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THE CHOLINESTERASES: ANALYSIS BY PHARMACOGENOMICS IN MAN

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Abstract

We have undertaken a study on variations in cholinesterase (ChE) genes in relation to cardiovascular function and the metabolic syndrome. Peripheral and central nervous system control of cardiovascular (CV) function mediated through cholinergic pathways is critical in homeostatic maintenance of blood pressure and responsiveness to stress. For acetylcholinesterase (AChE; EC 3.1.1.7) our focus is to identify single nucleotide polymorphisms (SNPs) in the gene that are linked to cardiovascular function. For butyrylcholinesterase (BChE; EC 3.1.1.8) we examined whether BChE activity correlated with parameters of the metabolic syndrome and cardiovascular function. ChE can be found in whole blood enabling a characterization of biochemical phenotype in addition to correlating genotype with phenotypic physiologic responses. Analysis of enzymatic activity was determined spectrophotometrically in blood samples from twin and other subject registries. Correlation analysis revealed significant relationships between enzyme activity and certain CV endpoints. Linkage analysis with data from a dizygotic twin set showed a suggestive linkage at the *BChE* locus and statistical analysis revealed a high correlation between BChE activity and variables associated with cardiovascular risk and the metabolic syndrome. Pattern of within-pair twin correlations by zygosity and the ACE model-fitting findings suggest the major source of this variation (65%) is attributable to an additive genetic component. To date nineteen SNPs have been identified by the re-sequencing of *AChE* including 4 nonsynonymous coding SNPs (cSNPs).

Introduction

Peripheral and central nervous system control of CV function mediated by cholinergic neurotransmission is critical in homeostatic maintenance of blood pressure and responsiveness to exercise, postural alterations, and stress as demonstrated in animal models [1] [2]. Central cholinergic pathways in the spinal cord and higher brain centers modulate CV responses to influence basal blood pressure and baroreflex pressor responses via the cholinergic neurotransmitter, acetylcholine (ACh). Increased arterial pressure activates baroreceptors in the aortic arch and carotid sinus initiating afferent impulses to the vasomotor center (VMC) in the medulla. Stimulation of the vagus nerve in the VMC increases vagal parasympathetic release of ACh causing change in potential in the sinoatrial node (pacemaker cell of the heart) and resulting in a decrease in heart rate that diminishes cardiac output [3]. In turn peripheral responses are controlled by nicotinic receptors in ganglia and the adrenal medulla as well as

muscarinic receptor sites in postganglionic parasympathetic systems. Rapid ACh turnover is essential in the regulation of cholinergic neurotransmission and cholinergic control of CV function. AChE plays a key role in modulating cholinergic transmission by catalyzing the rapid hydrolysis of ACh. In the cholinergic control of CV function, it has been demonstrated using neostigmine, an inhibitor of AChE, that the rate of ACh degradation plays a greater role in determining the properties of transduction from vagus nerve activity to heart rate rather than the concentration of available ACh at the neuroeffector junction [4].

AChE and BChE are closely related belonging to the superfamily of α/β-hydrolase fold proteins but differing in their substrate and inhibitor specificity. The mammalian *AChE* is encoded within 7.5 kilobases in the human genome and is located on chromosome (Ch) 7q22 [5]. *BChE* spans over 70 kilobases and is located on Ch 3q26 [6]. AChE and BChE share about 54% amino acid sequence identity and are present in most tissues, although BChE is usually more abundant than AChE except in brain and muscle [7].

Human Subjects

San Diego

Participants included 80 unrelated subjects recruited by advertisement and referral from a general population in the San Diego area. Twin subjects were recruited by access to a population birth record-based twin registry [8], as well as by newspaper advertisement. We recruited n = 478 twin individuals: n =167 monozygotic (MZ) pairs, and n = 72 dizygotic (DZ) pairs. Twin zygosity assignment was based on self-identification, with further confirmation by the presence or absence of heterozygosity at the TH gene microsatelite [9]. Twin ages were 14 to 84 years. Family histories for hypertension [in a first-degree relative before the age of 60 yr] were as follows: 111 pairs were positive (one or both parents); 102 pairs were negative; and 26 pairs were indeterminate/unknown. There were 429 individuals that were normotensive, and 49 were hypertensive. Subjects were volunteers from urban southern California and each subject gave informed, written consent; protocol was approved by the Institutional Review Board.

Australia

Participants in this study were described in a previous report [10]. They completed a questionnaire in 1989 and a telephone interview in 1993–1994, and provided a blood sample in 1993–1996. All participants were twins, born between 1903 and 1964. Zygosity was determined from responses to questions about physical similarities and the inability of others to tell them apart, supplemented by blood group information and (for pairs included in linkage studies) extensive microsatellite genotyping. Participants gave informed consent to the questionnaire, interview, and blood collection; studies were approved by appropriate Ethics Review Committees.

Results

In vivo cholinesterase activity from whole blood was ascertained for the San Diego panel of 80 unrelated and 478 twin samples. AChE and BChE enzyme activities in whole blood were determined using 1mM AChE, yielding nearly maximal activity of AChE but less then maximal activity of BChE. The AChE activity mean and standard deviation (SD) for the unrelated samples was 42.9 ΔA/min/ul (7.2) and the BChE activity mean was 9.8 ΔA/min/ul (2.4). AChE activity mean and (SD) for the twin samples was 42.0 ΔA/min/ul (7.2) and the BChE activity mean was 9.8 ΔA/min/ul (2.3). BChE activity mean ascertained for the Australian panel of 2192 twins (569 monozygotic and 527 dizygotic twin pairs) was 15.3 ΔA/min/ul (4.0). Linear regression and correlation studies using GraphPad Prism software (Graphpad Inc., San Diego,

CA) on the intra-twin monozygotic and dizygotic pairs revealed a significant shared relationship of ChE activity particularly within the intra-twin monozygotic pairs.

Exploratory analysis on the Australian twin panel using SPSS, Ver. 13 (SPSS Inc.) revealed significant correlation between BChE activity and certain CV risk factors and variables associated with the metabolic syndrome (Table 1). More detailed analysis of the source of variation was performed using the Mx program, Ver. 1.50 [11] with results indicating that 65% of the variation in serum BChE was attributable to additive genetic components (Table 2) [12]. The results of linkage analysis on 368 dizygotic twin pairs revealed two peaks with LOD scores of 3.0 or greater on Ch 3 and 5 [12]. The peak on Ch 3 at GATA3H01 (168.7 Mb from the p-terminal end) coincided with the *BChE* locus, and the peak on Ch 5 at GATA12G02 (82.0 – 114.1 Mb) may encompass up to 70 genes.

Discussion

The observed ChE activity values covered a 3-fold range with an approximate Gaussian distribution for both the San Diego and Australian panels. Regression and correlation analysis by twin pairwise zygosity revealed a significant intra-twin pair relationship for both twin panels particularly in the case of BChE activity. We have shown significant correlations between ChE activity and variables associated with CV disease and the metabolic syndrome. Linkage analysis of the Australian panel identified the appropriate region of the *BChE* locus. Given the high heritability of BChE activity, defining variation(s) in this area could potentially reveal causal SNPs that might affect the protein product either through gene expression and/or enzyme activity. Searching for and identifying variations in a gene(s) that may encode a protein (e.g. transcription factors) affecting the *BChE* locus will be labor intensive. Analysis of this region may be best conducted when this linkage result is replicated or candidate genes in this area identified.

Our AChE polymorphism study is on-going, but initial findings have revealed significant correlations between enzyme activity and certain CV endpoints such as systolic blood pressure.

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***Table 1**

Correlations between plasma cholinesterase activity (adjusted for method variation) and variables related to cardiovascular risk.[a]

	Males	Females
Age	0	0.24[b]
Apo (apolipoprotein) A1	0	−0.13[c]
Apo A2	0.27[b]	0.06
Apo B	0.30[b]	0.34[b]
Apo E	0.28[b]	0.27[b]
Cholesterol (total)	0.28[b]	0.32[b]
HDL-C	−0.13[c]	−0.23[b]
LDL-C (calculated)	0.19[c]	0.29[b]
Triglycerides (log)	0.33[b]	0.34[b]
Urate	0.19[c]	0.25[b]
BMI (body mass index)	0.26[b]	0.28
BP (blood pressure), systolic	0.28[b]	0.24[b]
BP, diastolic	0.32[b]	0.23[b]
GGT (log)	0.27[b]	0.24[b]
AST (log)	016[c]	0.11[c]
ALT (log)	022[b]	0.17[b]
Glucose	0.07	0.15[c]
Insulin (log)	0.12[d]	0.18[b]
Alcohol intake (previous week)	0.06	−0.11[c]
Smoker (Yes/No)	−0.01	−0.04

[a] Triglyceride, glucose, & insulin values adjusted for reported time of last meal.

[b–d] *P* values are calculated on the highlyconservative assumption that the effective number of cases is one half the actual number to allow for any effects of the twin status of the participants. Unless indicated, the correlation is not significant:

[b] $P < 0.0001$;

[c] $P < 0.01$;

[d] $P < 0.05$.

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***Table 2**

Correlation of BChE Activity and the ACE Model

A.	**MZF**	**MZM**	**DZF**	**DZM**	**DZOS**
N of pairs	408	158	224	77	217
Correlation of plasma cholinesterase activity	0.73	0.65	0.40	0.44	0.43

B.						**Percentage of variance due to:**		
Models	**−2LL**	**df**	**$\Delta\chi^2$**	**Δdf**	**p**	**A**	**C**	**E**
ACE	7922.5	2157	-	-	-	65 (50–75)	7 (0–21)	28 (25–32)
AE	7923.5	2158	1.01	1	0.315	72	-	28
CE	8007.6	2158	85.2	1	$<10^{-18}$	-	58	42

MZF = monozygotic female
DZF = dizygotic female
LL = log likelihood
MZM = monozygotic male
DZM = dizygotic male
df = degrees of freedom
DZOS = dizygotic other sex

A. Pairwise similarity of twin plasma cholinesterase results, by zygosity, after adjustment for method variation, sex and sex-specific age effects.

B. Results of fitting the data to models containing sources of variation due to: A, additive genetic effects, C, shared-environmental effects, and E, non-shared environmental effects. The 95% confidence intervals for estimates of A, C and E under the ACE model are also shown. Comparison of the goodness-of-fit between the data and the models shows that the AE model is not significantly worse than the full ACE model, but that the CE models is strongly rejected.

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