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Butyrylcholinesterase: Association with the Metabolic Syndrome and Identification of 2 Gene Loci Affecting Activity

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Background: **Plasma cholinesterase activity is known to be correlated with plasma triglycerides, HDL- and LDL-cholesterol, and other features of the metabolic syndrome. A role in triglyceride metabolism has been proposed. Genetic variants that decrease activity have been studied extensively, but the factors contributing to overall variation in the population are poorly understood. We studied plasma cholinesterase activity in a sample of 2200 adult twins to assess covariation with cardiovascular risk factors and components of the metabolic syndrome, to determine the degree of genetic effects on enzyme activity, and to search for quantitative trait loci affecting activity.**

Methods and Results: **Cholinesterase activity was lower in women than in men before the age of 50, but increased to activity values similar to those in males after that age. There were highly significant correlations with variables associated with the metabolic syndrome: plasma triglyceride, HDL- and LDL-cholesterol, apolipoprotein B and E, urate, and insulin concentrations; γ-glutamyltransferase and aspartate and alanine aminotransferase activities; body mass index; and blood pressure. The heritability of plasma cholinesterase activity was 65%. Linkage analysis with data from the dizygotic twin pairs showed suggestive linkage on chromosome 3 at the location of the cholinesterase (*BCHE*) gene and also on chromosome 5.**

Conclusions: **Our results confirm and extend the connection between cholinesterase, cardiovascular risk factors, and metabolic syndrome. They establish a substantial heritability for plasma cholinesterase activity that might be attributable to variation near the structural gene and at an independent locus.**

Plasma cholinesterase (EC 3.1.1.8; butyrylcholinesterase, pseudocholinesterase) has been studied extensively because of the associations between low activity and delayed metabolism of the muscle relaxant succinylcholine *(1–3)*, and because it is a marker of exposure to organophosphate chemicals *(4)*. The physiologic function of plasma cholinesterase remains unresolved, however, and it is not clear whether the known sequence variations in the butyrylcholinesterase gene (*BCHE*)[5] account for all of the genetic variations in the population. The causes of variation take on an increased importance in the light of reports that cholinesterase is involved in triglyceride metabolism; that its activity is correlated with plasma LDL-cholesterol (LDL-C),[6] HDL-cholesterol (HDL-C), and triglyceride concentrations *(5–9)*; and that it shows significant associations with components of the metabolic syndrome *(10)*.

Although most of the evidence points to plasma cholinesterase acting as a marker (rather than a cause) of

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[5] Human genes: *BCHE*, butyrylcholinesterase; *CHE2*, cholinesterase (serum), 2.

[6] Nonstandard abbreviations: LDL-C and HDL-C, LDL- and HDL-cholesterol, respectively; GGT; γ-glutamyltransferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; cM, centimorgan(s); and LOD, logarithm of odds.

cardiovascular risk, metabolic syndrome, or diabetes, its proposed role in triglyceride metabolism might mean that natural variations in cholinesterase activity contribute to variations in risk. In addition to associations with cardiovascular risk factors, the metabolic syndrome, and possibly, type 2 diabetes *(11, 12)*, there are conflicting reports of a causative role for the low-activity K variant in Alzheimer disease *(13–20)*. The biomedical importance of plasma cholinesterase is therefore wider than the pharmacogenetic phenomenon of delayed metabolism of succinylcholine or other drugs. The gene for plasma cholinesterase (*BCHE*) is on chromosome 3, at bp 166973394–167037952, and many comparatively rare genetic variants leading to low activity are now known *(21)*. In addition to the known gene variants leading to low activity, a proportion of individuals show an additional cholinesterase band on electrophoresis and increased enzyme activity. This occurs with a frequency of 8% to 10% among Europeans *(22)*, and is ascribed to the effects of another gene, cholinesterase (serum), 2 (*CHE2*), whose location is uncertain *(23)*. A recent report *(24)* has shown that the increased activity is not attributable to increased cholinesterase protein concentration, but rather to increased specific activity.

Variations in plasma cholinesterase activity are therefore associated with variations in risk factors for cardiovascular and metabolic disease and are at least partly under genetic control. We studied variations in plasma cholinesterase activity in a sample of adult twins to assess the covariation with cardiovascular and metabolic disease risk factors, the magnitude of genetic effects on variation within the population, and the location of genes that determine or modify cholinesterase activity.

Participants and Methods

Participants in this study were described in a previous report *(25)*. 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, but in some cases only one member of a twin pair provided blood. 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, and the studies were approved by appropriate Ethics Review Committees.

We collected blood samples from 1134 men and 2241 women. Plasma and serum were separated and stored at −70 °C until analyzed. Immediately before blood collection, participants completed a brief questionnaire reporting their alcohol consumption over the previous week. They also reported the time of their last meal, and the time of blood collection was noted. At the same visit, their height and weight were measured. Body mass index was calculated from weight and height as weight (kg)/[height (m)]^2. Systolic and diastolic blood pressures were measured, with the participants sitting, by use of an automated blood pressure recorder (Dynamap 845 Vital Signs Monitor; Critikon Inc.). The mean of 2 results taken at 1-min intervals was calculated. Blood pressure results were available for 1666 of the participants.

Plasma samples were analyzed for cholinesterase activity by measurement of the absorbance increase at 412 nm on addition of the substrate acetylthiocholine at a final concentration of 0.5 mmol/L, according to the colorimetric method of Ellman et al. *(26)*. 5,5′-Dithio-bis(2-nitrobenzoic acid) at a final concentration of 0.3 mmol/L was used as the chromogenic indicator of thiocholine formation. Samples were either measured in 1-cm optical-path cuvettes in a spectrophotometer (Response; Gilford Instrument Laboratories) for 5 min or in a 0.73-cm optical-path microtiter plate reader (Safire; Tecan Systems Inc.) for 3 min and 35 s. In either case, activity was recorded as a change in the absorbance at 412 nm in 1 min per microliter of serum. Butyrylcholinesterase activity was determined by including the specific acetylcholinesterase inhibitor BW284c51 (1 μmol/L final concentration) in the reaction mixture. Activity was expressed in international units as μmoles of acetylthiocholine hydrolyzed per milliliter of sample per minute.

Serum γ-glutamyltransferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, glucose, triglycerides, and urate were measured by Boehringer Mannheim reagents and methods on a Hitachi 747 analyzer. HDL-C was measured by precipitation of non-HDL lipoproteins with dextran/$MgSO_4$ followed by enzymatic cholesterol assay. Apolipoproteins A-I, A-II, B, and E were measured by immunonephelometry on a Behring nephelometer using Behring reagents. Plasma insulin was measured by RIA (Diagnostic Products Corporation).

Several of the measured variables were log-transformed because their frequency distributions were skewed. All references to serum GGT, AST, ALT, triglycerides, and insulin and to the quantity of alcohol consumed per week are to the log-transformed values unless specified otherwise. LDL-C was calculated from the total cholesterol, HDL-C, and triglyceride values by the Friedewald equation if triglycerides were <4.5 mmol/L. If the serum triglyceride concentration was above this limit, LDL-C was treated as missing. The samples were not taken in the fasting state, but participants reported the time of their last meal, and the triglyceride, glucose, and insulin results were adjusted for the elapsed time between the last meal and blood collection.

Exploratory analysis was carried out with SPSS, Ver. 13 (SPSS Inc.). Because the participants were twins and therefore not genetically independent, the effective number of individuals for any characteristic with substantial heritability would be less than the actual number of participants, and the significance (but not the magnitude) of correlations may be overestimated. More detailed ex-

amination of the effects of covariates and the sources of variation in cholinesterase was performed with the Mx program, Ver. 1.50 *(27)*, which is designed for analysis of twin and family data and overcomes this problem.

We performed a genome-wide linkage analysis for loci affecting plasma cholinesterase activity on the dizygotic twin pairs. DNA was extracted from blood or buccal swabs according to standard procedures *(28)*. Genotype data were assembled from 4 genome scans that had been done previously for other projects by the Mammalian Genotyping Service (Marshfield, WI), Leiden University Medical Centre (Leiden, The Netherlands) *(29)*, Sequana Inc., and Gemini plc (United Kingdom). Pedigree structures for each scan were examined to identify inconsistencies between the genotypic data and pedigree relationships. Once any discrepancies were resolved, data for the 4 scans were merged and then checked again for pedigree errors. The combined genome scan data included 458 markers that were typed in 2 or more scans, which were included separately on the genetic map for the scan, separated by a very small distance [0.001 centimorgans (cM)]. The consistency of genotype information among these 458 markers was checked via cross-tabulations of allele calls between different scans. Markers with genotypic data inconsistent between different genome scans were removed from further analysis. Map positions were in Kosambi cM, estimated via locally weighted linear regression from the National Center for Biotechnology Information build 34.3 physical map positions and from published deCODE and Marshfield genetic maps. The procedures for combining and checking the genotype data and for the linkage analysis are described in Ref. *(30)*.

Trait-specific empirical genome-wide suggestive and significant thresholds were calculated through use of 1000 gene-dropping simulations as described by Abecasis et al. *(31)*. Details are given in Ref. *(30)*. The empirical genome-wide thresholds for suggestive or significant linkage *(32)* were defined as the thresholds for which we observed, on average, 1 or 0.05 peaks per simulation with a logarithm of odds (LOD) score at or above the threshold, respectively. After the initial simulation results, which produced unusually high significance thresholds, we applied winsorization to reduce the impact of outliers on the linkage analysis *(33)*. This was done by setting values for all cholinesterase residuals greater than 3 SD above or below the mean to values equivalent to 3 SD above or below the mean, respectively. Linkage analysis and the results of simulations to determine the genome-wide empirical *P* value are reported for the winsorized dataset.

Results

Cholinesterase results were obtained for 2237 samples, including 564 complete monozygotic and 518 dizygotic twin pairs. The mean (SD) activity was 954 (272) U/L, with a nonparametric 95% range of 505-1539 U/L. Because the method was changed from measurement of absorbance with a spectrophotometer to use of a microtiter plate reader during the course of the study, we examined the running mean of the results across time and also calculated and graphed the cumulative sum of the differences from the mean (cusum) *(34)*. This plot (see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol52/issue6/) showed evidence of 5 periods with different mean values during the analysis of the samples, which was conducted over 15 months from January 2003 to April 2004. To reduce the effects of this analytical variation, dummy variables were created for the second to fifth periods and included as definition variables in an analysis using Mx. The results are summarized in Table 1 of the online Data Supplement. Significant effects of variation in the method over time were confirmed, together with significant effects of sex, and of age in women but not men.

Exploratory analysis of the correlations between cholinesterase activity, adjusted for variation over time, and other variables gave the results shown in Table 1. There were multiple significant correlations with known cardiovascular risk factors and variables associated with the metabolic syndrome. There was no significant correlation, in either men or women, with alcohol intake or smoking.

Table 1. Correlations between plasma cholinesterase activity (adjusted for method variation) and variables related to cardiovascular risk.[a]

	Males	Females
Age	0.00	0.24[b]
Apo[e] A1	0.00	−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	0.26[b]	0.28
BP, 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)	0.16[c]	0.11[c]
ALT (log)	0.22[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, and insulin values are adjusted for the reported time since the last meal.

[b–d] *P* values are calculated on the highly conservative assumption that the effective number of cases is one half the actual number, to allow for any effects of the twin status of participants. Unless indicated, the correlation is not significant: [b] $P < 0.0001$; [c] $P < 0.01$; [d] $P < 0.05$.

[e] Apo, apolipoprotein; BMI, body mass index; BP, blood pressure.

The pairwise correlations by zygosity after adjustment for method variation, sex, and age are shown in Table 2 of the online Data Supplement, together with the results of testing models of genetic and environmental sources of variation. We found that 65% (95% confidence interval, 50%–75%) of the variation in plasma cholinesterase activity was attributable to additive genetic effects. Although the model including only additive genetic and nonshared environmental sources of variation fitted the data satisfactorily, a small shared environmental effect cannot be excluded.

The results of linkage analysis on 368 dizygotic twin pairs with genome-scan data are shown in Fig. 1. Two peaks with LOD scores of 3.0 or greater were found, on chromosomes 3 and 5. The empirical significance thresholds determined by simulation on these data were 3.7 (for 1 occurrence in every 20 simulations, genome-wide; $P = 0.05$) and 1.8 (for an average of 1 occurrence per simulation), however; therefore, both peaks must be considered suggestive by the criteria of Lander and Kruglyak *(32)* (expected to occur less than once per genome scan) rather than significant. The peak on chromosome 3 (peak LOD score, 3.00; empirical genome-wide significance, $P = 0.241$) at GATA3H01 (172.3 cM, or 168.7 Mb from the p-terminal end of the chromosome) coincided with the location of the *BCHE* gene (167.0 Mb). The peak on chromosome 5 (peak LOD score, 3.34; empirical genome-wide significance, $P = 0.135$) was at GATA12G02 (106.1 cM, or 91.0 Mb), and the 1-LOD interval ran from 98.4 to 123.1 cM (82.0–114.1 Mb). No other linkage peaks exceeded the suggestive threshold of LOD score (>1.8).

Discussion

The observed plasma cholinesterase values covered a 3-fold range, from 505 to 1539 U/L, and the mean values varied by sex and, for women, by age (see Table 1). We confirmed that there are many significant correlations between cholinesterase activity and variables associated with cardiovascular disease, metabolic syndrome, and diabetes. We also established a significant degree of heritability for plasma cholinesterase activity in the general population and identified 2 chromosomal locations that contribute to this heritability. We detected variation in the method across time by cusum analysis to define the points at which changes had occurred. This permitted adjustment of the results for method variation as well as for age and sex effects. This was done before assessment of correlations with other variables, sources of variation, and linkage. Residual analytical variation would attenuate the correlations (which were nevertheless highly significant) and inflate either nonshared or shared environmental sources of variation. Because both samples from twin pairs were generally analyzed at the same time, estimates of shared environment are more likely to be affected, and we did in fact find that if method variation was not taken into account the shared environmental effects were significant (data not shown). Because linkage analysis is based on within-pair differences, it will not be

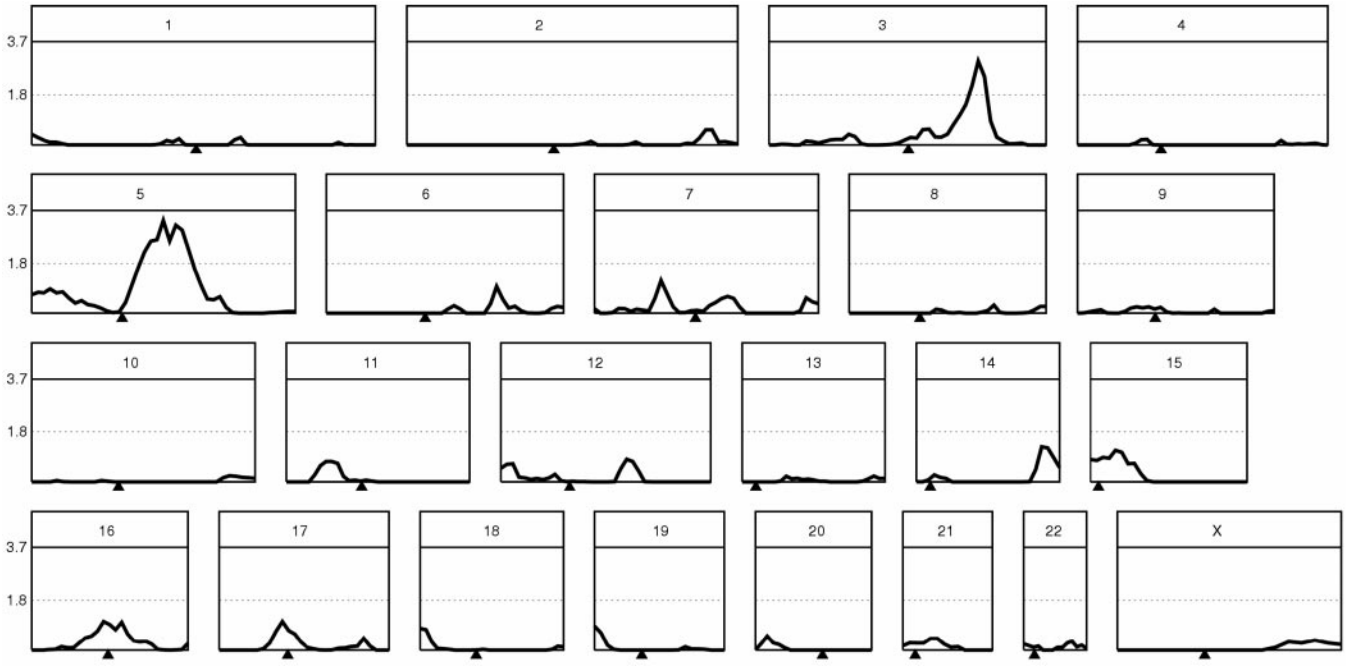

Fig. 1. Results of linkage analysis for the detection of loci affecting plasma cholinesterase, after adjustment for method variation, sex, and sex-specific age effects.

For each chromosome panel (chromosome number listed at the *top*), the LOD score is plotted on the *y axis* and the genetic distance from the p-terminal end of the chromosome on the *x axis*.

substantially affected by between-batch analytical variation.

As noted above, several previous investigators have found significant relationships between cholinesterase activity and triglycerides, HDL-C, and LDL-C *(5, 6, 8, 9)*. A recent report *(10)* has extended this to a wider range of variables associated with the metabolic syndrome. Our results agree with this, and it is now clear that plasma cholinesterase clusters with a wider range of characteristics, including body mass index, apolipoprotein concentrations, insulin, liver enzymes, and blood pressure. Although many of these associations can be related to lipid or lipoprotein metabolism, the association with blood pressure does not easily fit into a concept of cholinesterase as an esterase involved in the metabolism of triglycerides and VLDL. There seems to be a broader involvement of cholinesterase with the metabolic syndrome, extending to normal variation between people in their blood pressure and (in the extreme case) to hypertension. The associations with AST, ALT, and GGT activities, which are known to be associated with insulin resistance *(35)* and increased in the metabolic syndrome *(36)*, probably reflect an association between cholinesterase activity and the metabolic syndrome, of which fatty liver is a feature.

One important issue arising from these findings, and previous similar ones, is whether higher cholinesterase activity is a cause or a consequence of dyslipidemia and metabolic syndrome. Several published reports of animal *(7, 37, 38)* or human *(9)* studies are relevant, but unfortunately, the results do not give a clear answer. Interventions that primarily increase or decrease lipids tend to have the same effect on cholinesterase, but inhibition of cholinesterase activity in vivo has been shown to decrease lipid concentrations. For example, mice with streptozotocin-induced diabetes showed increased serum LDL, triglycerides, and cholinesterase *(7)*, which decreased with insulin treatment, suggesting that the insulin-deficient state led to changes in cholinesterase activity. However, in the same study, inhibition of cholinesterase activity with tetraisopropylpyrophosphoramide led to a decrease in serum LDL and triglyceride concentrations. These results appear to place cholinesterase in the chain of events leading to changes in lipid values, rather than being a consequence. If this is the case, and particularly if this reasoning also applies to the other features of the metabolic syndrome that showed significant correlations with cholinesterase activity, then the sources of variation in cholinesterase activity between people take on an added significance. Blood pressure, as well, might be influenced causally by cholinesterase because the cholinesterase substrate acetylcholine causes vasodilation when infused into the human vasculature, triggering nitric oxide release via endothelial muscarinic cholinergic receptors *(39)*. Thus, an excess of cholinesterase activity in the metabolic syndrome could also adversely affect endothelial function and ultimately increase blood pressure.

The pattern of within-pair twin correlations by zygosity and the model-fitting results, shown in Table 2 of the online Data Supplement, suggest contributions to variation from additive genetic effects and nonshared environmental effects. Some shared environmental effects, possibly related to batch effects on the measurement of cholinesterase activity, cannot be excluded but are estimated at only 7% of the total variance. The genetic effects are substantially greater, at 65% (95% confidence interval, 50%–75%). Therefore, the major source of variation is genetic, and the location of the relevant genes can be assessed from the linkage analysis carried out on a subset of the dizygotic pairs.

This linkage analysis revealed 2 suggestive peaks, on chromosomes 3 and 5. The localization of a gene or genes whose variation affects plasma cholinesterase activity to chromosome 3 is to be expected, as this is the location of the *BCHE* gene itself. Nevertheless, it is gratifying to be able to identify linkage in the appropriate region, and this shows that linkage analysis for genes affecting quantitative traits can be done with comparatively small numbers of sibling pairs. Several variants of the *BCHE* gene that affect cholinesterase activity are already known; these include the common K variant, which produces an ~20% decrease in activity, and the much rarer variants with major effects. Given the high heritability of cholinesterase activity and the linkage peak on chromosome 3 at the *BCHE* locus, it is likely that there are other sequence variations in or near the *BCHE* gene that affect activity, and a search using single-nucleotide variations and haplotype analysis will help to define them.

The chromosome 5 linkage peak is less readily explained. There are ~70 genes or possible genes in the region indicated by this peak, but none has obvious relevance to plasma cholinesterase activity. A search through this region with current techniques would be time-consuming and expensive and not justified until the linkage result in this region has been replicated. One possibility that should be considered is that this region of chromosome 5 contains a gene for a protein that binds to cholinesterase and increases its activity *(24)*; efforts to identify the nature of this protein by conventional biochemical means have been unsuccessful to date. Another possibility is that genes in this region affect the risk of insulin resistance and metabolic syndrome and that the linkage for cholinesterase activity is a consequence of this. However, other linkage studies on the metabolic syndrome *(40, 41)* do not support linkage to this region of chromosome 5, nor do our own results on metabolic syndrome components using this cohort of persons [Ref. *(42)* and our unpublished data].

In summary, our results emphasize the relevance of cholinesterase activity to cardiovascular risk and extend knowledge of its sources of variation. Further characterization of the chromosome 3 *BCHE* locus might elucidate the effects of known variants against the overall heritability and the linkage peak. Such investigations might also

determine whether genetic causes of low activity lead to lower values for the cardiovascular risk factors, a finding that would clarify the practical significance of plasma cholinesterase for cardiovascular risk and cardiovascular disease. The larger task of detailed examination of genes under the chromosome 5 peak must await identification of candidate genes in this region, or replication of our linkage result.

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