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Rapid Identification of Atypical Variant of Plasma Butyrylcholinesterase by PCR

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Human butyrylcholinesterase is the enzyme responsible of mivacurium and succinylcholine metabolism, which may be significantly impaired when mutation Asp70Gly is found in patients. We describe a simple PCR method for the detection of this variant. Thirteen out of sixteen patients tested after prolonged apnea were positive for the presence of this mutation (50.0% homozygotes and 31.3% heterozygotes), suggesting that this test contributes to the explanation of some clinical events and to their prevention in relatives of these patients. Clin Chem Lab Med 2002, 40(8):799–801

Key words: Pharmacogenetics; Succinylcholine; Mivacurium; Butyrylcholinesterase; Plasma cholinesterase; Polymerase chain reaction.

Abbreviations: BChE, butyrylcholinesterase; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

Introduction

Succinylcholine and mivacurium are short-acting muscle-relaxant drugs that are commonly administered to patients undergoing surgery to facilitate tracheal intubation. In most patients, the effect of muscle relaxation disappears in 3–5 min: these drugs are short-acting because of quick hydrolysis by plasma butyrylcholinesterase (BChE, EC 3.1.1.8). BChE is secreted into plasma by liver cells, and has no defined associated physiological function. Though several genetic variants of this esterase have been related to the prolonged apnea in patients treated with the above muscle relaxants, dibucaine-resistant variant, called atypical variant, is most frequently involved in this delayed response. Patients who are homozygotes for the mutation, with the AA genotype (atypical/atypical), have the most prolonged response, from 1 hour to more than 10 hours (1). Heterozygotes with usual/atypical (UA) genotype have normal to moderately prolonged response (2, 3). The genetically determined prolonged response to succinylcholine or mivacurium is a classical example of pharmacogenetics.

In Caucasian populations, the frequency of heterozygotes for the usual and atypical genes is about 1 in 25, and the frequency of homozygotes for the atypical gene is about 1 in 2500. Traditional tests that have been used to determine phenotypes, inhibition with dibucaine or fluoride, are not sufficient to differentiate BChE variants. Molecular studies on BChE have permitted precise identification of alterations in the *BChE* gene (4, 5). Atypical variant is associated with mutation Asp70Gly (A→G at 209). In this work, we describe a simple method using loss of a *Mbo*I restriction site to detect this atypical mutation. We relate the phenotypes based on both dibucaine numbers and activity values to the genotypes determined by this PCR-RFLP method in 28 patients.

Materials and Methods

Forty six patients (17 males and 29 females) were tested for Asp70Gly (A→G at 209), either after prolonged response to succinylcholine or mivacurium (n=16), or within the context of a familial survey (n=30). All persons gave their informed consent prior to their inclusion in the study.

Reaction was carried out with 0.3–1.0 µg of genomic DNA in a total volume of 50 µl containing (final concentration) 1.5 mmol/l $MgCl_2$, 0.2 mmol/l of the four deoxynucleotide triphosphates, 1.25 U of *Taq* polymerase (Perkin Elmer, Norwalk, USA) and 0.6 µmol of each of the primers. PCR conditions were as follows: preliminary denaturation at 94 °C for 5 min, followed by 30 cycles of 30 s at 94 °C, 30 s at 50 °C, and 30 s at 72 °C. The reaction was ended with 7 min at 72 °C.

For amplification of 157 bp DNA fragment containing the atypical mutation site in the *BChE* gene, we used the forward primer I (5′-TTC AAA AAG CCA CAG TCT CT-3′) and the reverse primer II (5′-GAT ATA AAC AGT CTT CAC TG-3′) (Genaxis, Montigny le Bretonneux, France), derived from primers AP1 and AP2 described by McGuire *et al.* (5).

The digestion reactions contained 15 µl of PCR product, 0.5 of *Mbo*I (Promega, Madison, USA), 2 µl of 10X Buffer (supplied with the enzyme) and 100 mg/l bovine serum albumin, in a final volume of 20 µl. These components were incubated for 3 hours (or overnight) at 37 °C. After the reaction, 15 µl of the PCR mixture was mixed with a loading buffer and then electrophoresed in a 4% Resophor-agarose gel (3% and 1%, respectively) (Eurobio, Les Ulis, France) for 1.3 hours at 70 V. Bands were made visible by ethidium bromide staining of the gel and results were recorded with molecular imager Gel Doc 1000 (Bio-Rad, Ivry-sur-Seine, France).Control samples of known genotype were run in each gel, along with *Hae*III-digested pBR 322 size marker (Eurobio).

Digestion by *Mbo*I restriction endonuclease generated two fragments (106 and 50 bp) in the homozygous (AA) allele and three fragments (81, 51 and 25 bp) in the wild-type allele (UU).

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A heterozygote for the mutation (UA) theoretically shows four bands of 106, 81, 51 and 25 bp, which correspond to the two alleles (Figure 1).

The catalytic activity of cholinesterase in serum and its sensitivity to inhibition by dibucaine (6) were measured by a slide method developed by Ortho Clinical Diagnostics (Rochester, USA) on Vitros 250 analyzer for 35 patients. Dibucaine was from Sigma Chemical Co. (St. Louis, USA) and dibucaine number (DN) was determined with a concentration of dibucaine of 15 mmol/l.

Results

Out of the population tested, 10 patients were homozygotes and 20 were heterozygotes (21.8% and 43.5%, respectively) for this mutation, with an allele frequency of 43.5%. When considering only patients who presented prolonged apnea, 50.0% were homozygotes and 31.3% were heterozygotes for mutation Asp70Gly (Table 1).

It is worth noting that results of BChE genotyping were closely related to phenotype for the 35 patients tested, based on inhibition with dibucaine (Table 1). We emphasize that butyrylcholinesterase activity in serum was significantly decreased in heterozygotes who experienced prolonged apnea.

Discussion

Atypical variant was found in 81.3% of patients with prolonged apnea, reasserting its high frequency in such patients. For the two remaining patients, the observed symptomatology may be associated with other mutations of BchE which are less easy to detect, such as BCHE*F54, BCHE*24M, *100S, *250P, *267R, *330I, *365R, *418S, *515C, *539T, BCHE119STOP, *465STOP (7), or may occur for another reason, such as central respiratory depression, hyperventilation or overdose (1).

In conclusion, the detection of Asp70Gly mutation at nucleotide 209 of the *BchE* gene is a first step in the diagnostic strategy after prolonged apnea as a result of treatment with mivacurium or succinylcholine. Even though molecular biology techniques have revealed the precise location of nucleotide alterations in most of the variants, DNA sequencing is time consuming and is still not commonly available. There is no doubt that future studies will reveal new cheap, rapid and accurate methods which will facilitate the phenotyping/genotyping procedures, and improve our understanding of the physiological and clinical significance of BChE and its variants.

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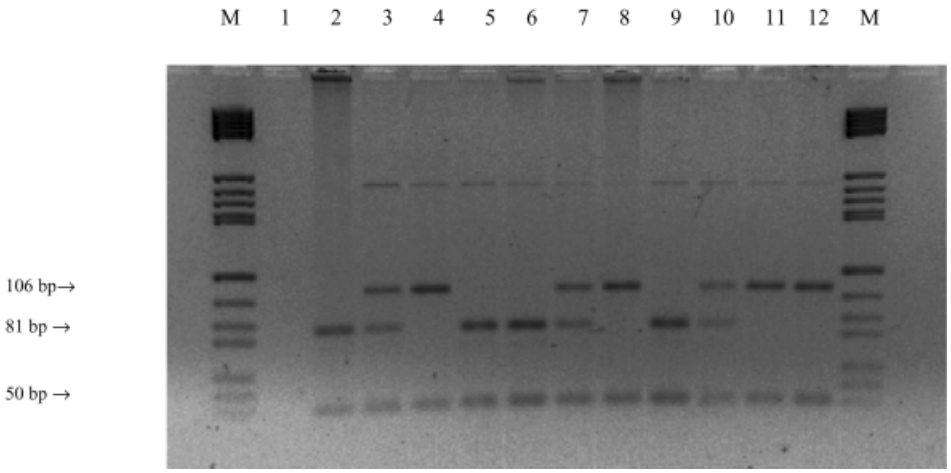

Figure 1 PCR amplification and restriction digestion of DNA employed to determine the presence of atypical variant of plasma cholinesterase. Lane M: *Hae* III-digested pBR 322 size marker; lane 1: negative control, lane 2, 5, 6, 9, wild-type (UU); lanes 3, 7, 10: heterozygous for atypical variant (UA); Lanes 4, 8, 11, 12: homozygous for atypical variant (AA).

Table 1 Serum cholinesterase phenotypes and genotypes for the Asp70Gly mutation on the nucleotide at position 209.

Activity				
Patients with prolonged apnea	Units/l (usual values: 5500–12000)	DN	Phenotype	Genotype
1	7714	88	UU	Wild-type
2	4831	85	UU	Wild-type
3	3401	73	UA	Heterozygote
4	2662	65	UA	Heterozygote
5	2072	63	UA	Heterozygote
6	1480	78	UA	Heterozygote
7	4282	53	AA	Homozygote
8	3593	50	AA	Homozygote
9	3587	51	AA	Homozygote
10	3031	41	AA	Homozygote
11	2231	47	AA	Homozygote
12	1996	40	AA	Homozygote
13	1622	49	AA	Homozygote
Relatives				
1	8663	90	UU	Wild-type
2	7793	90	UU	Wild-type
3	7640	84	UU	Wild-type
4	7616	88	UU	Wild-type
5	7332	87	UU	Wild-type
6	6588	86	UU	Wild-type
7	5309	91	UU	Wild-type
8	5046	85	UU	Wild-type
9	4570	92	UU	Wild-type
10	7505	80	UA	Heterozygote
11	7022	80	UA	Heterozygote
12	6697	79	UA	Heterozygote
13	6508	78	UA	Heterozygote
14	5800	77	UA	Heterozygote
15	5792	83	UA	Heterozygote
16	5744	69	UA	Heterozygote
17	5487	78	UA	Heterozygote
18	3639	79	UA	Heterozygote
19	3532	77	UA	Heterozygote
20	2780	86	UA	Heterozygote
21	2650	41	AA	Homozygote
22	2076	42	AA	Homozygote
	UU	UA	AA	
Mean results for activity:	6646±1445	4675±1922	2598±894	
Mean results for DN:	88±3	78±6	45±5	

Phenotypes: U, usual; A, atypical; DN, dibucaine number.

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