A new variant of butyrylcholinesterase gene detected in two patients in Yamaguchi Prefecture

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ABSTRACT

Two unrelated Japanese patients, a 56-year-old male and a 59-year-old female, 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 sera by an immunological method. DNA sequence analysis identified a point mutation at codon 100 (CCA → TCA), resulting in a Pro → Ser substitution. A new *Mva* I restriction site was introduced into the PCR product of a normal subject by PCR-primer introduced restriction analysis (PCR → PIRA) using a specific mismatched primer. This PCR-PIRA method thus enabled us to distinguish clearly this mutation from the normal allele.

Introduction

Human serum butyrylcholinesterase (BChE) is characterized clinically because of its function of hydrolysis of the muscle relaxant succinylcholine (SCC), used in surgical operations as an anesthetic. Individuals homozygous for hereditary serum BChE deficiency have an abnormal response to administration of SCC in a standard dose, resulting in prolonged apnea, which may be due to the reduced affinity of BChE variant for this substrate, but it is harmless to the carrier in daily life.

A single BCHE gene has been located on chromosome 3(3q26) and is to be at least 73kb long and to contain four exsons interrupted by three introns. The BChE enzyme protein is a tetramer of identical monomeric subunit, which is glycoprotein made of 574 amino acids[1].

The molecular basis of several genetic variants of BCHE has been reported, including an atypical gene[2], a fluoride resistant gene[3] and a silent gene[4]. Several genetic variants have been found in the Japanese population[5,6]. This paper aims to report a new case of BChE deficiency found in two unrelated families.

MATERIALS AND METHODS

The first case was a 56-year-old male(patient KK) 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 KA) who visited Yamato General Hospital because of multiple artlcular rheumatism. She showed also lower level of BChE activity.

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

The serum of each patient was subjected to electrophoresis on an 8% acrylamide slab gel. Then it was transfered 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, staining for BChE activity and immunological staining for BChE protein. For the former, one membrane was stained with 2-amino-5 chlorotoluene diazotate after incubation in α-naphthylacetate solution. For the latter, the other membrane was incubated with antihumanBChE rabbit serum (DAKO, Glostrup, Denmark) as the first antibody and then with horseradish peroxides 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.) Genomic DNA was amplified by the polymerase chain reaction (PCR) according to the method of McGuire et al[2].

The oligonucleotides used as the primers for the PCR were 5'-TACGGTACC-TTTGGATAATGGCCTAGTAGCTGTTGAC-3' in exon 2 for the sense and 5'-AGT-CCTGATGGTAAACCTGTTGCCGATCTGCTTTGAAGC-3' for the anti sense. The PCR product was cloned into the pT7Blue T-cloning vector (TA Cloning Kit, Novagen Co.). Individual positive clones were identified and plasmid DNA was isolated and sequenced by a dye terminator cycle sequencing kit (ABI) and a DNA sequencer (model 373 : ABI)

The DNA was also amplified by PCR with one specific pair of primers sense 5'-GATTCAATGCAATTGTACGGATCCGATTAGC-3', antisense 5'-CAATACAGTG-GCATTTTTTGGTTTAGGTCCTG-3', which binds and contains a single base mismatch at the fourth position from the 3' end (asterisk). The amplified DNA fragment (184bp) was digested with *Mva* I restriction enzyme according to the manufacturers's instructions. The digest was separated by electrophoresis on 3.5% agarose gel.

RESULT AND DISCUSSION

The patients (KK and KA) had unusually low BChE activity and inhibition numbers, DN and FN could not be calculated.

The C_4 band, which is a major component of the usual BChE isozyme, was not identified in the serum of the patients KK and KA and furthermore, the immunoreactive

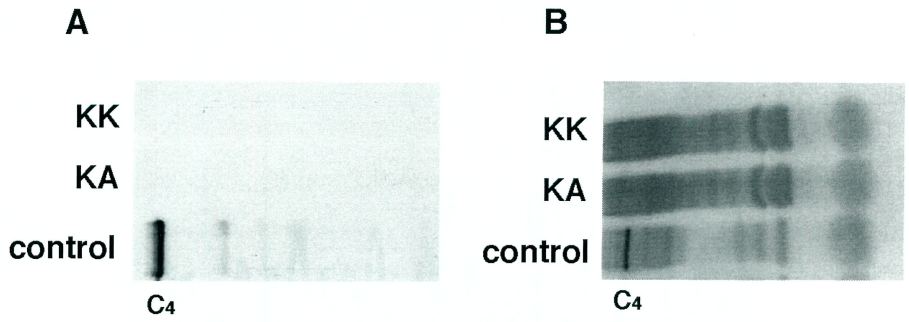

Fig. 1. Butyrylcholinesterase zymogram.
A : BChE activity staining of patients KK and KA on nylon membrane. Note that the pateints showed no the absence of the C_4 band, while the control showed the C_4 band.
B : Peroxidase staining of immunoreactive BChE protein on nylon membrane.

BChE protein band was not seen in thier serum(figure 1).

The PCR product of 184bp obtained from the normal subject was digested with *Mva* I into two fragments, 32bp and 152bp, while the PCR product from patient KK was found to be resistant to *Mva* I digestion(figure 2).

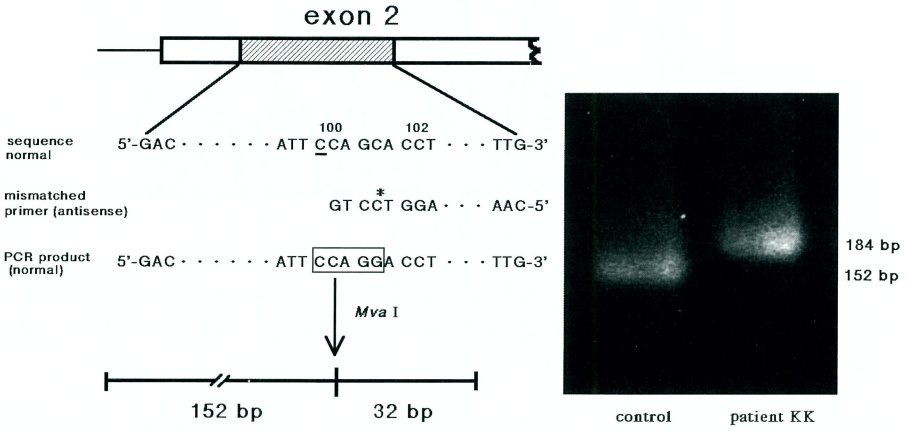

Fig. 2. Scheme for detection of the Pro100Ser mutation of the BCHE variant. The specific primer is composed of 32 mer and is designed a single base mismatch at the fourth position from the 3'end (asterisk). The PCR product of 184bp, which was derived from a normal subject at codon 100(under bar), has a new *Mva* I recognition site(square box) and digested two fragments of 32bp and 152bp. Right : separation of *Mva* I restriction fragments on 3.5% agarose gel.

Sequence analysis of the BChE gene of patient KK revealed a transition mutation of C to T in nucleotide 298, which converted codon 100 from (CCA Pro) to TCA (Ser), making him a homozygote of this mutation(figure 3). Similarly, thesame mutation was found in patient KA by sequencing of the entire region of the BChE gene.

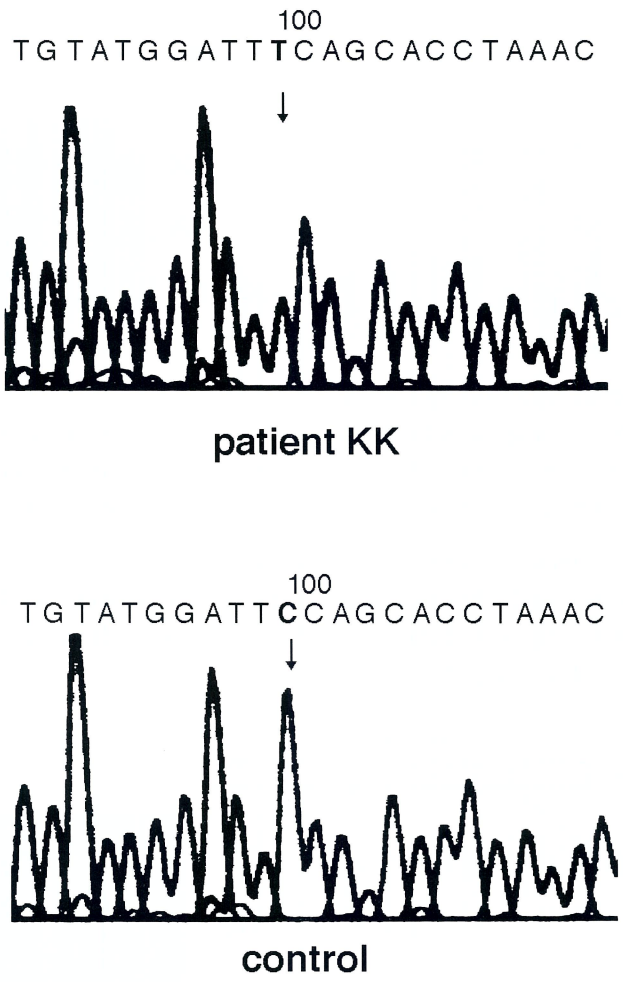

Fig. 3. Sequence analysis of the automated DNA sequencer for the region of the mutation. Pateint KK is homozygous for the substitution CCA → TCA at codon 100 (Pro → Ser).

Although they have been living in the same prefecture, no consanguineous relationship between them has been established.

The amino acid Pro at residue 100 and the surrounding region is well conserved among some vertebrates as can be seen below[10].

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

Therefore, substitution of Ser for Pro may prevent stability or folding of the BChE protein.

In our investigation, we found four single base substitutions and one base insertion in the BCHE genes of individuals homozygous for a silent gene. They included one nonsense mutation (TGC to TGA at codon 400)[11], three missense mutations(TAT to TGT at codon 128, GCA to GTA at codon 199[12], GGA to CGA at codon 365[5]), and one base insertion(ACC to AACC at codon 315)[13]. Two of these four single base substitutions were transversions.

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