Two novel mutations in the *BCHE* gene in patients with prolonged duration of action of mivacurium or succinylcholine during anaesthesia

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Background **Butyrylcholinesterase (BChE) hydrolyses the neuromuscular blocking agents, succinylcholine and mivacurium used during general anaesthesia. Hereditary low BChE activity may result in an extensively prolonged duration of action of these drugs, especially in patients who are homozygous for the atypical or silent variants. We present three novel mutations in the butyrylcholinesterase gene (*BCHE*) identified in three families in which a member had experienced severely prolonged duration of action of succinylcholine.**

Methods **As the phenotypes of the three probands could not be established with certainty using conventional biochemical tests, DNA samples were collected from two of the probands and four relatives. Genotypes were determined using complete nucleotide sequencing.**

Results **Three novel mutations were identified: *BCHE*FS126, BCHE*I3E4-14C* and *BCHE*328D*. The proband in family 1 was genotyped as *BCHE*115D*I3E4-14C/BCHE*FS126*, whereas the proband in family 3 was compound heterozygous for *BCHE*328D* and *BCHE*142M*. In both patients, BChE activity was below detection limit, and they experienced an extensively prolonged duration of action of succinylcholine. The proband in family 2 was not sequenced, but a relative was heterozygous for *BCHE*FS126*. *BCHE*I3E4-14C* was in linkage with a known silent variant.**

Conclusions **Two novel variants of *BCHE* are silencing the enzyme function. *BCHE*FS126* results in a truncated protein lacking the active site and is therefore inactive. The second variant is *BCHE*328D*, also resulting in an inactive protein, as this change in amino acid is radical and furthermore situated in the gorge harbouring the active site. These variants result in extensively prolonged duration of action of succinylcholine. *Pharmacogenetics and Genomics* 17:995–999**

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Introduction

Two types of cholinesterases exist, the acetylcholinesterase (AChE, EC 3.1.1.7) and the butyrylcholinesterase (BChE, plasma cholinesterase, EC 3.1.1.8). They differ in their tissue distribution, substrate specificity and sensitivity to various inhibitors. AChE is mainly present in muscles and nervous tissue, whereas BChE is produced in the liver and is present in all tissues. In addition, BChE hydrolyses the two neuromuscular blocking agents, succinylcholine [1] and mivacurium [2] used during general anaesthesia. Hereditary low BChE activity may result in an extensively prolonged duration of action of succinylcholine [3] and mivacurium [4], especially in patients who are homozygous for genetic variants such as the atypical or silent variants. Following a normal intubation dose of mivacurium, the time to full spontaneous recovery is 30 min in phenotypically normal patients [5] compared with 6–8 h in patients homozygous for low activity variant alleles [6–10] requiring postoperative ventilation in the intensive care ward. Recent studies of BChE knockout mice suggest that BChE also has a role in neurotransmission and that deficiency in BChE activity might lead to intolerance of standard doses of the anti-Alzheimer drugs such as the AChE inhibitors, huperzine A and donepezil [11].

In the Danish Cholinesterase Research Unit (DCRU), which is a register for patients and their families with genetic variants in BChE, 13 different phenotypes have been determined [12]. In 25% of patients referred because of prolonged duration of action of succinylcholine, it was, however, not possible to establish a phenotype using conventional biochemical methods [13]. Therefore, molecular genetic methods were established.

The butyrylcholinesterase gene (*BCHE*) is located at chromosome 3, 3q26.1-q26.2 [14–16]. The gene consists of four exons separated by three large introns [17]. The coding sequence is 1722 bp corresponding to the 574 amino acid sequence of BChE. Till now more than 58 different variants are reported [18]. We report the identification of two novel mutations in *BCHE* identified in three families, a member of which has experienced severely prolonged duration of action of succinylcholine.

Methods

Patients

The probands of the three unrelated families were referred to DCRU because of prolonged duration of action of succinylcholine at a time, when it was not possible to perform genotyping. The biochemical tests, however, indicated that two of the patients were homozygous for a silent variant and one was heterozygous for the atypical and a silent variant (AS). When genotyping was established in the department, complete nucleotide sequencing of members of the three families was performed to establish the genotypes of the patients with certainty. In the meantime, some members had died and genotyping was not possible. The genotyping was part of a larger study, the aim of which was to determine mutations in the *BCHE* gene and to evaluate the clinical significance of the mutations.

The local Ethics Committee for Frederiksberg and Copenhagen counties as well as the Danish Medicines Agency and the Data Protection Agency approved the study.

Determination of the phenotypes

BChE activity was measured using the method of Kalow and Lindsay [19]. Four different inhibitors of the BChE activity were used for determining the phenotypes: dibucaine number [20], fluoride number [21], urea number [22] and the ρ number [23], but only the results of the dibucaine inhibition are given (Table 1). History of prolonged duration of action of mivacurium or succinylcholine as well as a pedigree analysis was included when possible. The reference intervals for enzyme activity and relative inhibition for the different phenotypes found in DCRU were used [12].

Genotyping

Genotyping was performed using complete nucleotide sequencing of the entire cDNA-coding region with flanking intron–exon boundaries [24]. In brief, genomic DNA was extracted from leucocytes using QIAamp DNA Blood Mini kit (QIAGEN, KEBO Lab A/S, Ballerup, Denmark). The four exons and intron–exon boundaries of the *BCHE* gene were amplified in five polymerase chain reactions using primers located within introns, noncoding flanking regions of *BCHE*, or the coding region itself. Amplified DNA samples showed single strong bands of the expected size, after agarose gel electrophoresis. The products of the polymerase chain reactions were purified and following cycle sequencing with dye terminators, the products were analysed on an automatic ABI Prism DNA 377 sequencer (Applied Biosystems, Copenhagen, Denmark). The nucleotide sequence was compared with the normal *BCHE* sequence. Moreover, mutations were confirmed by sequencing in the opposite direction. Furthermore, pedigree analysis was performed whenever possible.

Computer analysis and software

Protein sequences were obtained through Genbank. From the website of European Bioinformatics Institute: *www.ebi.ac.uk*, the software program CLUSTAL W (1.81) for multiple sequence alignment of AChE and BChE was used with the default settings. The crystal structure of human BChE was from NCBI Entrez Structure, entry 1P0M. Three-dimensional structure was analysed using the Rasmol (*http://www.umass.edu/microbio/rasmol/index.html*) programme.

Results

Three novel mutations were identified: *BCHE*FS126*, *BCHE*328D* and *BCHE*I3E4-14C*.

Table 1 Biochemical characteristics, phenotypes, and genotypes in families with novel mutations. Individuals are numbered according to pedigrees. For comparison, reference values for wild-type phenotype are given [12]

Family	Patient	BChE activity (U/l)	DN (%)	Phenotype	BCHE genotype	Dose of succinylcholine (mg/kg)	Duration of paralysis (min)
1	1–1	1244	82	UU	Wild-type/wild-type		
	1–2	0	nd	SS	**115D*I3E4-14C/*FS126*	1.8	240
	1–3	0	nd	SS	Not sequenced		
	1–4	371	83	US	Not sequenced		
	1–5	654	85	US	**115D*I3E4-14/wild-type*		
	1–6	439	80	US	**FS126/wild-type*		
2	2–1	257	28	AS	Not sequenced	2.0	360
	2–2	756	59	UA	Not sequenced		
	2–3	246	93	US	**FS126/wild-type*		
3	3	0	nd	SS	**142M/*328D*	1.3	90
Reference values for wild-type		660–1660	79–86	UU		1.0	5–10

BChE, butyrylcholinesterase; DN, dibucaine number; nd, not done. Phenotypes: AS, heterozygous for the atypical variant and a silent variant; SS, homozygous for silent variants; UA, heterozygous for the atypical variant; US, heterozygous for a silent variant; UU, wild-type.

*BCHE*FS126*

*BCHE*FS126* is a deletion of two nucleotides, 376–377 (CAT→_ _T), resulting in a shift in the translational reading frame. Furthermore, the deletion introduces a nonsense codon in the third codon after the frameshift leading to a truncated protein.

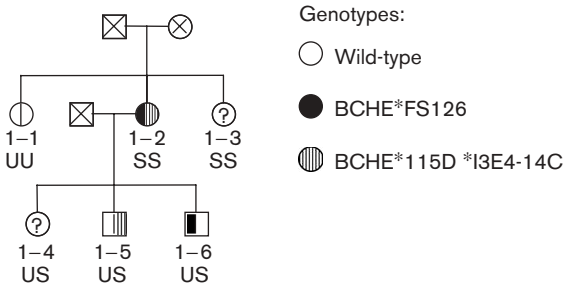
The proband in family 1 (Fig. 1, patient 1–2) had the genotype *BCHE*115D*I3E4-14C/BCHE*FS126* and displayed prolonged duration of action of succinylcholine resulting in 3 hours of apnoea and hence postoperative artificial ventilation at the intensive care unit. *BCHE*115D* is a known silent mutation [25,26], and this mutation was inherited by a son (1–5) in linkage with another novel mutation, *BCHE*I3E4-14C* (see later). *BCHE*FS126* was inherited by another son (1–6). In this family (Fig. 1 and Table 1), two patients had BChE activity below detection limits (patients 1–2 and 1–3). Patient 1–3, who was not sequenced, presumably carries the same genotype as her sister (patient 1–2).

The mutation *BCHE*FS126* also occurred in the unrelated family 2 (Table 1). One patient with a low BChE activity was genotyped as heterozygous for *BCHE*FS126* (Table 1, patient 2–3). The proband (2-1) was not sequenced, but had previously been ascribed a phenotype as compound heterozygous for the atypical variant and a silent variant using biochemical assays. This patient experienced prolonged duration of action of 360 min following succinylcholine.

*BCHE*328D*

*BCHE*328D* is the result of a nucleotide substitution at position 983 (GCT→GAT) leading to an amino acid substitution of alanine by aspartate.

Fig. 1



Pedigree of family 1. ○ = Female, □ = male, ⊗ = not tested, ? in symbol indicates not sequenced. The symbols represent the genotypes. Beneath the symbols, the number of the individual and the corresponding phenotype are given. BChE, butyrylcholinesterase; SS, homozygous silent variant; US, heterozygous for a silent variant; UU, wild-type.

The proband in family 3 (Table 1, patient 3) was compound heterozygous for *BCHE*328D* and *BCHE*142M*, the latter mutation also known as the H-variant. BChE activity was below detection limit, and the patient had experienced a prolonged duration of action of succinylcholine of 90 min, which is six times the normal duration. Furthermore, as a part of a cumulative dose–response study [27], this patient had been given mivacurium 0.016 mg/kg (1/5 of ED95 in a phenotypically normal patient), a dose that caused complete neuromuscular block. In the same study, patients homozygous for the atypical variant (D70G) were given comparable doses that likewise caused complete neuromuscular block. This indicates that the patient had severely affected enzyme activity similar to patients homozygous for the common atypical variant.

*BCHE*I3E4-14C*

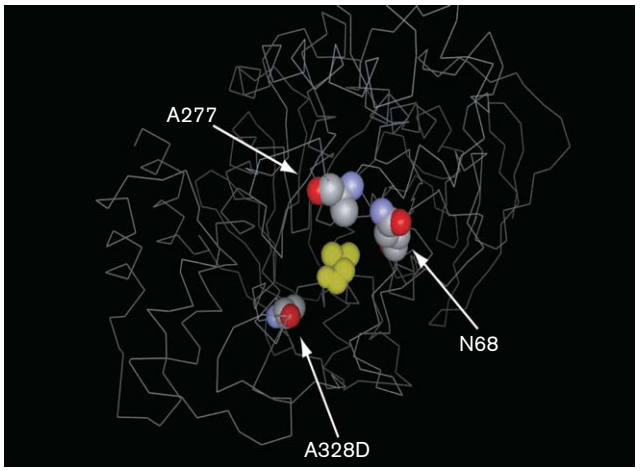
This mutation is a nucleotide substitution of T to C in intron 3, 14 nucleotides upstream of the intron 3/exon 4 junction. It was identified in two patients (Table 1, patients 1–2 and 1–5) in linkage with a known silent mutation *BCHE*115D* [25] and the possible significance of this mutation is therefore not possible to assess in this study.

Discussion

We have identified two new inactive BChE variants. One deletion mutant, *BCHE*FS126*, leads to a truncated protein lacking the active site and is therefore inactive.

The second new inactive variant is *BCHE*328D*. From the phenotype of the patient carrying this novel variant (Table 1, patient 3), this mutation also appears to be silencing the BChE protein. The activity of the second allele of this compound heterozygous patient is suppressed by a well-characterized amino acid substitution, *V142M*. This mutation is also called the H-variant with a reported BChE activity of less than 10% [28]. Amino acid number *A328* of BChE is an important residue of the enzyme. The cholinesterase enzymes have the active site in a gorge and A328D is situated in that gorge close to the active site (Fig. 2). The differences in substrate specificity of AChE and BChE relate to the size of the gorge, which is determined by the side chains of hydrophobic residues in the gorge. Thus, in BChE, a number of bulky side chains of the gorge in AChE have been substituted with smaller, aliphatic side chains. The differences in dimensions and local environment have profound impact on substrate and inhibitor selectivity of cholinesterases [29]. One example of this is the changes of residue A328 in BChE (a phenylalanine in AChE), which is evolutionary conserved and involved in the substrate activation. Site-specific mutagenesis of residue A328 to the bulky residues tyrosine or phenylalanine affected substrate activation [30] or even increased the hydroxylation rate of cocaine four-fold or 15-fold [31].

Fig. 2



Position of the *BCHE*328D* substitution in the crystal structure of BChE. The view is from the entrance of the active site gorge where *N68* and *A277* are situated in the opening of the gorge. Residue 328 is located deep in the gorge in proximity of the active site. Active site S198 is in yellow. BChE, butyrylcholinesterase.

The substitution found in our patient, *A328D*, introduces not only a hydrophilic residue, but also a negative charge in that important position in the enzyme. It is, therefore, very likely that this mutation renders the enzyme silent.

Finally, we report the mutation *BCHE*I3E4-14C*, which is of unknown significance in itself as it is only seen in linkage with a known silent variant.

The physiological role of BChE is unclear, but recent studies suggest different important functions. Animal studies suggest that BChE serves as backup for AChE in nerve transmission. Surprisingly, knockout mice that lack AChE activity are viable, but they die when their BChE is inhibited [32]. Even more important is a recent study of *BCHE* knockout mice that develop severe adverse drug reactions and die of convulsions when exposed to AChE inhibitors. BChE might have a protective effect, because it hydrolyses excess acetylcholine. Thus, BChE apparently has an important function in neurotransmission [11]. It is suggested that Alzheimer patients with silent *BCHE* will suffer from severe adverse drug reactions when they are given an anti-Alzheimer drug as donepezil. No studies of these serious adverse events in patients with low or no BChE activity, however, exist so far. The incidence of silent *BCHE* is 1 in 100 000 in European and American populations and therefore the acetyl cholinesterase drugs have to be used in many patients before an event is seen.

In conclusion, we report the identification of two novel inactive variants of BChE causing severely prolonged duration of action of succinylcholine. Recent reports among which are data from *BCHE* knockout mice, indicate that these variants may also play a role for patients treated with other drugs such as the anti-Alzheimer drugs [11].

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