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EDITORIAL COMMENT

Genetic Polymorphisms of Hepatic Lipase and Cholesteryl Ester Transfer Protein, Intermediate Phenotypes, and Coronary Risk

Do They Add Up Yet?*

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Hepatic lipase (HL) and cholesteryl ester transfer protein (CETP) are key enzymes of plasma lipid/lipoprotein metabolism (1,2).

BACKGROUND

Hepatic lipase. The HL gene (or *LIPC*), located on chromosome 15 (15q21-23), spans over 60 kb, contains 9 exons and 8 introns (3), and has substantial homology with lipoprotein lipase (LPL). Together with endothelial and pancreatic lipases, they process ≈150 g of dietary triglyceride daily (1,2). In contrast to LPL, the synthesis, location, and function of HL are restricted to the liver. The LPL is responsible for the first phase of lipolysis of very-low density lipoproteins (VLDL) and chylomicrons. As particle size decreases, HL plays an increasing role; HL also hydrolyzes core triglycerides and phospholipids in HDL_2 and HDL_3 (high-density lipoprotein), being most efficient for Lp(AI, AII)-containing particles. The HL activity negatively correlates with HDL cholesterol (HDL-C) levels.

CETP. The CETP gene, located on chromosome 16 (16q21) (4), specifies a 66 to 74 kDa hydrophobic glycoprotein, which is expressed in liver, spleen, adipose tissue, kidney, and skeletal muscle (1,2). The CETP is localized primarily on larger, Lp(AI)-containing HDL particles, and its principal role is to catalyze the exchange of triglycerides from apoB-containing particles (e.g., LDL, VLDL) for cholesteryl esters from HDL (1).

Common reduced-function variants of HL and CETP. Both loss of function mutations, which are rare, and the more common reduced-function allelic variants of HL and CETP structural or regulatory domains have been described (3,4). As Andersen et al. (5) summarize in this issue of the *Journal,* four linked single nucleotide polymorphisms (SNPs) in the HL promoter have been discovered and are associated with reduced HL activity. These variant alleles are common, such that almost 40% of Caucasians are heterozygous or homozygous carriers (5).

A common SNP for CETP has been extensively studied, which creates a *TaqI* restriction site. Although this SNP is located within intron 1, the *TaqI*B2 allele has been associated with reduced CETP activity. Strong linkage association with the C−629A CETP promoter polymorphism, which influences gene expression and CETP activity, may provide an explanation (6). In this issue of the *Journal*, Blankenberg et al. (7) examine further the C−629A polymorphism and a linked structural-domain variant, I405V.

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Consistent intermediate (HDL) phenotype accompanies loss-of-function variants. Plasma HDL-C shows an inverse relationship with atherosclerosis in the general population, which may be explained (at least in part) by the role of HDL in mediating reverse cholesterol transport (RCT). Accordingly, HDL-C is widely used as a biomarker for coronary risk. As noted, several common HL and CETP variants have been associated with reduced enzymatic mass and activity (6,8–15). The HL gene accounts for one-fourth of genetic variation in HDL-C levels (16). Both HL (5,8,10,11,17–20) and CETP (7,12–14,21–27) loss-of-function variant carriers consistently have been associated with higher HDL-C levels (and higher apolipoprotein AI levels, when measured).

Inconsistent effect on clinical (disease) phenotype. Despite the consistent impact of genetic variation in HL and CETP on lipids and lipoproteins, their effect on clinical phenotype is controversial (Table 1). Hypothetically, if HDL-C is a surrogate for RCT, variant allele carriage should be antiatherogenic. Conversely, higher HDL_2 may signal reduced RCT flux due to reduced enzymatic function; in this case, allele carriage would be proatherogenic (5,28). Into this controversy step the studies of Andersen et al. (5) and Blankenberg et al. (7).

CURRENT STUDIES

Andersen et al. (5) investigated an association between three SNPs in the HL promoter, levels of HDL-C, and risk of ischemic heart disease (IHD). A large (N = 9,121) representative sample of Copenhagen residents was genotyped, of which 957 had IHD. To expand the disease population, 921 additional IHD patients were added. The three variant HL alleles were common (frequencies, 0.21 to 0.22) and tightly linked. Levels of HDL-C and apolipoprotein AI increased in a stepwise fashion from wild-type to triple heterozygous to triple homozygous status. Clinical IHD, defined as previous myocardial infarction (MI) and/or cardiologist-diagnosed angina pectoris, was more prevalent, with an odds

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Table 1. Representative Literature Studies of Reduced-Function Variants of HL and CETP Genes

Gene/Study	Variant	Population (N)	1°/2° Risk	Intermediate Phenotype*	Clinical Outcome*	Statin/LL Interaction*
HL (*LIPC*)						
Zambon et al. (11)	−514T	U.S. (49)	2° (angio)	↑ HDL-C ↓ HL activity	↑ CAD progression on LL rx	↓ Benefit on HDL, LDL, angio CAD
Dugi et al. (28)	−514T	German (200)	1° (angio)	→HDL-C ↓ HL activity	↑ CAD extent	NR
Andersen et al. (5)	−480T (−514T)	Danish (10,042)	1°	↑ HDL-C, ↑ apo AI	↑ IHD (esp. ε43)	NR
Whiting et al. (20)	−514T	U.S. (3,868)	1° (angio)	↑ HDL-C	→angio CAD	NR
CETP						
Kuivenhoven et al. (21)	*Taq*IB2	Dutch males (807)	2° (angio)	↑ HDL-C, ↓ CETP activity	Relative ↓ CAD progression	angio CAD: ↓ B1B1, →B2B2
Zhong et al. (22)	D442G	Jap-Am males (3,469)	1°	↑ HDL-C, ↓ CETP	↑ IHD, esp. with nl. HDL	NR
Brousseau et al. (26)	*Taq*IB2	U.S. veterans (852)	2°	↑ HDL-C	↓ IHD events	NR (gemfibrozil)
Ordovas et al. (14)	*Taq*IB2	U.S. (2,916)	1°	↑ HDL-C	↓ →IHD males; →IHD females	NR
Agerholm-Larsen et al. (23)	I405V	Danish (10,014)	1°	↑ HDL-C	↑ IHD females; →IHD males	NR
Arca et al. (29)	*Taq*IB2	Italian (812)	1° (angio)	↑ HDL-C (controls)	→angio CAD	NR
Liu et al. (25)	*Taq*IB2	U.S. (768)	1°	↑ HDL-C	→MI (? ↓ MI if low HDL)	NR
Blankenberg et al. (7)	−629A	German (1,211)	2°	↑ HDL-C, ↓ CETP	↓ CV D	CV D: ↓ CC, →A with statin
Carlquist et al. (27)	*Taq*IB	U.S. (2,531)	2°	↑ HDL-C	→D/MI	D/MI: ↓ B2, →B1B1 with statin

*With variant, less common allele, associated with ↓ enzyme activity unless otherwise stated.
angio = angiography; CAD = coronary artery disease; CETP = cholesteryl ester transfer protein; CV = cardiovascular; D = death; HDL-C = high-density lipoprotein-cholesterol; HL = hepatic lipase; IHD = ischemic heart disease; Jap-Am = Japanese-Americans; LL = lipid-lowering; MI = myocardial infarction; nl. = normal; NR = not reported.

ratio (OR) of 1.5 ([confidence interval] CI 1.0 to 2.2) for homozygotes (≈5% of subjects) compared with wild-type subjects. Increased IHD prevalence persisted after adjustment for age, gender, and HDL-C (OR = 1.4, CI 1.1 to 1.9). The impact of HL variant homozygosity on disease was amplified in the presence of the relatively atherogenic ε43 apolipoprotein E genotype, the adjusted OR increasing to 2.0 (CI 1.2 to 3.2).

This "paradoxic" association with IHD despite higher HDL-C finds support in a recent German trial (28). Among 200 men undergoing elective coronary angiography, Dugi et al. (28) found the presence of the −514T HL promoter variant to be strongly associated with lower HL activity ($p < 0.001$) and greater angiographic coronary artery disease (CAD) extent ($p < 0.05$). The association was accounted for mainly by patients with normal HDL-C levels (>37 mg/dl). This HDL-C "paradox" was attributed to loss of RCT functions dependent on HL, such as formation of nascent, pre-β HDL particles and HL-enhanced uptake of cholesteryl ester from HDL by the hepatic SR-B1 receptor. Low-variant HL activity was proposed to be atherogenic primarily in the setting of normal HDL-C, whereas high-variant HL activity might be insufficient to compensate for reduced HDL with associated loss of the anti-inflammatory and antithrombotic properties.

Andersen et al. (5) also ascribe to this explanation: higher HDL-C associated with HL variants might mark reduced flux through the RCT system, whereas in most other contexts it reflects increased capacity. In further support, they draw an analogy to previous studies from Copenhagen (23) (and elsewhere [22]) where CETP loss-of-function variants resulted in increased HDL-C but were associated with increased IHD risk.

In contrast to the antiatherogenic view of HL is an angiographic study of lipid-lowering in 49 men with mixed dyslipidemia (11). Treated subjects with the wild-type genotype had the greatest decrease in HL activity, improvement in HDL_2-C and LDL buoyancy, and angiographic regression. Given differing patient populations, study size and design, therapeutic interventions, and end points, the implications of this smaller study in the present context remain unclear (11). However, these divergent results highlight the complexity of lipid/lipoprotein metabolism associated with HL and the dependence of clinical outcomes on both multiple interacting environmental (14) and genetic factors.

Blankenberg et al. (7) investigated associations among CETP C−629A and I405V polymorphisms, CETP activity, HDL-C, and the risk of fatal cardiovascular events. The study population included 1,211 German CAD patients in the AtheroGene study who were genotyped and prospectively followed for a median of four years, during which time 82 fatal cardiovascular events occurred. The two polymorphisms studied were found to be linked, with C−629A better explaining the outcomes. The variant −629A allele

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(38% prevalence) was associated with lower CETP activity (with an allele dosage effect) and higher HDL-C. Mortality, but not other cardiovascular outcomes, was substantially lower for carriers of one or two A alleles (4.6%, 4.0%) than for wild-type homozygotes (10.4%, $p < 0.0001$). Statin therapy was of benefit only in the high-risk CC (wild-type) patients, in whom it neutralized the genotype-associated hazard.

Given the tight linkage between the −629A and the *TaqI*B2 variant alleles (6,15), the Blankenberg et al. study (7) supports earlier observations from REGRESS, which found an effect of pravastatin on atherosclerosis progression only in B1B1 (homozygous wild-type) subjects (21). The intermediate (↑HDL-C, ↓CETP) phenotype-by-genotype result also is consistent with several earlier studies. Mechanistically, it might be hypothesized that statins act by decreasing CETP activity and cholesteryl ester transport from protective HDL to atherogenic VLDL. However, differences in survival by genotype were shown to be *independent* of HDL-C, CETP activity, and clinical covariables (7). Statin therapy was not randomized, and change in lipids by genotype with therapy was not assessed. Hence, the mechanism of survival advantage is unclear.

The AtheroGene clinical result is in contrast to several other studies, that found either no relationship of genotype to IHD risk or an opposite association (higher risk, greater treatment benefit for variant allele carriers) (Table 1) (14,22,23,27,29). Indeed, directionally different results for CETP I405V risk were reported from the neighboring Danish group (relative risk 1.4; CI 1.0 to 1.9 for women carriers of the 405V variant) (23). Of course, the studies differ in design, including baseline disease, gender, HDL-C, and therapy. But the example is illustrative of the lack of a consistent correlation of CETP genotype with clinical outcome.

Brown et al. (2) proposed that apparently conflicting findings could be reconciled if CETP activity were either protective or harmful depending on the atherogenicity of the apoB particles receiving cholesteryl ester from HDL. Genetically increased CETP would be protective and reduced CETP atherogenic in populations at low cardiovascular risk (low LDL-C, high HDL-C) and with low CAD prevalence (22,24), whereas the reverse would occur in high-risk (high LDL-C, low HDL-C), high-CAD-prevalence groups. This hypothesis deserves further investigation, but it does not appear to reconcile all reported studies (7,27,29).

DISCUSSION

The discovery of common genetic diversity within the human genome, including over four million SNPs (≈1% functionally active), has raised the hope that there will be increased understanding of disease pathogenesis, improved individual risk prediction, and customized preventive and therapeutic measures (pharmacogenomics). This promise has not yet been realized. The relation of high versus low levels of HL and CETP activity to HDL-C levels and overall risk is complex and likely situation-dependent. Accurate, readily measured markers of RCT flux are not available (HDL-C alone appears inadequate) but are certainly needed. Despite relatively consistent biomarker associations, inconsistent disease associations are a major impediment to the clinical application of genetic polymorphism determinations.

What might explain these discrepancies? Despite their promise, genetic association studies have been fraught with inconsistencies and failures of replication (30). Proposed explanations include chance associations (or missed associations) in populations of small size, publication bias (toward positive studies), population stratification artifacts (and other design issues in the selection of cases and controls) (28), imprecision in phenotyping and outcome assessment, and the use of SNPs themselves as genetic markers (31). Moreover, it appears unlikely that common genetic variants, allowed by natural selection to become highly prevalent, will have strong independent risk associations.

Rather, *multiple* interacting genetic and environmental factors (diet, exercise, drugs) likely will need to be accounted for to predict risk reliably. We recently proposed the concept of "genetic burden" (32). Individual dysfunctional polymorphisms not associated individually with discernible excess risk might progressively increase aggregate risk if considered together. Redundancy within metabolic pathways might allow for compensation for deficiency in one enzyme, but a combination of deficiencies in a series of proteins in a critical pathway (e.g., among genes for RCT) could progressively overwhelm compensatory mechanisms.

Finally, it recently has been proposed that "haplotype blocks" rather than individual SNPs may be the preferred unit of genetic risk. The SNPs do not occur in random combinations but in a relatively few fixed patterns within variably sized domains of deoxyribonucleic acid ("haplotype blocks") delimited by hot spots of meiotic recombination (33). Determining the net effect on disease risk of all co-inherited genetic polymorphisms within a haplotype block is an appealing avenue for clinical investigation.

In summary, the studies of Andersen et al. (5) and Blankenberg et al. (7) highlight the potential of HL and CETP polymorphisms to influence coronary heart disease (CHD) risk in carefully defined populations. However, taken together (Table 1) (34,35) association studies continue to defy simple characterization, and before clinical application can be considered, many questions still must be answered. Certainly, a need for replication exists, including prospective studies in very large and well-defined populations (with >500 to 1,000 events). Interventions (e.g., with statin therapy) should be randomized by genotype. Genetic and environmental modifiers should be carefully controlled. Combinations of polymorphisms in multiple genes in critical pathways ("genetic burden") should be assessed, and haplotypes (not just SNPs) should be evaluated. With

substantial effort and patience, the vision of gene-based medicine may yet be realized (36).

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