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Activity and polymorphisms of butyrylcholinesterase in a Polish population

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ABSTRACT

Butyrylcholinesterase (BChE) activity assay and inhibitor phenotyping can help to identify individuals at risk of prolonged paralysis following the administration of neuromuscular blocking agents, like succinylcholine, pesticides and nerve agents.

In this study, the activity of BChE and its sensitivity to inhibition by dibucaine and fluoride was evaluated in 1200 Polish healthy individuals. In addition, molecular analysis of all exons, exon-intron boundaries and the 3′UTR sequence of the *BCHE* gene was performed in a group of 72 subjects with abnormal BChE activity (<2000 U/L and >5745 U/L) or with DN (Dibucaine Number) or FN (Fluoride-Number) values outside the reference range (DN < 78 and FN < lower than wild type).

In a studied group, BChE activity range was similar to those observed in other populations. BChE activity screening allowed to detect UA and UF phenotypes in 26 (2.2%) and 15 (1.2%) individuals, respectively. Observed UA or UF phenotypes were confirmed by direct sequencing and heterozygous c.293A > G or c.1253G > T substitutions were identified in all cases. Nine out of 18 (50%) individuals with BChE activity below 2000 U/L had a mutation in 5′UTR (32G/A), intron 2 (c.1518-121T/C) or exon 4 (c.1699G/A; the K variant mutation). Majority of the individuals with BChE activity ≥6000 U/L were wild type.

To summarize, the range of BChE activity in a Polish population is similar to those observed in other countries. We conclude that the BChE phenotyping assay is a reliable method for identification of individuals with the UA and UF genotypes.

1. Introduction

Human butyrylcholinesterase (P06276; BChE) is a serine hydrolase widely distributed throughout the body with the highest levels detected in plasma and liver [1,2]. The enzyme hydrolyzes not only acetylcholine but also longer-chain chemicals containing choline ester bonds e.g., succinylcholine, and other non-choline esters, such as aspirin, cocaine and many others [3]. The exact physiological function is unclear although it acts as an endogenous bioscavenger against AChE (P22303) inhibitors. BChE provides protection against administrated or inhaled poisons by hydrolyzing or sequestering the toxic compounds before they reach their targets – synaptic AChE, and cause neurological damage [4].

The *BCHE* gene is located on the long arm of chromosome 3 at q26.1-q.26.2 (GRCh38/hg38). It consists of 64569 bp spanning 4 exons and 3 introns. The gene encodes a transcript of 2447 nt in size and a protein of 602 amino acids residues which includes 574 residues in the mature protein and 28 residues in the signal peptide. Human BChE (huBChE) is a globular, tetrameric serine esterase with a molecular mass of 340 kDa. Each monomer carries sialylated glycans on Asn residues at positions 17, 57, 106, 241, 256, 341, 455, 481, and 486, which increase the molecular mass of the protein from 66 to 85 kDa [5,6].

To date, more than 100 genetic variants of the *BCHE* gene have been described. Point mutations, small insertions and deletions have been identified. Some of these genetic alterations are molecular basis of phenotypic variants of *BCHE*, such as: atypical, fluoride-resistant or silent gene and K, J or H variants. *BCHE* genetic

Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; BMI, body mass index; BTC, *S*-butyrylthiocholine iodide; DN, dibucaine number; FN, fluoride number; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

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alterations can affect the function of the enzyme, which leads to the prolonged neuromuscular blockade by succinylcholine [7–10]. Moreover, there is a number of *BCHE* genetic variants called "silent" which expression result in a production of a protein with reduced or without enzymatic activity [11–17].

The characterization of BChE phenotypes in humans is performed by biochemical methods, which include measurement of the enzyme activity, DN (dibucaine number) and FN (fluoride number) values. DN value is defined as the percent of BChE activity that is inhibited by dibucaine. It is used to differentiate individuals who are resistant to dibucaine inhibition due to alterations of the anionic site of the BChE [18,19]. The DN and the BChE enzyme activity measurement can help to identify subjects at risk for prolonged paralysis following the administration of muscle relaxants such as succinylcholine or mivacurium. Such individuals, with decreased enzyme activity and DN values less than 30 are called Atypical (A) BChE phenotype [20–23]. The fluoride (F) variant of BChE enzyme is resistant to inhibition by 0.050 mM sodium fluoride in *in vitro* assay. Individuals who are compound fluoride and atypical heterozygous (AF phenotype) have prolonged response to succinylcholine. Therefore, DN, FN and total BChE activity have to be used in a combination to assign a biochemical phenotype of the individuals [21,24]. Many studies have shown that high serum BChE activity is associated with obesity, insulin resistance, metabolic syndrome, hyperlipidemia, coronary artery disease or hypertension [25–27]. It was also reported that butyrylcholinesterase K (BChE-K) is associated with increased risk of developing Alzheimer's disease [28–32]. BChE has been shown to inactivate ghrelin and affects the circulating levels of this peptide hormone, with consequences for weight gain, fat metabolism, and aggression levels [33–35]. Data from the BChE knockout mice, which on a fat diet are obese and significantly heavier than wild types, indicate a role for BChE in fat utilization [25]. Furthermore, SNPs of the human *BCHE* gene have been associated with body mass index (BMI) [26,36].

The most common assay used for the determination of BChE activity and enzyme phenotyping is based on Ellman's colorimetric method developed in 1961 and universally used until now [40]. It is rapid, simple and cheap method which can be easily adapted for high-throughput analysis. In this study, the BChE activity and phenotype was determined by methods and protocols developed and described by our group previously [41].

2. Materials and methods

S-butyrylthiocholine iodide (BTC, cat. no. 20820), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent cat. no. D8130), dibucaine hydrochloride (cat. no. D0638), sodium fluoride (cat. no. 201154) were purchased from Sigma-Aldrich (Germany). 0.1 M sodium phosphate buffer (PB, pH 7.4), stock and working solutions of BTC, DTNB, dibucaine and sodium fluoride were prepared as previously described [41]. All solutions were prewarmed to 25 °C prior to use.

2.1. Subjects

Blood and serum samples from 1200 anonymized healthy individuals (556 males and 644 females, aged from 2 to 94 (mean age 48) years) were obtained from a collection of the Central Clinical Laboratory of the Medical University of Gdansk. The local Ethical Committee approved the study (NKBBN/304/2013) and waived the need for informed consent from donors. The selected individuals enrolled to the study were asymptomatic and designated as a healthy, with complete blood count and serum activity of hepatic enzymes ALT and AST in the reference range. All blood and serum samples were stored in 0.3 mL aliquots at − 80 °C prior to analysis.

2.2. Micro-ellman assay for measuring BChE activity and phenotyping

Butyrylcholinesterase activity was determined spectrophotometrically by modified Ellman's method with the use of BTC as a substrate. Briefly, 10 μl of serum was added to 190 μl of PB (100 mM, pH 7.4) to achieve initially 20-fold dilution. 400-fold dilutions were prepared in 96-well microtiter plates in a final reaction volume of 200 μl of PB (100 mM, pH 7.4) with 0.5 mM DTNB and 5 mM BTC. All samples were mixed thoroughly by repetitive pipetting. 10 μl of diluted serum samples were added to the wells of a microtiter plate containing 40 μl of PB (100 mM, pH 7.4). Then, 50 μl of DTNB (2 mM in PB) was added, and incubated (25 °C, 10 min) in a microplate-reader. For BChE phenotyping, dibucaine hydrochloride or sodium fluoride were also added to final concentrations of 100 μM and 50 μM respectively. The reactions were initiated by addition of 100 μl BTC (10 mM in PB). The absorbance was monitored at 412 nm by a Tecan Infinite M200Pro (1 min intervals for 5 min, 25 °C). One unit (U) of enzyme activity is defined as the amount that hydrolyzes 1 μmol of butyrylthiocholine per 1 min. The molar absorption coefficients of TNB at 412 nm is 14150 M(−1)cm(−1) at 25 °C. The pathlength in the microtiter plates used in the assays was estimated experimentally as 0.58 cm. The detailed protocol was described previously [41]. All experiments were performed in triplicates and for measurements and calculations Tecan Magellan V7.0 software was applied.

2.3. DNA extraction, PCR amplification and sanger sequencing

Genomic DNA was extracted from 1 mL of peripheral blood leukocytes using Blood Mini kit in accordance with the manufacturer's protocol (A&A Biotechnology, Poland). All four exons, exon-intron boundaries and 3′UTR of the *BCHE* were amplified by PCR. A 25-μL PCR mixture contained 60 ng of extracted DNA, 10 pmoles of each forward and reverse primers (Table 1), dNTPs, buffer, and Marathon *Taq* DNA polymerase (A&A Biotechnology, Poland). Primer sequences, amplicons size and position within *BCHE* gene are presented in Table 1 and Fig. 1. Amplification was performed with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s with a final extension at 72 °C for 10 min. PCR products were purified using Clean Up (A&A Biotechnology, Poland). Bidirectional DNA sequencing of PCR amplification products was performed using BigDye Terminator v.3.1 cycle sequencing kit and 3130 Genetic Analyzer according to the manufacturer's

Table 1
PCR primer sequences and amplicons size for *BCHE* sequencing analysis.

Primer	Exon	Amplicon size (bp)	Sequence 5′-3′
M1F	1	344	F- AGACTACCTGCAATTGTAAAGCA
M1R			R- TCTCATCCCACAGAATGAGC
M2-1F	2	452	F- CCTATGTAGGCCTTTACAGAAGC
M2-1R			R- TTGATCTATGTTCTGACAGCAAG
M2-2F	2	593	F- GCCACAGTCTCTGACCAAGTG
M2-2R			R- TTCTGTTCCTAGCTTCATAAAGAG
M2-3F	2	532	F- TGTTCACCAGAGCCATTCTG
M2-3R			R- ACAACATCACCCAAGGCCTC
M2-4F	2	420	F- AGTGAGTTTGGAAAGGAATCC
M2-4R			R- AGAGACCAAGCAAAGCTAAGC
M3F	3	415	F- CACTAAGCCCAGTTCACATACG
M3R			R- CATCACCGTGCCTTGGAG
M4-1F	4	381	F- TGTACTGTGTAGTTAGAGAAAATGGC
M4-1R			R- TACTAAGTTAAAGATGTGAGGAATC
M4-2F	4	517	F- AGATCAAGGCAAAAATATCAGGAGC
M4-2R			R- ATAAGGTGTTTTAAAGTGGCTGAG

NCBI Reference Sequence: NC_000003.12.