Familial Hypocholinesterasemia Found in a Family and a New Confirmed Mutation

Weidong LIU, Toshikazu HADA, Kohya FUKUI, Hiroyasu IMANISHI,
Noboru MATSUOKA*, Arata IWASAKI and Kazuya HIGASHINO

A 45-year-old man was hospitalized because of acute hepatitis. His serum cholinesterase (ChE) was below 10 IU/l (normal range: 105–240 IU/l) during the disease course and after his recovery. The patient was suspected of having familial hypocholinesterasemia. His family members were healthy except that his father had hypertension and gall stones. Analysis of ChE gene in the proband and his family revealed three point mutations at nucleotides 298 (CCA to TCA), 1,410 (CGT to CGG) and 1,615 (GCA to ACA). The first mutation caused an amino acid change at codon 100 from proline to serine, which was a new mutation not previously reported, but the second one was a silent mutation. The third mutation resulted in an amino acid alteration from alanine to threonine at codon 539 in exon 4 of the ChE gene. The mode of transmission of these mutations is described.

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Introduction

Patients with a- or hypocholinesterasemia generally have no signs or symptoms and can have a healthy daily life. However, when these patients must be injected with a muscle relaxant, succinylcholine, which is broken down by serum cholinesterase (ChE; EC 3.1.1.8, also called butyrylcholinesterase), they may develop prolonged apnea because of the lack of this enzyme. Thus, it is important to evaluate the levels of serum ChE in all patients. DNA sequencing of serum ChE has already been accomplished (1, 2) and several genetic variants causing a- or hypocholinesterasemia have been reported (3–7). In the present study, we carried out gene analysis of familial hypocholinesterasemia in a family of five.

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Materials and Methods

Patients

The proband, a 45-year-old man, was hospitalized with A-type acute viral hepatitis. On admission, serum aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) were

2,601 and 3,321 IU/l, respectively. The titer of IgM antibody to hepatitis A virus was 4.4 (normal range: <1.0 C.I.). Total bilirubin was 4.6 mg/dl. His serum ChE fell below 10 IU/l (normal range: 105–240) during the disease course and persisted at that level after recovery. He was suspected of having familial hypocholinesterasemia, and his family members were examined for their serum ChE levels. The family tree is illustrated in Fig. 1. All family members were healthy except his father had hypertension and gall stones which were well controlled by his doctor. None of the family members were receiving any medications affecting serum ChE activity.

The proband and his elder brother had serum ChE levels below 10 IU/l, and were suspected to be homozygous for the silent ChE gene. Serum ChE levels of the patient's father, mother and younger brother were around or below the normal lower limits and they were suspected to be heterozygous for the same gene.

Enzyme assay

Serum ChE activity was determined using a Choline C “Kokusai” kit (Kokusai Shiyaku, Tokyo) in which the substrate was dimethoxybenzoylthiocholine. Dibucaine and fluoride numbers were determined using benzoylcholine as a substrate as described previously (6).

From the Third Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya and *Matsuoka Clinic, Yatsushiro

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Reprint requests should be addressed to Dr. Toshikazu Hada, the Third Department of Internal Medicine, Hyogo College of Medicine, 1-1, Mukogawa-cho, Nishinomiya, Hyogo 663

Figure 1. Family tree of the patients with hypocholinesterasemia. Boxes indicate males and circle indicates female.

Blood collection and plasma phenotyping

Whole blood samples were collected from 5 members of the family. Plasma phenotypes were determined from ChE activity using benzoylcholine as a substrate, with inhibition by dibucaine and sodium fluoride. The dibucaine and fluoride numbers were determined using 10 μ M dibucaine and 50 μ M NaF, respectively, according to methods previously reported (8, 9).

DNA amplification and sequencing

White-blood cell DNA from 5 persons of the family was extracted using a DNA extraction kit (Microprobe Corporation, CA, USA). Exons, 2, 3 and 4, which encode the entire mature protein of ChE, were individually amplified by a polymerase chain reaction (PCR) method using a HPLC-purified primer set and Taq DNA polymerase (Takara Shuzo Corporation, Shiga). The primers for exon 2 were the same as those described by Maekawa et al (7). The primers for exons 3 and 4 were newly designed for this study (Table 1). A total of 35 rounds of amplification were performed, each round consisting of a denaturation step at 94°C for 1 minute, an annealing step at 55°C for 1 minute and extension step at 72°C for 1.5 minutes. After confirming the sizes and homogeneity of the PCR products, direct sequencing of the entire coding region of the ChE gene

was performed using a dye terminator cycle sequencing core kit (Perkin-Elmer Corporation, CA, USA) and an ABI 373A DNA sequencer (Perkin-Elmer Corporation).

Results

Plasma ChE activity and phenotyping

As shown in Table 2, the proband (II-2) and his elder brother (II-1) had very low serum ChE activity. The proband's father (I-1) had serum enzyme activity around the normal lower limit. The proband's mother and his younger brother (I-2, II-3) had serum enzyme activity below the normal lower limits. The dibucaine and fluoride numbers examined for two of the persons (I-1, I-2) were within the normal range.

DNA analysis within the pedigree

As shown in Fig. 1 and Table 2, the proband and his elder brother both had a homozygous missense mutation at nucleotide 298 (CCA to TCA) which caused the replacement of proline by serine at codon 100 (Pro 100 Ser) in exon 2 of the ChE gene. Figure 2A shows the results of direct sequencing of the amplified products of the proband, demonstrating a C to T transversion at nucleotide 298. This mutation has not previ-

Table 1. Primer Sequences Used for Amplification of Cholinesterase Gene Newly Designed for This Study

Primer	Nucleotide location	Sequence (5' to 3')	Amplified region
AP 11'	Intron 2	AGCTCTGTGAACAGTGTAGAA	exon 3
AP 12'	Intron 3	CACCGTGCCTTGGAGAGTATAC	
AP 5'	Intron 3	CTGTGTAGTTAGAGAAAATGGC	exon 4
AP 6'	Intron 4	CCTTCTGGCATTTCCTTTCAGC	

Table 2. ChE Activity and the Location of Point Mutations in Each Individual Family Member

	I-1	I-2	II-1	II-2	II-3	II-4
Age (year)	73	70	48	47	45	42
ChE (105–240 IU/l)	109	77	10	10	86	nd
P1 (exon 2)	hetero	hetero	homo	homo	hetero	
P6 (exon 4)	normal	hetero	normal	normal	hetero	

nd: not determined, hetero: heterozygous, homo: homozygous, P1: point mutation at nucleotide position 298 (CCA to TCA), which causes an amino acid alteration at codon 100 from proline to serine, P6: point mutation at nucleotide position 1615 (GCA to ACA), which causes an amino acid alteration at codon 539 from alanine to threonine.

ously been reported. In addition, another homozygous nonsense mutation was observed at nucleotide 1,410 (CGT to CGG) for both the proband and his elder brother (Fig. 2B). In this case, no amino acid substitution occurred. We performed further DNA analyses of the ChE gene for the remaining three family members (I-1, I-2 and II-3). The proband's father (I-1), mother (I-2) and younger brother (II-3) also had a heterozygous missense mutation at nucleotide 298 (CCA to TCA) which resulted in an amino acid change from proline to serine at codon 100 in exon 2 of the ChE gene. Another heterozygous missense mutation was found in the proband's mother and younger brother at nucleotide 1,615 (GCA to ACA) which caused an amino acid change from alanine to threonine at codon 539 in exon 4 of the ChE gene. These results indicated that the proband's mother and younger brother were compound heterozygotes: CCA (Pro) to TCA (Ser) at codon 100 and GCA (Ala) to ACA (Thr) at codon 539 (Figs. 1, 2C, 2D and Table 2). A homozygous nonsense mutation observed at nucleotide 1,410 (CGT to CGG) for the proband and his elder brother was also found in the other family members. Since a homozygous nonsense mutation was observed in all of the family members, we examined whether this mutation occurred in normal individuals. Twenty normal individuals with normal ChE activity all had a homozygous nonsense mutation at nucleotide 1,410 (CGT to CGG).

Discussion

Serum ChE is generally considered to be synthesized in the liver and released into the blood stream. Serum ChE is routinely measured as a test of liver function. Low serum ChE activity has been reported in acute and chronic liver diseases (10), and has also been noted in several genetic hypocholinesterasemia (3–7). Therefore, discrimination of liver diseases and genetic hypocholinesterasemia is very important. The present proband was suspected of having genetic hypocholinesterasemia because of low serum ChE activity after recovery from acute hepatitis. Thus, the possibility of genetic hypocholinesterasemia should be considered in patients with liver disease.

The phenotyping method introduced by Kalow and Genest

(8), based on the dibucaine number, is a useful and reliable method for determining whether a person has atypical ChE. Atypical ChE has a special feature in that the atypical allele can be detected in homozygotes as well as heterozygotes (3). It has been reported that most of the other variants can not be identified when they occur in combination with the usual allele (3). It is therefore difficult to discriminate by phenotyping tests whether a person is a carrier of the silent, fluoride, H, J, K, or some other variants (3). Our proband's father (I-1) and mother (I-2) had normal dibucaine and fluoride numbers. Therefore, neither the point mutation at codon 100 nor the point mutation at codon 539 of the amino acid sequence seemed to affect the dibucaine number. However, it is not clear at present whether either or both mutations affect the fluoride number. In the present study, we identified three point mutations, two of which were located in exon 2 and the other one was in exon 4 of the ChE gene. The first point mutation at codon 100 of the amino acid sequence is newly identified and appears to cause low ChE activity (Fig. 1 and Table 2). We already found this mutation in another family with genetic hypocholinesterasemia in addition to the present family, as well as two sporadic cases (unpublished data). However, the mechanism which causes hypocholinesterasemia remains to be elucidated. The second mutation at codon 470 of the amino acid sequence is a silent mutation and does not result in amino acid alteration. As this mutation could be detected in all family members as homozygotes, we suspected that this mutation occurs in normal persons, and this was confirmed in 20 persons with normal ChE activity. Considering our data in comparison with the original report concerning the DNA sequence of the ChE gene, this mutation may occur only in Japanese. Ethnic distribution of this mutation should be clarified in the future. The third mutation at codon 539 of the amino acid sequence was previously reported by Bartels et al (11) and is called the K variant. Rubinstein et al (12) found that the K variant is associated with a 33% reduction in serum ChE activity. Considering the enzyme activities of our subjects (I-2, II-3), our findings are consistent with those of Rubinstein et al. We found this mutation in another family in addition to the present family and in one sporadic case (unpublished data).

As reported by Neville et al (13), if plural point mutations occur simultaneously in one ChE molecule, these point mutations may interact with each other intramolecularly. Then, in one case, one point mutation magnifies the effect of another point mutation. In another case, the reverse phenomenon can be observed. Although at least 10 different allelic forms have been described (13), each phenotype, which is expressed with a characteristic peptide sequence, can be modified by interaction with another mutation and cannot be identified by conventional tests such as ChE activity, inhibition rate by several inhibitors and so on. In the present family, homozygous mutation at nucleotide 298 in patients II-1 and II-2 caused an anomalously low serum ChE activity and a heterozygous mutation in patient I-1 caused a moderate reduction of the enzyme activity. The reduction of ChE activity observed in patients I-2 and II-3 seemed to occur in an additive fashion of each effect of two heterozygous mutations. Further investigation is needed to

Figure 2. A) DNA sequencing from the proband (II-2) representing a homozygous missense mutation at nucleotide 298 (C to T). B) DNA sequencing of the proband's father (I-1) representing a homozygous point mutation at nucleotide 1,410 (T to G). C) and D) DNA sequencing from the proband's mother demonstrating a heterozygous missense mutation at nucleotides 298 (C to C+T) and 1,615 (G to G+A), respectively.

clarify the intramolecular relationships between ChE activity and point mutations.

Sequencing the DNA of family members demonstrated that the propositus' father carried two point mutations at codons 100 and 470 on one DNA strand and one mutation at codon 470 on the other DNA strand. The propositus' mother carried two point mutations at codons 100 and 470 on one DNA strand and two point mutations at codons 470 and 539 on the other DNA strand. In summary, we described familial hypocholinesterasemia, and confirmed a new mutation which causes reduced serum ChE activity. However, the mechanism which resulted in hypocholinesterasemia remains to be elucidated. In addition, another nonsense mutation was observed in all members examined in the present study. Further investigation is needed to clarify the ethnic distribution of this mutation.

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