KAWASAKI MED J VOL 24: NO 2 61-67 (1998)

Missense mutation (BCHE P100S) of the butyrylcholinesterase gene in two patients with cholinesterasemia

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Accepted for publication on September 25, 1998

ABSTRACT. A missense mutation which resulted in a silent phenotype of human plasma butyrylcholinesterase (BChE) was identified in the DNA of two Japanese patients, a 56-year-old male and a 59-year-old female. They showed extremely low butyrylcholinesterase (BChE) activity in their sera and seemed to be homozygous for a silent type of the BChE gene. Production of the BChE protein could not be found in their plasma by an immunological method. DNA sequence analysis identified a point mutation at codon 100 (CCA → TCA), resulting in a Pro → Ser substitution. An artificial restriction site was introduced into a PCR product with a specific mismatched primer by the use of the PCR-primer introduced restriction analysis (PCR-PIRA). This PCR-PIRA method enabled us to clearly distinguish this mutation from the normal allele.

Key words: missense mutation — silent phenotype — butyrylcholinesterase

Human serum butyrylcholinesterase (BChE) deficiency is a rare autosomal recessive disease characterized by resistance to hydrolysis of several drugs, particuiarly succinylcholine (SCC), a short acting muscle relaxant. When this drug is injected intravenously into homozygous individuals for this disease, a dangerous prolonged apnea will occur as a result of muscle paralysis, but the disease is harmless to the carrier in daily life.[1]

Several genetic variants of BChE have been reported, including an atypical gene,[2] a fluoride resistant gene[3] and a silent phenotype gene.[4] Several genetic variants have also been found in the Japanese population.[5,6] When it was impossible to obtain fresh blood we used plasma as a starting material for amplification of DNA. The aim of this paper is to report a case of BChE deficiency determined by sequencing of the PCR products from plasma in two unrelated families.

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MATERIALS AND METHODS

Case report

The first case was a 56-year-old male (patient I) who visited Yamaguchi Prefectural Central Hospital complaining of borderline hypertension. Laboratory data revealed markedly decreased BChE activity.

The second case was a 59-year-old female (patient II) who visited Yamato General Hospital because of multiple articular rheumatism. She also showed an extremely low level of BChE activity.

The results of hematological and chemical examinations of two patients were within normal range.

BChE isozyme analysis and phenotype analysis

BChE activity in plasma and phenotyping with inhibition numbers as dibucaine number (DN) and fluoride number (FN) were determined by an enzymatic method using butyrylthiocholine iodide as a substrate according to the method of Iuchi *et al.*[7] The plasma of each patient was subjected to electrophoresis on an 8% acrylamide slab gel, and then was transferred onto nylon membranes with the help of electric semidry equipment according to the method of Hirano.[8] These membranes were used for two staining procedures as staining for BChE activity and immunological staining for BChE protein. The BChE activity was stained with 2-amino-5-chlorotoluene diazotate and α-naphthylacetate in 0.2M phosphate buffer (pH 7.1). Immunological reaction was carried out by the incubation with antihuman BChE rabbit serum (DAKO, Glostrup, Denmark) as the first antibody and then with horseradish peroxidease conjugated swine antirabbit IgG (DAKO, Glostrup, Denmark) as the second antibody according to the method of Hangaard *et al.*[9] The immunoreactive BChE protein was stained with Konica immunostain HRP-1000 (KONICA Co.)

DNA analysis

DNA was amplified directly from the original plasma samples by the polymerase chain reaction (PCR) according to the method of McGuire *et al.*[12] The oligonucleotides used as primers for the PCR were 5'-CAAGCATCAT-ATTTTAGG-TAATTATCATCAATAAAG-3', which binds to 90 nucleotide upstream of the splice site between intron 1 and exon 2 for the sense side and 5'-GGGACAACAAATGCTTCAT-TCAGAAGAATTTCTTGGGGA-3', which binds to codon 268-281 in exon 2 for the antisense side, and then a nested PCR was performed to obtain an adequate amount of the PCR product for sequencing. The pair of primers for the nested PCR were 5'-TGCTATATGCAGAAGGCTTA-3', which binds to 49 nucleotide upstream of the intron 1 and exon 2 junction, and 5'-TTGGGGATCTTTATTTCTA-AGACA-3' which binds to codon 263-270 in exon 2 as the oligonucletide of the sense and antisense primers, respectively.

Polymerase chain reaction-primer introduced restriction analysis (PCR-PIRA)

The PCR product obtained as described above was amplified again by PCR with one specific pair of primers, a sense 5'-GACCAAGTGGTCTGAT-

ATTT-3' and an antisense 5'-CAATACAGTGGCATTTTTGGTTTAGG-TC*CTG-3' which contains a single base mismatch at the fourth position from the 3' end (asterisk). The amplified DNA fragment (184 bp) was digested with the *Mva*I restriction enzyme according to the product manual (Takara, Kyoto, Japan). The digested DNA fragments were separated by electrophoresis on 3.5% agarose gel.

RESULTS

BChE activity

The patients showed an unusually low level of BChE activity and their inhibition numbers by DN and FN were not able to be measured (data not shown).

BChE isozyme and phenotype analysis by electrophoresis

The C_4 band on the BChE activity staining, which is a major component of the usual BChE isozyme, was not identified in the plasma of patients I and II and, furthermore, the immunoreactive BChE protein band was not seen, which is showing no BChE protein in thier plasma (Fig 1). These results suggest they were homozygous for the BChE mutation gene.

DNA analysis

Sequence analysis of the PCR product from the plasma of patient I revealed a transition mutation of C to T in nucleotide 298, which converted codon 100 from CCA (Pro) to TCA (Ser), showing him a homozygote of this mutation (Fig 2). Similarly, the same mutation was found in patient II by the sequencing of the PCR product from her plasma.

PCR-PIRA

Cleavage of the PCR product of 184bp obtained from a normal subject by *Mva*I yielded two fragments of 32 bp and 152 bp, whereas the PCR product from patient I was found to be refractory to *Mva*I digestion and showed only one band of 184 bp, making an easy assay for the detection of this mutation (Fig 3).

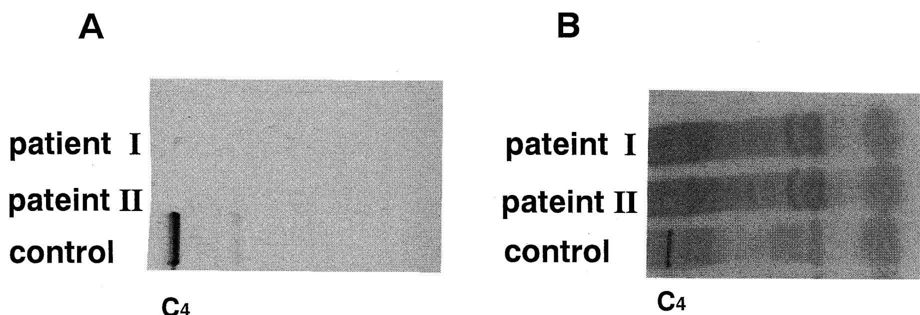

Fig 1. Butyrylcholinesterase zymogram.
A: Staining of BChE activity of patients I and II on nylon membrane. Note that the patients showed absence of the C_4 band, while it was present in the control.
B: Peroxidase staining of immunoreactive BChE protein on a nylon membrane.

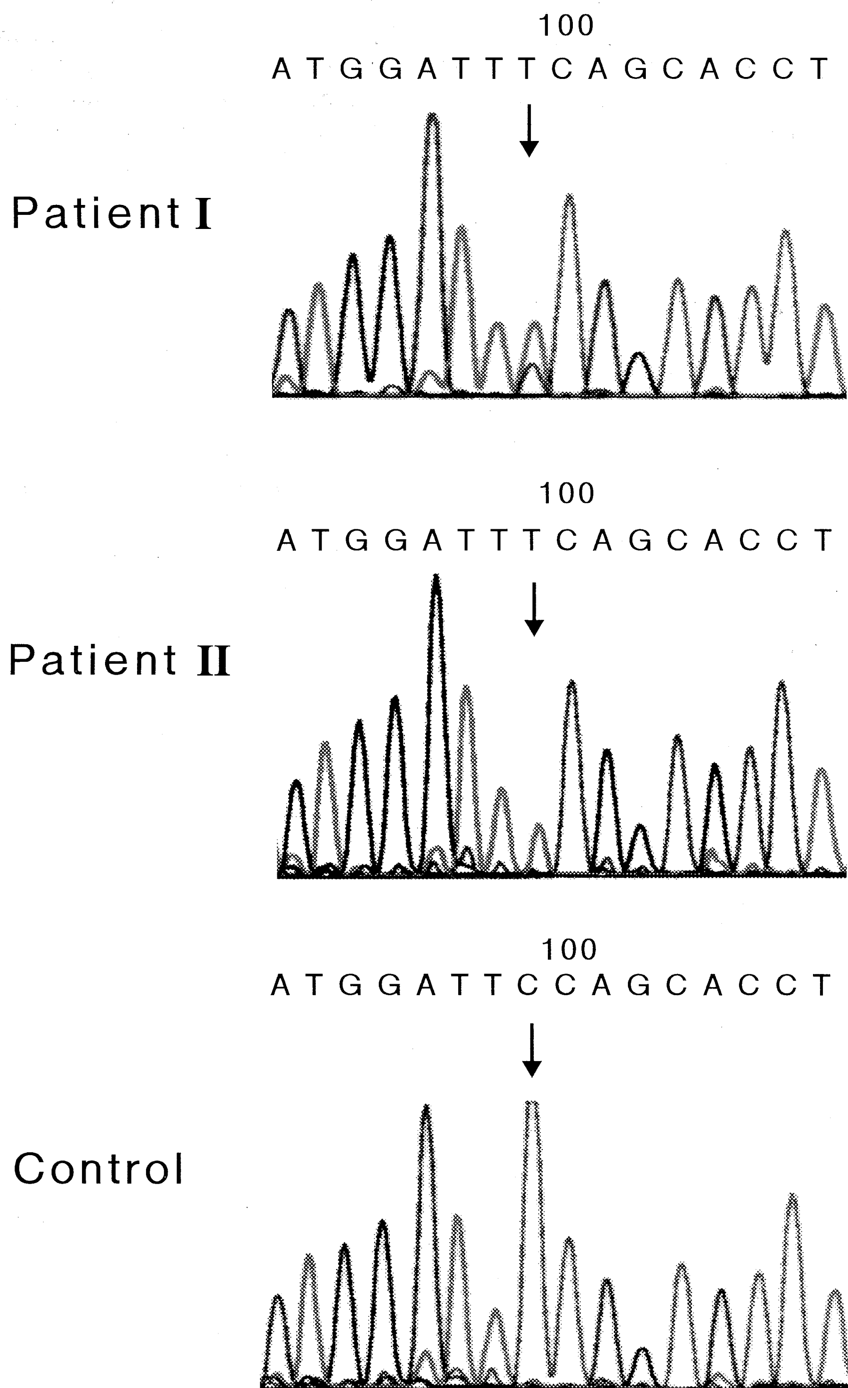

Fig 2. Sequence analysis for the region of the missense mutation. Patients I and II were homozygous for the substitution CCA → TCA at codon 100 (Pro → Ser). Each arrow shows T or C.

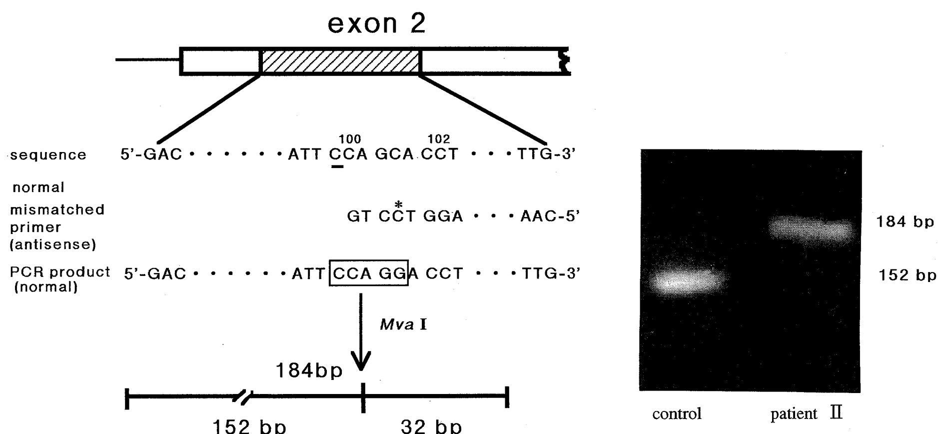

Fig 3. Scheme for detection of the P100S mutation of the BCHE variant on 3.5% agarose gel. The specific primer is composed of 32 mer and is designated as a single base mismatch at the fourth position from the 3'end (asterisk). The PCR product of 184bp, which was derived from a normal subject at codon 100 (under bar), has a new *Mva*I recognition site (square box) and was digested into two fragments of 32 bp and 152 bp. However, the C to T mutant at the codon 100 is not recognized by the *Mva*I.

DISCUSSION

This study was performed on plasma samples sent to our laboratory with a request arised by a BChE enzyme assay. We used plasma as a starting material for amplification of DNA and it was verified that plasma was a useful material when it was not possible to obtain fresh blood sample. In this study, we identified a missense mutation of C to T in codon 100 in two patients. Although they have been living in the same prefecture, no consanguineous relationship between them has been established. However, it is possible that their ancestors might be of the same lineage. This BChE variant was first described in a Japanese male by Liu *et al*,[10] but this first case appears to be unrelated to the present patients.

Proline at the residue 100 resides and the vicinity amino acids are highly conserved throughout the evolution among BChE proteins in six species (Fig. 4).[11]

These findings suggest a critical role for proline in the structure of BChE protein.

Therefore, substitution of Ser to Pro is expected to result in certain conformational changes and to prevent stability or folding of the BChE protein. The P100S mutation was responsible for the BChE deficiency in the two patients studied in this report.

We have previously reported four genetic mutations associated with the silent BCHE gene. They included one nonsense mutation [TGC (Cys) to TGA (termination codon) at codon 400],[12] three missense mutations [TAT (Tyr) to TGT (Cys) at codon 128,[13] GCA (Ala) to GTA (Val) at codon 199,[14] GGA (Gly) to CGA (Arg) at codon 365[5]], and one base insertion (ACC to AACC at codon 315).[15] Two of these four single base substitutions were transversions. The mutation C to T in the present study is not a transversion.

					100			
Human	BChE	V	W	I	**P**	A	P	K
Monkey	BChE	V	W	I	**P**	A	P	K
Pig	BChE	V	W	I	**P**	A	P	K
Dog	BChE	V	W	I	**P**	T	P	K
Bovine	BChE	V	W	I	**P**	T	P	K
Rabbit	BChE	V	W	I	**P**	T	P	K

The boldtyped residue represents the affected position

Fig 4. Amino acid sequence of BChE in the vicinity of amino acid position 100.

This work was supported in part by Research Project Grants (8-103 and 9-114) from Kawasaki Medical School. The authors thank Miss. M. Higashi and Miss. K. Abe for their excellent technical assistance.

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