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The Identification of Butyrylcholinesterase (BCHE) Polymorphisms in a Small Australian Defence Force Cohort

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ABSTRACT

Genetic variation in the plasma enzyme butyrylcholinesterase (BCHE) affects the response of humans to xenobiotic agents. Mutations in *BCHE* are responsible for the majority of cases of prolonged apnea following the administration of succinylcholine. In addition, genetic variation in *BCHE* is linked to sensitivity to both organophosphate and carbamate compounds, including the deployment related drugs pyridostigmine, physostigmine, heptyl physostigmine and SDZ-ENZ 713. The study described in this report successfully screened the four coding regions (and surrounding intronic regions) of *BCHE* for both novel and known polymorphisms. High Resolution Melt (HRM) and sequencing procedures revealed 12 different genetic polymorphisms, and 35/51 individuals were shown to carry at least one *BCHE* polymorphism. Eight of the polymorphisms had been documented previously, two of which have been reported in individuals with succinylcholine and pyridostigmine sensitivities. Of the four novel polymorphisms found, two are predicted to change an amino acid in *BCHE*. This study has demonstrated clearly that unique and important functional genetic variation may be found and there may be a small subset of individuals in the ADF with enhanced sensitivity to succinylcholine and and organophosphate and carbamate compounds.

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Executive Summary

Deployed military personnel may be exposed to a wide range of chemicals and drugs. Following the Gulf War, some veterans reported illnesses that might have been the result of chemical exposures, and significant correlations between perceived illness and chemical exposure have been reported. Reported chemical exposures include the insect repellent N, N-diethyl-m-toluamide (DEET), insecticides such as permethrin and chlorpyrifos used to protect against insect borne diseases and the neuro-protective agent pyridostigmine bromide used to protect against nerve agent attack.

There is a wide range of chemicals and drugs that interact with the cholinergic nervous system including: nerve agents, nerve agent pre-treatments, insecticides and therapeutic drugs. Sensitivity to cholinesterase inhibitors and effective nerve agent pre-treatment rely on the activity of the enzymes acetylcholinesterase (ACHE), butyrylcholinesterase (BCHE) and paraoxonase 1 (PON1), and these genes are subject to genetic variation. Genetic variations in these genes have been shown to alter the level and activity of their enzyme products, and effective drug treatment may therefore be influenced by the genotype of the individual.

BCHE hydrolyses chemicals containing ester bonds such as: drugs acting at the neuromuscular junction (succinylcholine), local anaesthetics (procain, chloroprocaine and cocaine) and heroin. It is also a biological scavenger against organophosphorus and carbamate compounds used as pesticides and nerve agents. Individuals carrying heterozygous or homozygous inactivating alleles have been shown to have a lower capacity to interact with and detoxify drugs. Variation in an individual's genetics may therefore result in failure to achieve a desired therapeutic effect with nerve agent pre- and post-treatments or development of adverse drug reactions. In a military setting, genetic variation in *BCHE* may result in altered response to deployment related drugs including pyridostigmine, physostigmine, heptyl physostigmine and SDZ-ENZ 713.

Our initial work has focused on characterisation of the genetic variation in *BCHE* in a small cohort (n=51) of ADF personnel. Using the LightCycler 480 system, a protocol was established using High-Resolution Melting Curve Analysis to screen the *BCHE* gene. Any PCR amplicons displaying altered melting profiles were subjected to direct sequencing, and variants were identified.

This study has identified, in a subset of ADF members, genetic variation in *BCHE* which may confer increased sensitivity to a range of chemicals and drugs of interest to Defence. Initial screening has identified 12 different polymorphisms, 8 known and 4 novel. Two of the known polymorphisms have known functional effects with individuals carrying these polymorphisms exhibiting abnormal sensitivity to succinylcholine.

Future work will involve the characterisation of the four novel variants to determine if they are associated with a functional effect on either protein levels or enzyme activity.

In summary, this work demonstrates clearly the existence in the ADF of genetic variations that may have a significant impact on the response of individuals to pharmaceuticals and their sensitivity to chemical warfare agents. Furthermore, these susceptible individuals may be identified using a simple, non-invasive test procedure. It is also likely that functional variants unique to Australian populations exist and that these will need to be identified in order to accurately predict chemical and drug response.

Authors

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Katherine Shields joined the Chemical and Toxin Medical Countermeasures group, DSTO in 2007. Prior to DSTO, Kate obtained a PhD at Department of Primary Industries and subsequently conducted post-doctoral research at the Baker IDI Heart and Diabetes Institute. The focus of her work at DSTO includes the transcriptional analysis of Phase II detoxification genes and the analysis of genetic variation in response to drugs of ADF interest.

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Justine Lewis graduated in 2005 from the University of Canberra with a Bachelor of Applied Science Human Biology/Forensic Biology (Honours) degree. Justine started work at DSTO in 2006 within the Chemical and Toxin Medical Countermeasures Group and has worked in the areas of chemical warfare agent toxicology and human pharmacogenomics.

Contents

List of Abbreviations

Synonymous mutation	change in DNA base which does not alter an amino acid
SNP	Single nucleotide polymorphism
gDNA	genomic DNA
PCR	Polymerase Chain Reaction
UTR	Untranslated region

1. Introduction

Organophosphorus compounds (OPs) are triesters of phosphoric acid and their major use is as insecticides. Chemical warfare agents that affect the nervous system and nerve agents (eg. soman and sarin) also belong to this class of chemicals. On acute exposure, OPs cause neurotoxicity in the form of a cholinergic syndrome (i.e. overstimulation of muscarinic and nicotinic acetylcholine receptors in the central and peripheral nervous systems) resulting from accumulation of acetylcholine in the synaptic cleft as a consequence of inhibition of acetylcholinesterase (AChE). In addition, both acute and repeated low-level exposure to some OPs is associated with delayed neuropathy and can result in long term cognitive effects [1, 2].

Human butyrylcholinesterase (*BCHE*) is an enzyme found in plasma and many other parts of the body. The enzyme hydrolyses drugs containing ester bonds such as drugs acting at the neuromuscular junction (succinylcholine), local anaesthetics (procaine, chloroprocaine and cocaine) and heroin [3]. It is also a biological scavenger for organophosphorus and carbamate compounds used as pesticides [4]. The rapid hydrolysis of succinylcholine by *BCHE* accounts for its short duration of action. Mutations in the *BCHE* gene affecting the function of this enzyme can lead to prolonged neuromuscular blockade by succinylcholine. In a military setting, abnormal *BCHE* enzyme levels may result in altered response to deployment related drugs including pyridostigmine, physostigmine, heptyl physostigmine and SDZ-ENZ 713 [5, 6].

The *BCHE* gene is located on chromosome 3q26.1-q.26.2. The genomic region for *BCHE* spans over 70 kb and has 4 exons and 3 large introns. The gene encodes a transcript of 2400bp in size and a protein of 574 amino acids. To date, more than 100 polymorphisms have been identified; however few have been studied fully. In general, *BCHE* polymorphisms have been shown to produce enzymes with varying levels of catalytic activity. The molecular bases of several genetic variants of *BCHE* have been reported, such as the Atypical gene, fluoride-resistant gene, silent gene, K variant, J variant and H variant [7-10]. In addition there are a number of additional *BCHE* polymorphisms which result in a protein with no enzymatic activity. Enzymes with activity below 10% of the wild type enzyme are called “silent” variants [11]. An example of the range of polymorphisms identified within *BCHE* are summarised in Appendix A.

Currently, little genetic analysis of *BCHE* has been performed in samples from an Australian population. A single study performed in 2003 screened the *BCHE* gene of 65 individuals suffering prolonged post-succinylcholine apnea. Overall the Atypical and K variant mutations were highly prevalent, however, six new *BCHE* mutations were identified, and it was found most individuals carried multiple homozygous mutations [12]. This demonstrated unique heterogeneity in a proportion of the Australian population and that novel functional variants exist in this population. There is little information available on the level of genetic variation in the Australian population, and the level of inter-individual variation in the expression of *BCHE* is unclear.

Therefore, combinations of phenotypes and expression levels of *BCHE* involved in OP detoxification might be expected to occur in military personnel and define a subgroup who are potentially more susceptible to adverse drug effects than the majority of ADF members.

Our approach to *BCHE* genotyping considered issues of time- and cost effectiveness.

The aim of this project was to;

- Screen for novel sequence variations within the coding regions of the *BCHE* gene.
- Identify the presence of any common polymorphisms of functional relevance to the ADF.
- Compare the genotype frequency of these polymorphisms and compare to the level and types of genetic variation with that found in other populations (US, European, Chinese).

2. Material and Methods

2.1 Population

The experimental procedures were approved by the Australian Defence Human Research Ethics Committee (ADHREC protocol 418/05), and all guidelines were adhered to. Participants were 51 Australian Defence Force soldiers who provided 10 ml of whole blood as part of a larger medical screening process. All samples were unlabelled at the time of collection to allow for a double-blind analysis, and no participant could be identified from their biological material. Samples used as controls were obtained from the Red Cross, agreement number 08-11VIC-05.

2.2 Preparation of Genomic DNA

Genomic DNA was isolated from whole blood using the QIAamp® DNA Blood Mini kit (Qiagen), using "Blood or Body Fluid Spin Protocol" with the following modifications. A total of 200 µl of whole blood was used in each extraction and samples were eluted in 200 µl of RNAase/DNAse free water and stored at 4°C.

2.3 High Resolution Melt Strategy

All primers were designed using Primer3 software which is freely available at http://frodo.wi.mit.edu/primer3/input.htm. The following parameters were applied to all primers; primer length 18–27 bp, GC% = 30–70%, Tm = 59–60°C and amplicon size = 100–250 bp. Amplicons were designed to cover all exonic regions of the *BCHE* gene and flanking intronic regions. Primer sets were designed so that multiple amplicons across an exon had at least a 30 bp overlap. Primer sequences and amplicon size are described in Technical report DSTO-TR-2498. Figure 1 describes the position of the 24 amplicons within the *BCHE* gene structure.

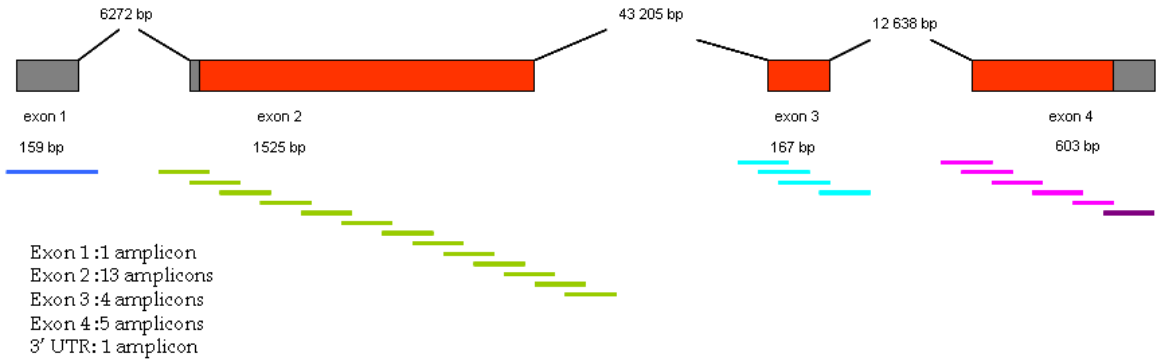


Figure 1: Schematic representation of the gene structure and relative position of amplicons used in analysis BCHE. BCHE consists of 4 exons, exon 1 is 159 bp, exon 2 is 1525 bp, exon 3 is 167 bp and exon 4 is 603bp. BCHE messenger RNA transcript is 2454 bp is size and the initiating ATG is located at base 9 of exon 2 (red) and stop codon is at position 125bp of exon 4. In total, exon 1 was covered by 1 amplicon (blue), exon 2; 13 amplicons (green), exon 3; 4 amplicons (aqua), exon 4; 5 amplicons (pink) and 3'UTR 1 amplicon, purple.

Real-time PCR amplification of the *BCHE* gene fragment was performed in a LightCycler® 480 High Resolution Melting Master mix containing a saturating HRM dye (Roche Diagnostics Co, Mannheim, Germany). Genomic DNA from unknown samples (5 ng) was added to a 20 µl reaction mixture containing 0.2 µM of each primer and 1 x High Resolution Master mix. $MgCl_2$ concentrations varied according to the primer pair and are described in technical report DSTO-TR-2498. Samples were also spiked with a 20% homozygous control sample as described in DSTO-TR-2498. PCR was carried out using a touchdown protocol, with annealing temperatures ranging from 62°C to 53°C. High-resolution melting curve data were obtained at a rate of 25 acquisitions per °C. PCR conditions are described in Table 1.

Table 1: LightCycler 480 Reaction conditions

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)
Pre-incubation				
95	none	00:10:00	4.4	0
Amplification 45 cycles				
95	none	00:00:10	4.4	0
62 – 53*	none	00:00:10	2.2	0
72	single	00:00:10	4.4	0
High Resolution Melting				
95	none	00:01:00	4.4	0
40	none	00:01:00	2.2	0
65	none	00:00:01	4.4	0
95	continuous		0.02	25
cooling				
40	none	00:00:30	2.2	0

* 53°C is second temperature target, with a step size of 0.5°C per cycle

Melting curves were analysed using the LightCycler 480 software. Briefly, melting curves for individual amplification products were normalised using a pre-melt signal set to a relative value of 100% and post melt signals set to a relative value of 0%. The melting shift was calculated automatically by the software using a default adjustment value of 5% for all analyses. Using a curve matching algorithm, samples were clustered into groups and difference plots examined. The sensitivity level was set on 0.3 or 0.6 for a high sensitivity setting. Higher sensitivity settings identify smaller fluorescent differences which are required when multiple melting domains exist within the amplicon examined. Amplicons displaying alternative melting profiles were confirmed by sequencing an independent PCR product.

2.4 DNA sequencing

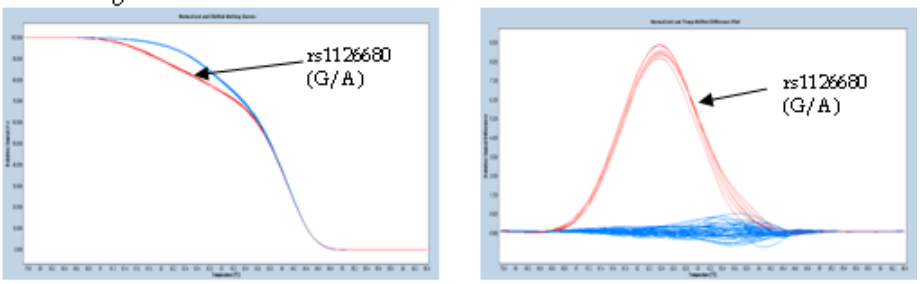
PCR amplifications were performed in a 20 µl reaction containing 0.5 µM of each primer, 5 ng gDNA, 0.8U FastStart Taq (Roche), 0.5 mM of each dNTP (Roche), 2.0 mM $MgCl_2$ and 1 x PCR buffer (Roche), using amplification conditions described in Table 3. Samples were analysed on 1.2% Agarose gels to confirm single products of correct size and subsequently purified using EXO-SAPIT (GE Healthcare). Samples were sequenced using the BigDye Terminator kit V3.1 (Applied Biosystems) and cleaned using Xterminator (Applied Biosystems) according to the manufacturer's instructions. Analysis of the sequencing reactions was performed on an ABI3130xl (Applied Biosystems). Sequence trace files were analysed for SNPs using Sequencher (GeneCodes, Genesearch).

3. Results

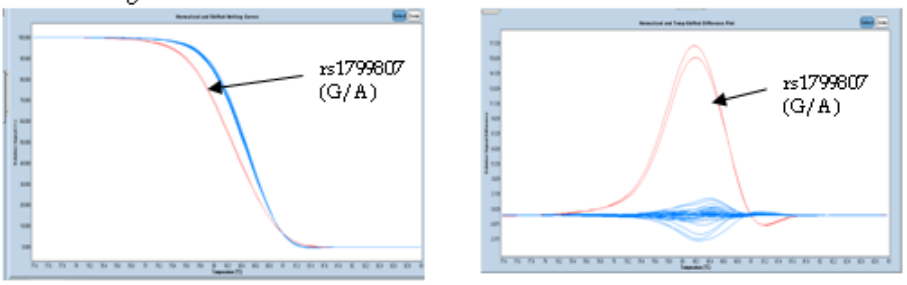
In this study, we tested the ability of HRM using the LightCycler 480 to screen for DNA variations in the coding regions of the *BCHE* gene. In total, 24 fragments in each of the 51 gDNA samples were scanned.

Figure 2 shows the melting curves and subtractive difference plots for all exons of the *BCHE* gene in which at least one variation was identified. The melting curves identified DNA samples with possible polymorphisms. Subtractive difference plots were obtained by subtracting the curve of the sample of interest from that of a control sample with normal DNA sequence. Abnormalities initially identified in the melting curves were confirmed and magnified by the subtractive difference plot. These samples were then amplified by PCR and sequenced (Appendix B).

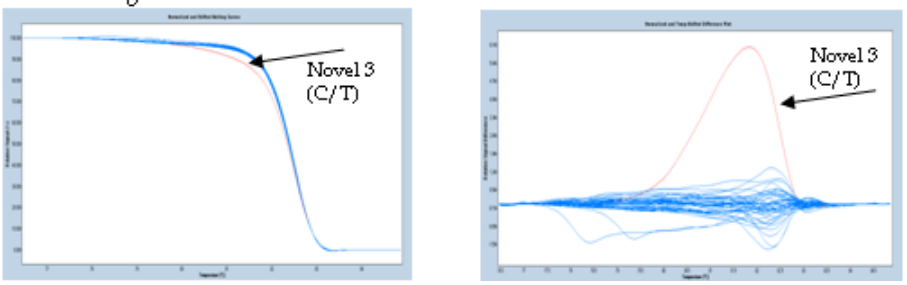
Exon 1 fragment 1



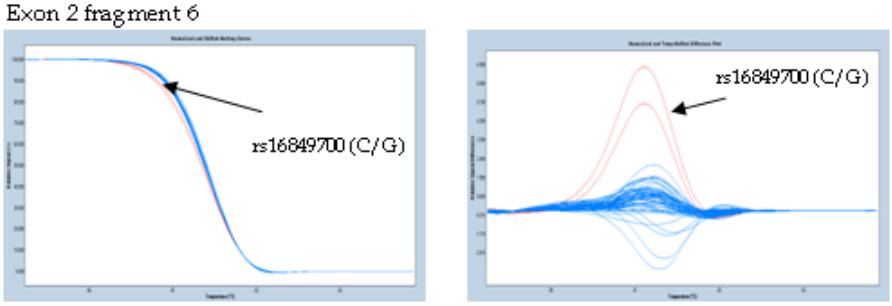
Exon 2 fragment 3



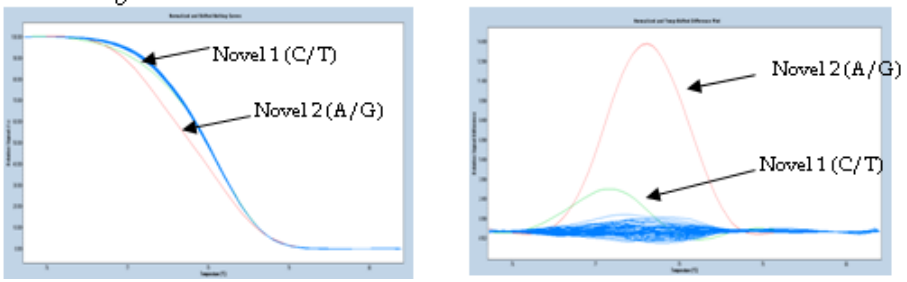
Exon 2 fragment 4



Exon 2 fragment 6



Exon 2 fragment 7.2



Refer to figure legend page 6

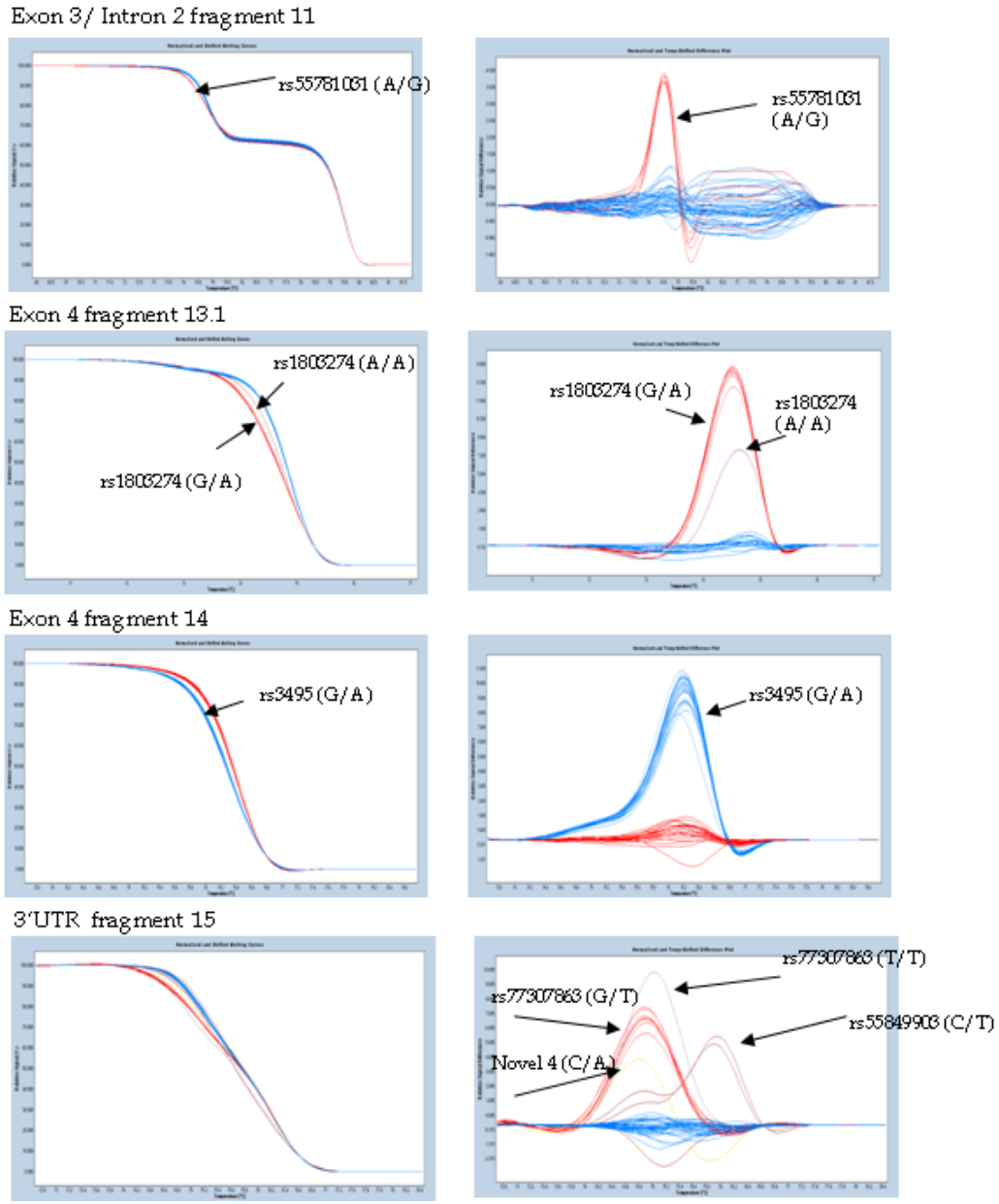


Figure 2: Melting (left) and subtractive difference plots (right) of normal and abnormal fragments of the BCHE gene. Note: the shifting of the plots is due to the indicated mutations. Fragments were analysed by PCR amplification with the HRM dye followed by a high-resolution thermal denaturation. Subtractive plots on the right were obtained by subtracting each curve from a non-polymorphic control. Note: each polymorphism produced a unique melting and difference plot

DNA variations in the *BCHE* gene identified in the 51 subjects are summarised in Table 2.

Of the 51 subjects, 35 had at least one SNP in the *BCHE* regions examined. Two polymorphisms are well described, K variant and Atypical variant. These alleles both have altered function compared to wild-type. K variant SNP results in a protein with 30% reduced activity and Atypical is a silent allele. In the case of K variant, 2 samples; SOTG8-68 and SOTG8-76 were

homozygous for the mutant allele. Samples RAN1-12 and RAN1-21 were shown to be heterozygous for both polymorphisms. Sequencing analysis uncovered 4 new *BCHE* mutations, not currently in public databases. Two of these Ile384Val and Ala212Val result in amino acid substitutions in exon 2. In summary 12 different polymorphisms were identified and these are summarised in Table 2.

Table 2: Summary of DNA variation in BCHE gene, (gDNA NCBI Reference Sequence: NG_009031.1; cDNA NCBI Reference Sequence: NM_000055.2, protein NP_000046.1m Map to genome build 37.1) identified in HRM analysis of 51 subjects.

Position in gene	Amplicon	Name of SNP	DNA variation	Number of positive samples
Exon 1 5' UTR	1	rs1126680	g.5129G>A c.-32G>A	G / G 45 / 51 G / A 6 / 51
exon 2	3	rs1799807 Atypical variant	g.11725A>G c.293A>G p.Asp98Gly	A / A 49 / 51 A / G 2 / 51
	4 and 5	Novel 3	g.12067 C>T c. 801 C>T p. Ala212Val	C / C 50 / 51 C / T 1 / 51
	6	rs16849700	g.12281G>C c.849G>C p.Glu283Asp	G / G 49 / 51 G / C 2 / 51
	7.1 and 7.2	Novel 1	g.15509 C>T c.1244 C>T p. Val384Val	C / C 49 / 51 C / T 1 / 51
	7.2 and 8.1	Novel 2	g.12582A>G c.1317A>G p.Ile384Val	A / A 50 / 51 A / G 1 / 51
exon 3 (intron 2)	11	rs55781031	g.56034T>C c.1518-121T>C	T / T 46 / 51 T / C 5 / 51
exon 4	13, 13.1, 13.2	rs1803274 K variant	g.68974G>A c.1699G>A p.Ala567Thr	G / G 29 / 51 G / A 21 / 51 A / A 2 / 51
exon 4 3'UTR	14	rs3495	g.69273G>A c.2159G>A	G / G 27 / 51 G / A 24 / 51
3'UTR	15	rs55849903	g.69433C>T c.2225C>T p.N / A	C / C 49 / 51 C / T 2 / 51
3'UTR	15	Novel 4	g.C>T 69474 c.2366 C>A p. N / A	C / C 50 / 51 C / T 1 / 51
near gene 3'	15	rs77307863	g.71985803C>A	C / C 42 / 51 C / A 8 / 51 A / A 1 / 51

Of the samples with polymorphisms, (24 / 35) had compound gene defects (i.e. compound heterozygotes and homozygotes) and this is summarised in Table 3. Five samples RAN1-18, RAN1-9, RAN1-4, RAN1-1 and SOTG8-45 identified 4 SNPs within the regions examined.

Table 3: Summary of polymorphisms found in BCHE in the regions examined for each individual

Sample	Exon 1	Exon 2	intron 2 (near 3')	Exon 3	Exon 4	3'UTR
SOTG8-45	NP	rs16849700 (G / C)	NP	NP	rs1803274 (A / G),rs3495 (G / A)	rs55849903 (C / T)
SOTG8-46	NP	NP	NP	NP	NP	novel 4 G / A
SOTG8-47	NP	NP	NP	NP	rs1803274 (A / G), rs3495 (G / A)	NP
SOTG8-48	NP	NP	NP	NP	NP	rs77307863 (G / T)
SOTG8-49	NP	NP	NP	NP	rs1803274 (A / G), rs3495 (G / A)	
SOTG8-50	NP	NP	NP	NP	rs1803274 (A / G), rs3495 (G / A)	NP
SOTG8-51	NP	NP	NP	NP	NP	NP
SOTG8-52	NP	NP	NP	NP	NP	rs77307863 (T / T)
SOTG8-53	NP	NP	NP	NP	rs1803274 (A / G), rs3495 (G / A)	NP
RAN1-1	rs1126680 A / G	NP	rs55781031 (A / G)	NP	rs1803274 (A / G), rs3495 (G / A)	NP
RAN1-2	NP	NP	NP	NP	rs1803274 (A / G), rs3495 (G / A)	NP
RAN1-4	rs1126680 A / G	NP	rs55781031 (A / G)	NP	rs1803274 (A / G), rs3495 (G / A)	NP
RAN1-5	NP	NP	NP	NP	rs1803274 (A / G), rs3495 (G / A)	NP
RAN1-6	NP	NP	NP	NP	NP	NP
RAN1-7	NP	NP	NP	NP	NP	NP
RAN1-8	NP	NP	NP	NP	NP	rs77307863 (G / T)
RAN1-9	rs1126680 A / G	NP	rs55781031 (A / G)	NP	rs1803274 (A / G), rs3495 (G / A)	NP
RAN1-10	NP	novel 3 C / T	NP	NP	NP	NP
RAN1-11	NP	NP	NP	NP	rs1803274 (A / G), rs3495 (G / A)	NP
RAN1-12	NP	rs1799807 A / G	NP	NP	rs1803274 (A / G), rs3495 (G / A)	NP
RAN1-13	NP	NP	NP	NP	NP	rs77307863 (G / T)
RAN1-14	NP	NP	NP	NP	rs3495 (G / A)	rs77307863 (G / T)
RAN1-15	NP	NP	NP	NP	rs1803274 (A / G), rs3495 (G / A)	NP
RAN1-16	NP	NP	NP	NP	rs1803274 (A / G), rs3495 (G / A)	rs77307863 (G / T)
RAN1-17	NP	novel 1 C / T	NP	NP	rs3495 (G / A)	NP
RAN1-18	NP	rs16849700 C / G	NP	NP	rs1803274 (A / G), rs3495 (G / A)	rs55849903 (C / T)
RAN1-19	NP	NP	NP	NP	NP	rs77307863 (G / T)
RAN1-20	NP	NP	NP	NP	rs3495 (G / A)	NP
RAN1-21	NP	rs1799807 A / G	NP	NP	rs1803274 (A / G) rs3495 (G / A)	NP

Sample	Exon 1	Exon 2	intron 2 (near 3')	Exon 3	Exon 4	3'UTR
RAN1-23	NP	NP	NP	NP	rs1803274 (A / G)	NP
SOTG8-55	NP	NP	NP	NP	NP	NP
SOTG8-56	NP	NP	NP	NP	NP	NP
SOTG8-57	NP	novel 2 A / G	NP	NP	rs3495 (G / A)	NP
SOTG8-58	NP	NP	NP	NP	rs3495 (G / A)	NP
SOTG8-59	NP	NP	NP	NP	rs3495 (G / A)	NP
SOTG8-60	rs1126680 A / G	NP	NP	NP	rs1803274 (A / G); rs3495 (G / A)	NP
SOTG8-61	NP	NP	NP	NP	NP	NP
SOTG8-63	NP	NP	NP	NP	NP	NP
SOTG8-65	NP	NP	NP	NP	NP	rs77307863 (G / T)
SOTG8-66	NP	NP	NP	NP	NP	NP
SOTG8-67	NP	NP	NP	NP	NP	NP
SOTG8-68	NP	NP	NP	NP	rs1803274 (A / A)	NP
SOTG8-69	NP	NP	NP	NP	NP	NP
SOTG8-70	NP	NP	NP	NP	NP	NP
SOTG8-72	NP	NP	NP	NP	NP	NP
SOTG8-73	NP	NP	NP	NP	NP	NP
SOTG8-75	NP	NP	NP	NP	NP	NP
SOTG8-76	rs1126680 A / G	NP	rs55781031 (A / G)	NP	rs1803274 (A / A)	NP
SOTG8-62	NP	NP	NP	NP	NP	NP
SOTG8-64	NP	NP	NP	NP	rs1803274 (A / G), rs3495 (G / A)	rs77307863 (G / T)
SOTG8-77	rs1126680 A / G	NP	rs55781031 (A / G)	NP	rs1803274 (A / G)	NP

NP: Non Polymorphic

Allele frequencies for SNPs were calculated when appropriate and this was compared to the literature, Table 4. Overall, this small ADF sample demonstrated genotype frequencies similar to AFD_EUR_PANEL and HapMap-CEU, both Caucasian panels (Appendix C). For detailed definitions of the populations shown refer: http://ccr.coriell.org/Sections/Collections/NHGRI/hapmap.aspx?PgId=266&coll=HG and a general description can be found in Appendix D.

Table 4: Allele frequencies

SNP		Population (NCBI)						
	ADF n=51	Caucasian AFD_EUR_PANEL	HapMap-CEU (European) HapMap-CEU	African American AFD_AFR_PANEL	HapMap-YRI HapMap-YRI	Asian AFD_CHN_PANEL	HapMap-HCB HapMap-HCB	HapMap-JPT HapMap-JPT
rs1126680	A 0.058 G 0.9411	A: 0.083 G: 0.917	A: 0.058 G: 0.942	1.00	1.00	1.00	1.00	1.00
rs1799807	A.0.980 G.0.200	A 0.979 G 0.021	0.992 0.008	0.978 0.022	1.00	1.00	1.00	1.00
rs16849700	C 0.98 G 0.02	C 1.00	1.00	C 0.935 G 0.065	C 0.925 G 0.075	1.00	1.00	1.00
rs55781031	A 0.951 G 0.049	A 0.917 G 0.083	N/A	N/A	N/A	N/A	N/A	N/A
rs1803274	A 0.235 G 0.765	A 0.167 G 0.833	0.175 0.825	0.152 0.848	0.167 0.833	0.083 0.917	0.200 0.800	0.205 0.795
rs3495	A 0.235 G 0.765	A 0.771 G 0.229	N/A	A 0.341 G 0.659	N/A	A 0.771 G 0.229	N/A	N/A
rs77307863	A 0.09 G 0.901	A 0.056 G 0.944	N/A	N/A	N/A	N/A	N/A	N/A

N/A- unknown, no frequency data

4. Discussion

BCHE is widely distributed throughout the body. The *BCHE* in plasma is synthesized in the liver. It is a biological scavenger against organophosphorus and carbamate compounds and has an important role in the hydrolysis of the muscle relaxant succinylcholine. Mutations in *BCHE* gene affect the function of this enzyme leading to prolonged neuromuscular blockade.

This study successfully screened the 4 coding regions (and surrounding intronic regions) of *BCHE* for both novel and known polymorphisms of interest. Overall, 35/51 individuals carry a *BCHE* polymorphism, although the functional relevance for many of these SNPs is unknown. HRM and sequencing procedures revealed 12 different genetic polymorphisms and this included the K variant and the Atypical gene, along with 4 novel mutations.

Besides the normal phenotype, the most frequent variations of *BCHE* are the Atypical and K variants. Both variants have been associated with prolongation of muscle relaxation after administration of succinylcholine and mivarcurium [13]. In Caucasian populations the allele frequency of the Atypical variant is about 2%, whereas the allele frequency of the K variant is between 16.7–17.5% with a homozygous incidence of 1 in approximately 63 individuals [13-15]. The K variant has been reported to be the most common variant in any population and *in vitro* enzyme activity studies have demonstrated a 33% reduction in enzyme activity. In the ADF

population examined, both the Atypical and K variant were identified. The K variant was observed in 23 individuals (21 heterozygous and 2 homozygous), representing a frequency of 25 of 102 alleles (24% allele frequency). This allele frequency is slightly higher than that found in the HapMap samples of randomly selected healthy individuals of Northern European (17.5%) and Asian (20%) decent. At present, the *BCHE* activity in the ADF samples has not been measured, although it has been reported that both heterozygous and homozygous individuals with the K variant have reduced serum *BCHE* activity [10]. As expected, the Atypical variant was found at a much lower frequency, with only 2 (1.8%) heterozygous individuals carrying the allele which is consistent with frequencies reported for Caucasian populations.

Previous studies have shown linkage disequilibrium between Atypical and K variants in which the K variant was found in 89–96% of Atypical variant carriers [9]. This has shown to be the case in both European and American populations however, the Atypical allele has been shown to be rare in Japanese [9, 10]. Genetic investigations have also provided evidence that while the K variant alone causes moderate prolongation of the action of mivacuirum, the effect is more pronounced in patients carrying both the Atypical and K variants [16]. Our results (although from a small population) support this as both samples RAN1-12 and RAN1-21 in which the Atypical variant was detected, were also K variant carriers.

HRM analysis identified a total of 4 novel *BCHE* mutations, 3 in exon 2 and one in the 3'UTR of *BCHE*. These were confirmed by DNA sequencing. The mutations named "novel 2" and "novel 3" describe the nucleotide substitution that leads to an amino acid change Ile384Val and Ala212Val, respectively. Further studies will compare the protein levels and activity of *BCHE* in these individuals to determine any possible functional consequence of these polymorphisms. The third mutation "novel 3" was a synonymous base pair change , a C-T at position 1244 and is unlikely to have any effect on the expression or activity of *BCHE*. The final mutation found in *BCHE* is located in the 3'UTR, a C-A substitution at position 2366 of the cDNA, may affect mRNA stability. Future work will compare the *BCHE* protein levels of this individual to control samples.

In conclusion, the outcomes of this study are:

- HRM is able to effectively screen for and identify polymorphisms in the *BCHE* gene.
- This approach detected eight known and four novel polymorphisms in a small ADF population.
- Two of the most commonly studied functional relevant polymorphisms the K variant and Atypical variant were identified in this subset of individuals from the ADF.
- Comparison to SNP databases, revealed the genotype frequencies of the 8 polymorphisms identified were similar to those found in Caucasian populations.
- Linkage studies in European and American populations identified the Atypical allele is often carried with the K variant. Our data support these findings.

These findings are significant as they provide a cheap and rapid method of obtaining prior knowledge of possible hypersensitivity to succinylcholine and various organophosphorus compounds in members of the ADF.

Further work will involve the generation of a larger data set to allow a more accurate determination of allele frequencies of polymorphisms of interest to the ADF. The measurement of *BCHE* protein and activity in serum samples will allow the determination of the functional relevance of the novel variation found.

5. References

1. **Beach, J.R., A. Spurgeon, R. Stephens, T. Heafield, I.A. Calvert, L.S. Levy, and J.M. Harrington**, Abnormalities on neurological examination among sheep farmers exposed to organophosphorous pesticides. Occupational and Environmental Medicine, 1996. 53(8): p. 520-525.
2. **Weiner, M.L. and B.S. Jortner**, Organophosphate-induced delayed neurotoxicity of triarylphosphates. NeuroToxicology, 1999. 20(4): p. 653-673.
3. **Girard, T. and C.H. Kindler**, Pharmacogenetics and anaesthesiology. Current Pharmacogenomics, 2004. 2(2): p. 119-135.
4. **Singh, S., M. Verma, C.O. Leelamma, K. Nain, R.C. Goel, N.K. Ganguly, and B.K. Sharma**, Red cell acetyl cholinesterase and plasma cholinesterase activity and genetic variants of plasma cholinesterase in Northwest Indian adults. International Journal of Clinical Pharmacology and Therapeutics, 1997. 35(9): p. 357-360.
5. **Loewenstein-Lichtenstein, Y., M. Schwarz, D. Glick, B. Norgaard-Pedersen, H. Zakut, and H. Soreq**, Genetic predisposition to adverse consequences of anti-cholinesterases in 'atypical' *BCHE* carriers. Nature Medicine, 1995. 1(10): p. 1082-5.
6. **Dunn, M.A., B.E.J. Hackley, and F.R. Sidell**, Pretreatment for Nerve Agent Exposure. Medical Aspects of Chemical and Biological Warfare. Textbook of Military Medicine, ed. R. Zajtchuk and R.F. Bellamy. 1997, Washington DC, USA: Office of the Surgeon General at TMM Publications, Department of Army. 181-196.
7. **Nogueira, C.P., C.F. Bartels, M.C. McGuire, S. Adkins, T. Lubrano, H.M. Rubinstein, H. Lightstone, A.F. Van der Spek, O. Lockridge, and B.N. La Du**, Identification of two different point mutations associated with the fluoride-resistant phenotype for human butyrylcholinesterase. American Journal of Human Genetics, 1992. 51(4): p. 821-8.
8. **Nogueira, C.P., M.C. McGuire, C. Graeser, C.F. Bartels, M. Arpagaus, A.F. Van der Spek, H. Lightstone, O. Lockridge, and B.N. La Du**, Identification of a frameshift mutation responsible for the silent phenotype of human serum cholinesterase, Gly 117 (GGT to GGAG). American Journal of Human Genetics, 1990. 46(5): p. 934-42.
9. **Bartels, C.F., F.S. Jensen, O. Lockridge, A.F. van der Spek, H.M. Rubinstein, T. Lubrano, and B.N. La Du**, DNA mutation associated with the human butyrylcholinesterase K-variant and its linkage to the atypical variant mutation and other polymorphic sites. American Journal of Human Genetics, 1992. 50(5): p. 1086-103.

10. **Maekawa, M.**, Genetic mutations of butyrylcholine esterase identified from phenotypic abnormalities in Japan. Clinical Chemistry, 1997. 43(6 Pt 1): p. 924-929.

11. **Primo-Parmo, S.L., C.F. Bartels, B. Wiersema, A.F. van der Spek, J.W. Innis, and B.N. La Du**, Characterization of 12 silent alleles of the human butyrylcholinesterase (BCHE) gene. American Journal of Human Genetics, 1996. 58(1): p. 52-64.

12. **Yen, T., B.N. Nightingale, J.C. Burns, D.R. Sullivan, and P.M. Stewart**, Butyrylcholinesterase (BCHE) Genotyping for Post-Succinylcholine Apnea in an Australian Population. Clin Chem, 2003. 49(8): p. 1297-1308.

13. **Levano, S., H. Ginz, M. Siegemund, M. Filipovic, E. Voronkov, A. Urwyler, and T. Girard**, Genotyping the butyrylcholinesterase in patients with prolonged neuromuscular block after succinylcholine. Anesthesiology, 2005. 102(3): p. 531-535.

14. **Babaoglu, M.O., T. Ocal, B. Bayar, S.O. Kayaalp, and A. Bozkurt**, Frequency and enzyme activity of the butyrylcholinesterase K-variant in a Turkish population. European Journal of Clinical Pharmacology, 2004. 59(12): p. 875-877.

15. **Levano, S., D. Keller, E. Schobinger, A. Urwyler, and T. Girard**, Rapid and accurate detection of atypical- and Kalow-variants in the butyrylcholinesterase gene using denaturing high-performance liquid chromatography. Anesthesia and Analgesia, 2008. 106(1): p. 147-151.

16. **Gatke, M.R., J. Viby-Mogensen, D. Ostergaard, and J.R. Bundgaard**, Response to mivacurium in patients carrying the K variant in the butyrylcholinesterase gene. Anesthesiology, 2005. 102(3): p. 503-508.

17. **Lockridge, O., C.F. Bartels, and T.A. Vaughan**, Complete amino acid sequence of human serum cholinesterase. Journal of Biological Chemistry, 1987. 262(2): p. 549-557.

18. **McGuire, M.C., C.P. Nogueira, C.F. Bartels, H. Lightstone, A. Hajra, A.F.L. Van der Spek, O. Lockridge, and B.N. La Du**, Identification of the structural mutation responsible for the dibucaine-resistant (atypical) variant form of human serum cholinesterase. Proceedings of the National Academy of Sciences of the United States of America, 1989. 86(3): p. 953-957.

19. **Souza, R.L.R., L.R. Mikami, R.O.B. Maegawa, and E.A. Chautard-Freire-Maia**, Four new mutations in the *BCHE* gene of human butyrylcholinesterase in a Brazilian blood donor sample. Molecular Genetics and Metabolism, 2005. 84(4): p. 349-353.

20. **Primo-Parmo, S.L., H. Lightstone, and B.N. La Du**, Characterization of an unstable variant (BChE115D) of human butyrylcholinesterase. Pharmacogenetics, 1997. 7(1): p. 27-34.

21. **Greenberg, C.P., S.L. Primo-Parmo, E.J. Pantuck, and B.N. La Du**, Prolonged response to succinylcholine: A new variant of plasma cholinesterase that is identified as normal by traditional phenotyping methods. Anesthesia and Analgesia, 1995. 81(2): p. 419-421.

22. **Manoharan, I., S. Wieseler, P.G. Layer, O. Lockridge, and R. Boopathy**, Naturally occurring mutation Leu307Pro of human butyrylcholinesterase in the Vysya community of India. Pharmacogenetics & Genomics, 2006. 16(7): p. 461-8.

23. **Gatke, M.R., J.R. Bundgaard, and J. Viby-Mogensen**, Two novel mutations in the BCHE gene in patients with prolonged duration of action of mivacurium or succinylcholine during anaesthesia. Pharmacogenetics and Genomics, 2007. 17(11): p. 995-999.

24. **Muratani, K., T. Hada, Y. Yamamoto, T. Kaneko, Y. Shigeto, T. Ohue, J. Furuyama, and K. Higashino**, Inactivation of the cholinesterase gene by Alu insertion: Possible mechanism for human gene transposition. Proceedings of the National Academy of Sciences of the United States of America, 1991. 88(24): p. 11315-11319.

25. **Hidaka, K., I. Iuchi, T. Yamasaki, M. Ohhara, T. Shoda, S. Primo-Parmo, and B.N. Ladu**, Identification of two different genetic mutation associated with silent phenotypes for human serum cholinesterase in Japanese. Rinsho byori. The Japanese journal of clinical pathology, 1992. 40(5): p. 535-540.

26. **Nogueira, C.P., C.F. Bartels, M.C. McGuire, S. Adkins, T. Lubrano, H.M. Rubinstein, H. Lightstone, A.F.L. Van der Spek, O. Lockridge, and B.N. La Du**, Identification of two different point mutations associated with the fluoride-resistant phenotype for human butyrylcholinesterase. American Journal of Human Genetics, 1992. 51(4): p. 821-828.

27. **Maekawa, M., K. Sudo, T. Kanno, K. Kotani, D.C. Dey, J. Ishikawa, M. Izumi, and K. Etoh**, Genetic basis of the silent phenotype of serum butyrylcholinesterase in three compound heterozygotes. Clinica Chimica Acta, 1995. 235(1): p. 41-57.

28. **Bartels, C.F., K.D. James, and B.N. La Du**, DNA Mutations Associated with the Human Butyrylcholinesterase J-Variant. American Journal of Human Genetics, 1992. 50: p. 1104-1114.

29. **Howard, T.D., F.-C. Hsu, J.G. Grzywacz, H. Chen, S.A. Quandt, Q.M. Vallejos, L.E. Whalley, W. Cui, S. Padilla, and T.A. Arcury**, Evaluation of Candidate Genes for Cholinesterase Activity in Farmworkers Exposed to Organophosphorous Pesticides-Association of SNPs in *BCHE*. Environ Health Perspect, 2010.

Appendix A: *BCHE* polymorphisms

It should be noted that the naming convention of SNP location has changed for *BCHE* over the years. Initial naming of SNPs used their position relative to N-terminus of the mature protein, listed as the alternative name [17]. Current existing convention is to number the effected amino acid from the signal sequence rather than the N-terminus of the mature protein (effect column). Where available, database reference numbers (rs numbers) are provided. Nucleotide change is relative to the full length cDNA, reference NM_000055.2 or the genomic sequence NT_005612.16 if the SNP is located within an intron.

Table A1. Description of the main polymorphism identified in the literature and public databases. Where available, the SNP ID, alternative name, nucleotide change and effect (if any) on the protein is listed.

dbSNP ID	Alternative name	Nucleotide change	Effect	Functional studies		Reference
				In vivo	*In vitro*	
rs55950599	N / A	A176T	T6S	Unknown	Unknown	dbSNP build 132
	Silent 2 BCHE*FS6	A259-	Frame shift at codon 6	Silent phenotype	deletion of A at position 259 of full length cDNA No protein expressed	[11]
rs114063013	N / A	A227G	I23V	Unknown	Unknown	dbSNP build 132
rs116047990	N / A	A279G	K40R	Unknown	Unknown	dbSNP build 132
rs56309653	BCHE*24M	C315T	T52M	Unknown	Reduced activity of enzyme	[10]
rs114355070	N / A	C324A	A55D	Unknown	Unknown	dbSNP build 132
	BCHE28I	T326A	F56I	Suggested pathogenic variant	BChE deficiency	[12]
rs116097205	BCHE*33C	A342G	Y61C	Silent phenotype	Normal protein levels in plasma enzymatically inactive in plasma.	[11]
	BCHE*37S	C353T	P65S	Silent phenotype	Protein expressed at low levels in culture. Poor activity 1-2% to BZ, BTC, ACT, PTA	[11]
rs75995351	N / A	C373A	F71L	Unknown	Unknown	dbSNP build 132

dbSNP ID	Alternative name	Nucleotide change	Effect	Functional studies		Reference
				In vivo	*In vitro*	
rs1799807	Atypical BCHE BCHE*70G	A453G	D98G Silent	Succinylcholine sensitivity Pyridostigmine sensitivity	Dibucaine resistant Predicted to have increased sensitivity to positively charged organophosphates, no diff with uncharged, eg. paraoxon and DFP	[18]
	BCHE*75R	G467C	G103R reduced	Unknown	45% of activity BTC as compared to wt in 293T kidney cells	[19]
	BCHE*90D	A514C	E118D	Silent phenotype	Protein rapidly degraded, no activity in 293T cells	[19]
	BCHE*N96Y	A530T	N124Y	Unknown	Unknown	Australian specific [12]
rs3732880	BCHE*100S	C542T	P128S	Silent phenotype	Reduced protein activity	[10]
	BCHE*106fs	A insertion at 562 of cDNA	N134FS	Silent phenotype	Insertion of a stop codon, 23 amino acids downstream	Australian specific [12]
	Silent 7 BCHE*115D	G591A	G115D	Silent phenotype		[20]
rs55703238	silent-1 BCHE*FS117	-594T	G117fs	Silent phenotype	Deletion of a T causes a shift in the reading frame from Gly117 where GGT(Gly)-GGAG (Gly and one base) to a new stop codon at position 129 No protein expressed	[8]
	BCHE*125F	A619T	L153F	Silent phenotype	Protein expressed at low levels in culture. Poor activity 1-2% to BZ, BTC, and PTA. ACT improved	[11]

dbSNP ID	Alternative name	Nucleotide change	Effect	Functional studies		Reference
				In vivo	*In vitro*	
	H variant BCHE*142M	G669A;	V170M	Succinylcholine sensitivity	Approximately 10% reduced concentration	[19]
	BCHE*170E	T745G	D198E	Silent phenotype	Protein expressed at low levels in culture. Poor activity 1-2% to BZ, BTC, ACT, PTA	[11]
rs114706984	BCHE*184V SC variant	C795T	A212V	Unknown	Plasma sample showed decreased ability to hydrolyse succinylcholine	[21]
	BCHE*198G	A836G	S226G	Silent phenotype	Normal protein levels, enzymatically inactive in plasma,	[11]
	BCHE*199V	C841T	A199V	Unknown	Same protein levels and enzyme activity as wt to BTC in 293T kidney cells	[19]
	BCHE*201T	G847A	A229T	Silent phenotype	Normal protein levels, enzymatically inactive in plasma	[11]
rs28933389	fluoride- 1 BCHE*243M	C972T	T271M	Partial succinylcholine sensitivity	Resistant to inhibition by 0.050 mM sodium fluoride in the in vitro assay	[7]
rs16849700		G1009C	R283D	Unknown	Unknown	dbSNP build 132
rs115624085		A1044G	K295R	Unknown	Unknown	dbSNP build 132
rs114991020		A1094T	T312S	Unknown	Unknown	dbSNP build 132
rs116607681		C1119T	P320L	Unknown	Unknown	dbSNP build 132
rs104893684	BCHE*335P	T1164C;	L335P silent	Silent phenotype	No protein in plasma	[22]
	BCHE*271X	G1055T	E299X	Silent phenotype	Absence of protein and activity in human plasma	[11]

dbSNP ID	Alternative name	Nucleotide change	Effect	Functional studies		Reference
				In vivo	*In vitro*	
	BCHE*328D	C1227A	A355D	Predicted silent phenotype	Unknown	[23]
	BCHE*K355insALU	1306- 1320 Alu insertion	K383Alu	Silent phenotype	Insertion of an ALU element at codon 383	[24]
	BCHE*FS315	ACC- AACC at position 1187	T343fs	Silent phenotype	A insertion at position 1187 of cDNA. Frameshift at codon 343	[25]
rs115129687	BCHE*365R	G1337C	G393R	Silent Phenotype	no protein	[25]
rs35979453		T1357-	F399X	Unknown	frameshift	dbSNP build 132
	BCHE*R386C	C1400T	R413C	Unknown	Unknown	[12] Australian specific
rs28933390	fluoride-2 F-2; BCHE*390V	G1413T	G418V	Patients have succinylcholine sensitivity	Fluoride resistant	[26]
	BCHE*418S	T1497V	F446S	Silent phenotype		[27]
	BCHE 424X	C1515T	R452X	Silent phenotype	Studies suggest non functional protein	Australian specific [12]
	BCHE*E460K	G1622A	E488K	Unknown	Unknown	[12]
	BCHE*465X	A1637T	R493X	Silent phenotype	Premature stop codon 593	[27]
rs115017300		C1652T	R498W	Unknown	Unknown	dbSNP build 132
	BCHE*471R	T1655	W499R	Silent phenotype	Protein expressed at low levels in culture. Poor activity 1-2% to BZ, BTC, ACT, PTA	[11]
	J variant BCHEE497V	A1734T; exon 3	E524V	Succinylcholine sensitivity	33% reduced concentration	[28]
	BCHE*500X	T1745A	528X	Silent phenotype	Absence of protein and activity in human plasma	[11]
rs77586249		T1773C	I538T	Unknown	Unknown	dbSNP build 132
	BCHE*515C	C1787T	R543C	Silent phenotype		[27]

dbSNP ID	Alternative name	Nucleotide change	Effect	Functional studies		Reference
				In vivo	*In vitro*	
	BCHE*518L	A1797T	Q546L	Silent phenotype	Protein expressed at low levels in culture. Poor activity (1-2%) to BZ, BTC, ACT, PTA	[11]
rs116333990		C1815T	S552L	Unknown	Unknown	dbSNP build 132
rs114166903		T1851C	I564T	Unknown	Unknown	dbSNP build 132
rs56325145	BCHE*I3E4-14C	T685-14C 165491308		In linkage with BCHE*115D (known silent mutation)	Unknown, nucleotide substitution in intron 3, 14 nucleotides upstream of exon 4	[23]
rs1803274	K variant BCHE*539K	G1859A	K567T	Succinylcholine sensitivity	66% reduced concentration	[9]
rs2668207		165493724* C/T		Association with lower cholinesterase levels independent of pesticide exposure	Located within intron 2, 4.3-9.5% effect on activity	[29]
rs2048493		165544302 C/G		Association with lower cholinesterase levels independent of pesticide exposure	Located within intron 2, 4.3-9.5% effect on activity	[29]

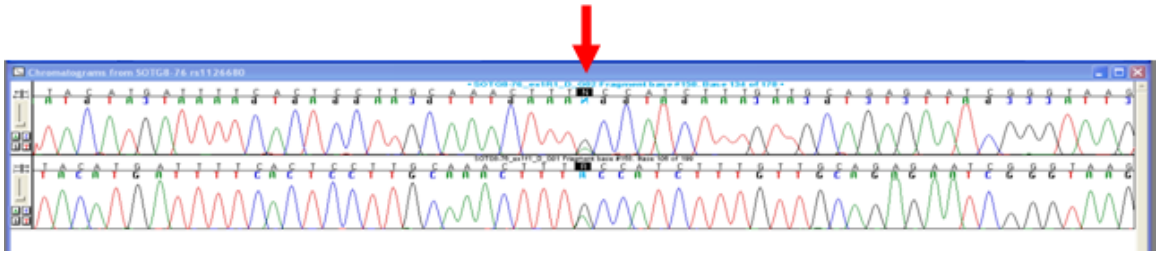
DN: the percent inhibition of activity caused by 0.03umol.L dibucaine BZ: benzoylcholine; BTC; butyrylthiocholine, ACT; acetylthiocholine, PTA; phenylthioacetate

Appendix B: Sequencing results of HRM Analysis

This appendix presents an example of the sequencing data obtained for each unique SNPs identified in this study. In most cases a SNP was deemed confirmed if a second independent PCR was performed and the SNP was observed both the sense and anti-sense directions, or using 2 different primers.

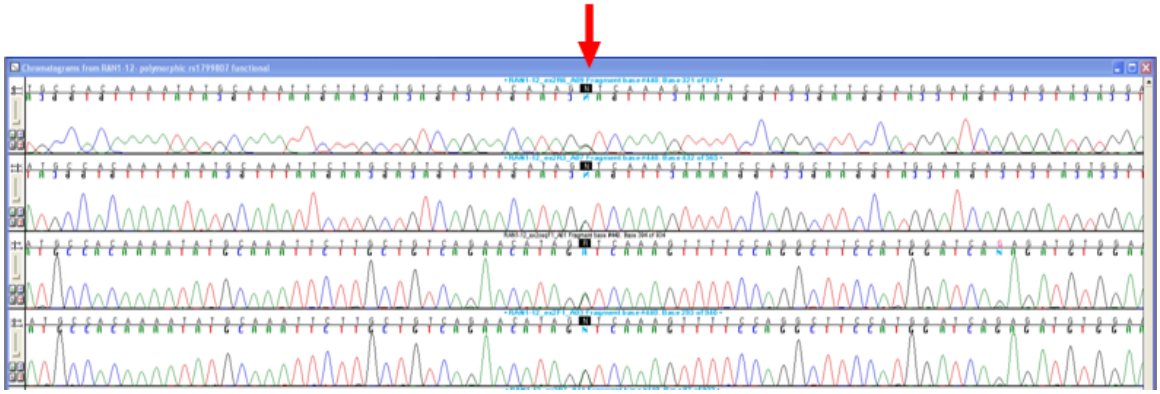
Exon 1 fragment 1 rs1126680 (G/A).

SOTG8-76 (G / A)



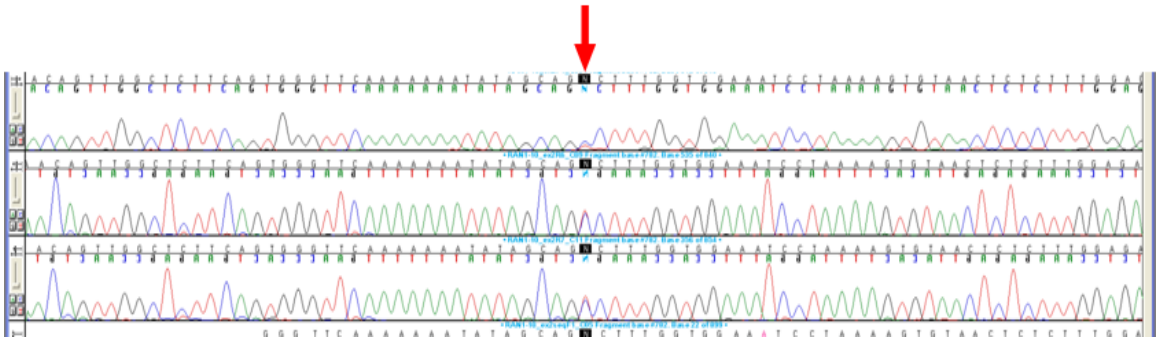
Exon 2 Fragment 3 r1799807 (G/A)

RAN1-21 (G / A)



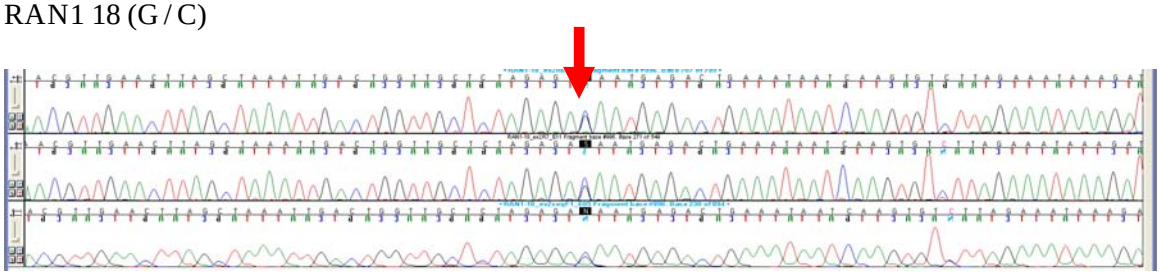
Exon 2 Fragment 4 Novel (C/T)

RAN1-10 (C / T)



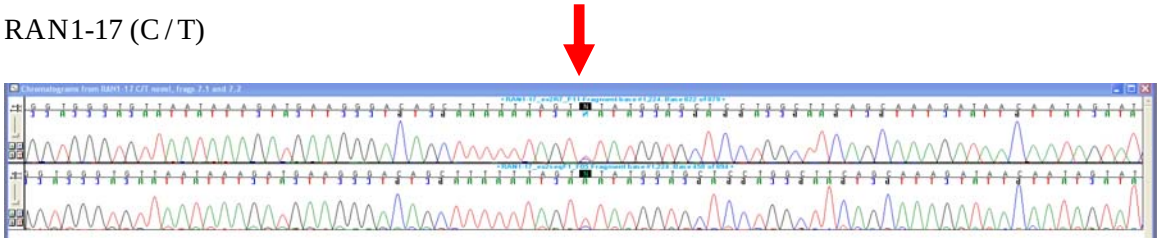
Exon 2 Fragment 6, rs16849700 (G/C)

RAN1 18 (G / C)



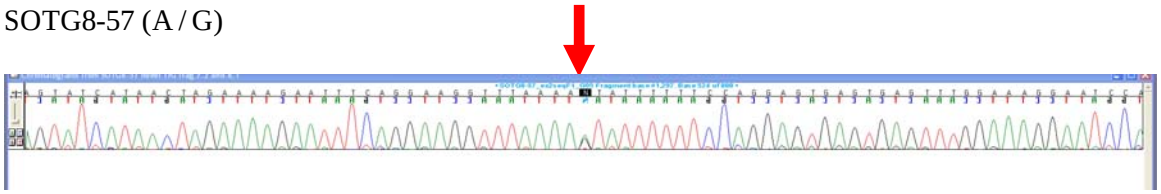
Exon 2 Fragment 7.2 Novel 1 (C/T)

RAN1-17 (C / T)



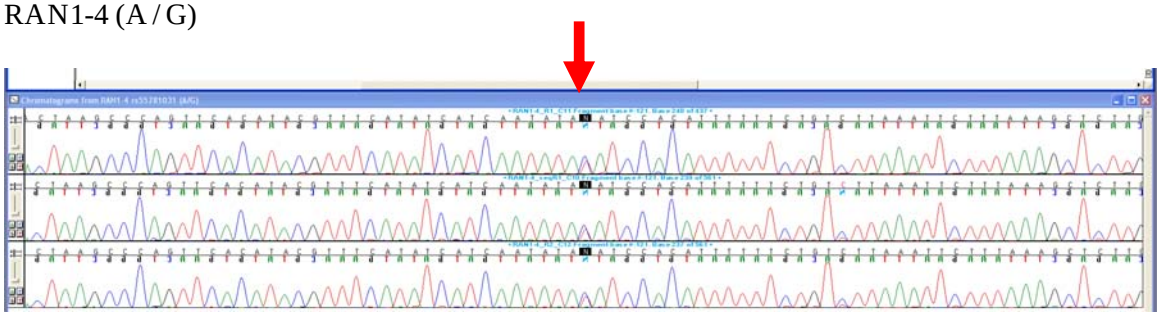
Exon 2 Fragment 7.2 Novel 2. (A/G)

SOTG8-57 (A / G)



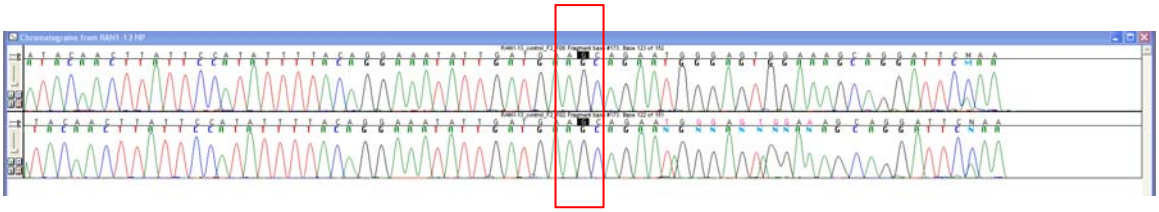
Exon 3 Fragment 11 rs55781031 (A/G)

RAN1-4 (A / G)

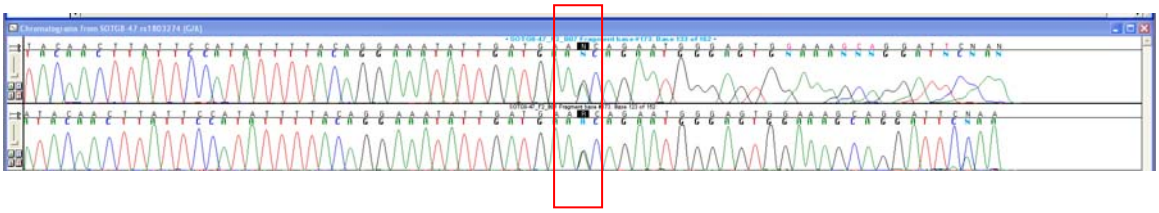


Exon 4 Fragment 13.1, rs1803274 (G/A). All Allele combinations detected.

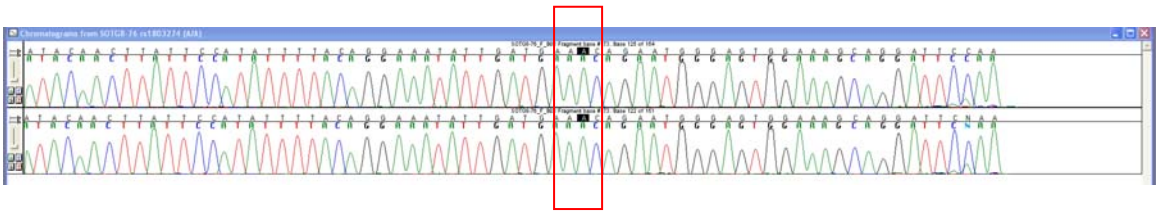
RAN1-13 (G / G).



SOTG8-47 (G / A)

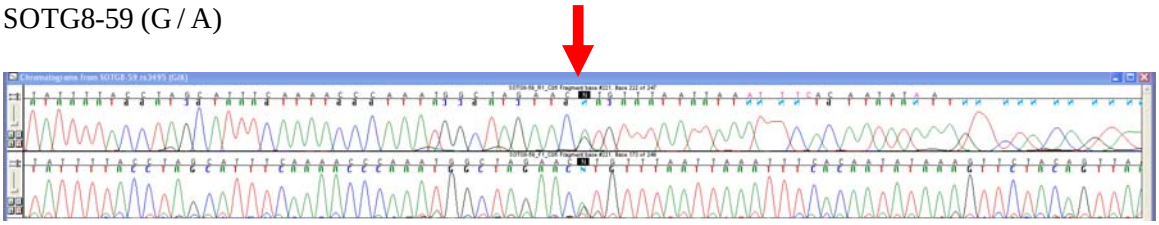


SOTG8-76 (A / A)



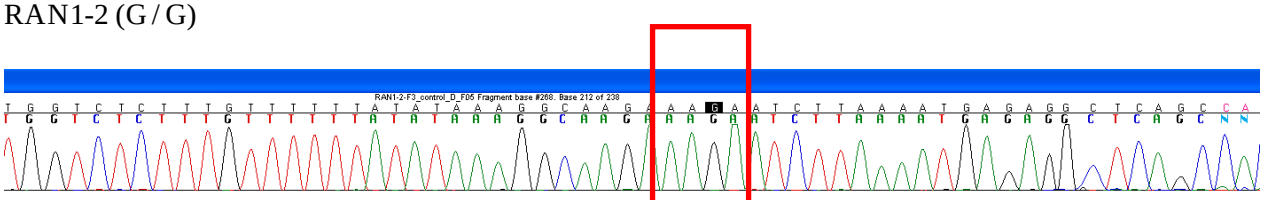
Exon 4 Fragment 14 rs3495 (G / A)

SOTG8-59 (G / A)

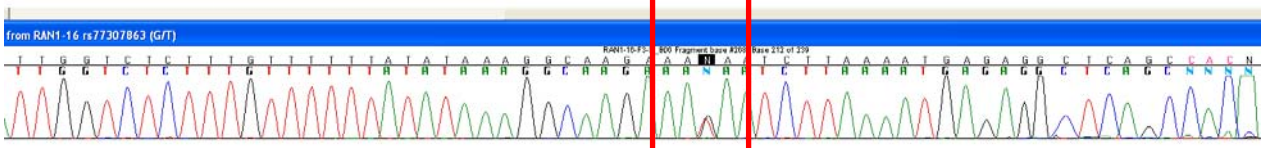


Exon 4 fragment 15, rs77307863 (G / T). All allele combinations found

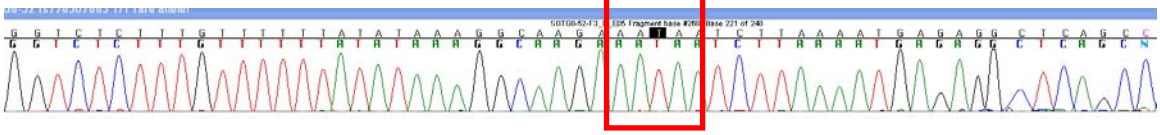
RAN1-2 (G / G)



RAN1-16 (G / T)

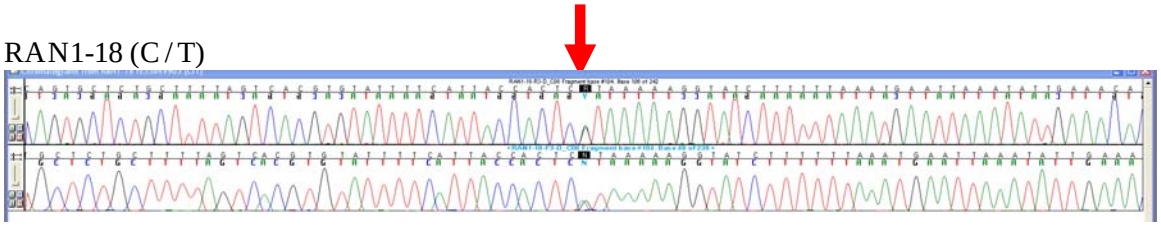


SOTG8-52 (T / T)



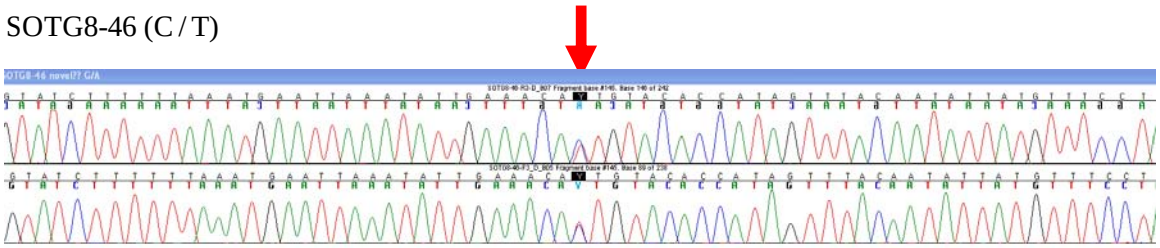
Exon 2 Fragment 15 rs55849903 (C / T)

RAN1-18 (C / T)



Exon 2 Fragment 15 novel 4 (C / T)

SOTG8-46 (C / T)



Appendix C: Genotype frequencies

This appendix describes the genotype frequencies of the SNPs identified in this study as compared to external databases.

Table C1: The genotype frequencies of SNPs of interested as compared to the NCBI and dbSNP 132 database

SNP		**Population (NCBI)**						
	ADF n=51	Caucasian AFD_EUR_PANEL	HapMap-CEU (European) HapMap-CEU	African American AFD_AFR_PANEL	HapMap-YRI HapMap-YRI	Asian AFD_CHN_PANEL	HapMap-HCB HapMap-HCB	HapMap-JPT HapMap-JPT
rs1126680		0.167 (A / G) 0.833 (G / G)	0.017 (A / A) 0.050 (G / A) 0.900 (G / G)	1.000 (G / G)	1.000 (G / G)	1.000(G / G)	1.000 (G / G)	1.000 (G / G)
rs1799807		0.958 (A / A) 0.042 (A / G)	0.983 (A / A) 0.017 (A / G)	1.000 (A / A)	1.000 (A / A)	1.000 (A / A)	1.000 (A / A)	1.000 (A / A)
rs16849700		1.000 (C / C)	1.000 (C / C)	0.870 (C / C) 0.130 (G / C)	0.850 (C / C) 0.150 (G / C)	1.000 (C / C)	1.000 (C / C)	1.000 (C / C)
rs55781031		N / A	N / A	N / A	N / A	N / A	N / A	N / A
rs1803274		0.042 (A / A) 0.250 (G / A) 0.708 (G / G)	0.017 (A / A) 0.317 (G / A) 0.667 (G / G)	0.304 (A / G) 0.696 (G / G)	0.317 (A / G) 0.683 (G / G)	0.167 (A / G) 0.833 (G / G)	0.022 (A / A) 0.356 (A / G) 0.622 (G / G)	0.067 (A / A) 0.267 (A / G) 0.667 (G / G)
rs3495		0.583 (A / A) 0.375 (A / G) 0.042 (G / G)		0.045 (A / A) 0.591 (A / G) 0.364 (G / G)		0.542 (A / A) 0.458 (A / G) 0.000 (G / G)		
rs55849903		N / A	N / A	N / A	N / A	N / A	N / A	N / A
rs77307863		N / A	N / A	N / A	N / A	N / A	N / A	N / A

Appendix D: Population descriptions

Table D1: Summary of populations and number of individuals

Population	Approved individual DNA samples	Approved Individual cell cultures
Yoruba in Ibadan, Nigeria [NRI]	220	220
Han Chinese in Beijing, China [CHB]	162	162
Japanese Tokyo, Japan, [YPT]	131	131
CEPH collection [CEU]	180	180

A brief description of the Hap Map (HapMap) populations is provided below. Additional information can be found at http://hapmap.ncbi.nlm.nih.gov/citinghapmap.html.en

Yoruba in Ibadan, Nigeria (YRI)
These samples were collected in a particular community in Ibadan, Nigeria, from individuals who identified themselves as having four Yoruba grandparents. Including the name of the city and country where these particular Yoruba samples were collected also reinforces the point that the sample set does not necessarily represent all Yoruba people, whose population history is complex. These samples should not be described merely as "African," "Sub-Saharan African," "West African," or "Nigerian," since each of those designators encompasses many populations with many different ancestral geographies.

Japanese in Tokyo, Japan (JPT)
These samples were collected in the Tokyo metropolitan area, from people who came from (or whose ancestors came from) many different parts of Japan. These samples are representative of the majority population in Japan, as it included samples from people whose grandparents were all from Japan.

Han Chinese in Beijing, China (CHB)
Samples were collected from individuals living in the residential community at Beijing Normal University who were self-identified as having at least three out of four Han Chinese grandparents. Although individuals of Beijing University were from many different parts of China, this set of samples was not drawn to be representative of all Han Chinese people.

CEPH (Utah Residents with Northern and Western European Ancestry) (CEU)
These samples were collected from people living in Utah with ancestry from northern and western Europe. The term "CEPH" stands for the Centre d'Etude du Polymorphisme Humain, the organisation that collected these samples in 1980. Because the importance of precision in assigning group membership to prospective donors based on ancestral geography was not well appreciated in 1980, it is unclear how accurately these samples reflect the patterns of genetic variation in people with northern and western European ancestry. These samples should not be described as "European," nor seen as representing people with ancestry from other parts of Europe (e.g., southern or eastern Europe). The samples also should not be described as "Caucasian," a term that carries racial overtones, and that technically refers only to people from the area between the Black and Caspian seas

The “panel” populations are described in detail at:
http://ccr.coriell.org/Sections/Collections/NHGRI/hapmap.aspx?PgId=266&coll=HG

AFD_EUR_PANEL
These 24 samples from the Coriell Cell Repository are primarily of European American descent. Samples consist of 23 unrelated CEPH parents selected by the SeattleSNPs Program for Genomic Applications, plus one sample (NA17201) from Coriell's human variation panel of 50 Caucasians (HD50CAU).

AFD_CHN_PANEL
These 24 samples of Chinese descent from the Coriell Cell Repository were selected from the Han People of Los Angeles Panel of 100 (HD100CHI).

AFD_AFR_PANEL
These 23 samples of African American descent from the Coriell Cell Repository were selected from the human variation panel of 50 African Americans (HD50AA).

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19. ABSTRACT

Genetic variation in the plasma enzyme butyrylcholinesterase (BCHE) affects the response of humans to xenobiotic agents. Mutations in *BCHE* are responsible for the majority of cases of prolonged apnea following the administration of succinylcholine. In addition, genetic variation in *BCHE* is linked to sensitivity to both organophosphate and carbamate compounds, including the deployment related drugs pyridostigmine, physostigmine, heptyl physostigmine and SDZ-ENZ 713. The study described in this report successfully screened the four coding regions (and surrounding intronic regions) of *BCHE* for both novel and known polymorphisms. High Resolution Melt (HRM) and sequencing procedures revealed 12 different genetic polymorphisms, and 35 / 51 individuals were shown to carry at least one *BCHE* polymorphism. Eight of the polymorphisms had been documented previously, two of which have been reported in individuals with succinylcholine and pyridostigmine sensitivities. Of the four novel polymorphisms found, two are predicted to change an amino acid in *BCHE*. This study has demonstrated clearly that unique and important functional genetic variation may be found and there may be a small subset of individuals in the ADF with enhanced sensitivity to succinylcholine and and organophosphate and carbamate compounds.