of the patient's family

Sequence analysis of the BCHE gene of the proband (II-3) revealed a transition mutation of G to C in nucleotide 1093, which changed codon 365 from GGA (Gly) to CGA (Arg), making her a homozygote of this mutation (Fig.3). Similarly, the same mutation was found in her son and daughter, suggesting they were heterozygous for this mutation (data not shown).

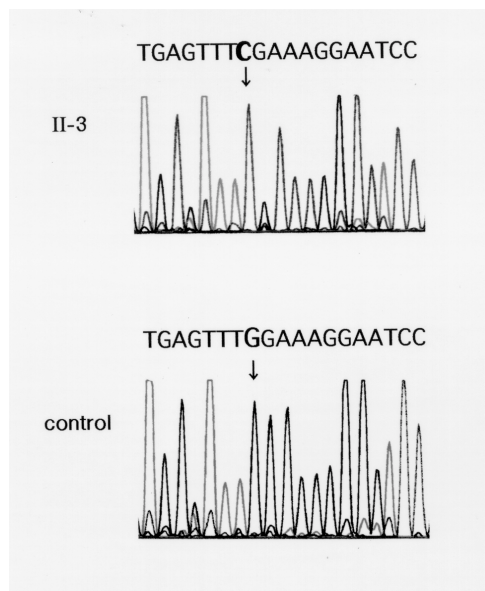


Fig. 3. Sequence analysis for the region of the mutation by the automatic DNA sequencer. The proband (II-3) is homozygous for the substitution GGA → CGA at codon 365 (Gly → Arg), while the son (III-1) and daughter (III-3) had a heterozygous condition for the same mutation (data not shown). The arrow indicates the mutation site of nucleotide 1093 (codon 365).

Restriction endonuclease analysis

The PCR product (992 bp) obtained from the propositus was completely digested with *TaqI* into two fragments, 609bp and 383bp, and that of the two children showed three fragments of 992, 609 and 383bp. That of the normal subject was found to be resistant to *TaqI* digestion. These results led to the same analytic consequences of the genotypes demonstrated by DNA sequencing (Fig.4).

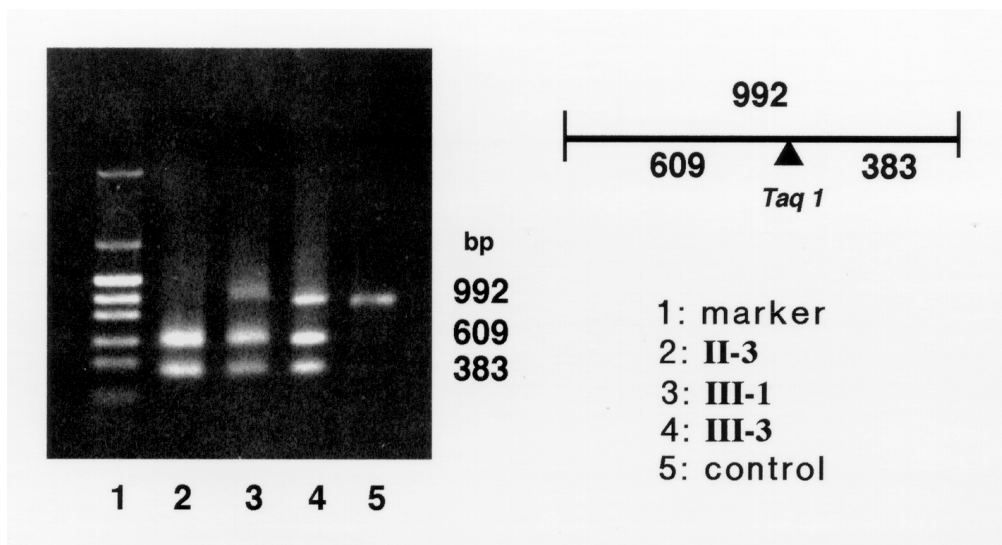


Fig. 4. Restriction endonuclease analysis of the propositus and other family members with *TaqI*. The electrophoregram shows that DNA from the propositus (II-3) gave only bands of 609bp and 383bp, whereas that from her son (III-1) and her daughter (III-3) gave bands of 609bp, 383bp, and 992bp. The normal subject gave a band of 992bp.

DISCUSSION

There are four genes recognized on the locus E_1 that participate in directing BChE biosynthesis: E_1^u (usual: normal gene), E_1^a (atypical: dibucaine-resistant type), E_1^f (fluoride-resistant type) and E_1^s (silent type). E_1^a , E_1^f , and E_1^s are allelic to E_1^u and these genes 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 the genes E_1^a , E_1^f , and E_1^s can be differentiated by representative inhibitors. The presence of the E_1^s gene is readily diagnosable in homozygotes in whom 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 BChE activity and phenotyping by DN and FN cannot distinguish between a normal individual (E_1^u/E_1^u) and a heterozygote (E_1^u/E_1^s). Therefore, to determine whether the subject is a heterozygote of E_1^s or not, genotype analyses are essential.

The nucleotide sequence of E_1^u -BChE was first reported by Arpagaus *et al.* in 1990 (1).

Recently, several genetic variants of BChE deficiency have been reported in the Japanese population, including BCHEALU355 (11), BCHE365R (2, 4), BCHEFS315 (4), BCHE418S, BCHE515C, BCHE210P and BCHE465P (9). In addition to these variations, we reported a new case of point mutation, BCHE199V (12). All of these variants were characterized as the

A CASE OF FAMILIAL CHOLINESTERASEMIA

silent phenotype of BChE.

DNA sequence analysis of the propositus identified a point mutation at codon 365 (GGA CGA), resulting in a Gly Arg substitution. A family study showed her son and daughter to have the same mutation. This mutation has been reported previously in homozygous individuals(2,4) and also in a number of heterozygous individuals(4,9,14). In addition, we found the BChE365R variant in 14 blood-unrelated families and Maekawa et al found it in 22 families(13). However, there are no reports of this variant except Japanese(5). Therefore, this mutation is so far the most frequently identified mutation that is found solely in the Japanese population.

Their PCR products derived from the mutant with G C substitution has a new *TaqI* recognition site at codon 365. In addition to DNA sequence analysis, the PCR products of Genomic DNA were digested with the *TaqI* restriction enzyme. The PCR product obtained from the propositus was completely digested with *TaqI* into two fragments, and that of the normal subject was found to be resistant to *TaqI* digestion. This restriction endonuclease analysis using *TaqI* may be the first choice for detecting a mutation in Japanese.

The frequency of the silent gene of BChE is estimated to be 1/100,000 individuals in the population investigated. In our hospital (Harima Hospital of Ishikawajima-Harima Heavy Industries, Health Insurance Society), two types of mutations responsible for BChE deficiency were incidentally found during a two year period(12). Therefore hypocholinesterasemia with BChE deficiency might not be such a rare genetic disease in this area.

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