A Common Promoter Polymorphism in the Hepatic Lipase Gene (*LIPC*-480C>T) Is Associated With an Increase in Coronary Calcification in Type 1 Diabetes

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Type 1 diabetes is associated with coronary heart disease (CHD) and coronary artery calcification (CAC), a measure of subclinical CHD. The hepatic lipase gene promoter polymorphism (*LIPC*-480C>T) is a common variant affecting lipid metabolism. This study examined the relation between the *LIPC*-480C>T and CAC in type 1 diabetes. In the type 1 diabetic patients studied, 56% had CAC >0 Agatston units (AU). These subjects had a longer duration of diabetes (26.2 ± 1.3 vs. 17.8 ± 1.4 years; $P < 0.001$), lower HDL cholesterol levels (55.7 ± 2.4 vs. 61.0 ± 2.5 mg/dl; $P = 0.05$), higher triglyceride levels (101 ± 17.3 vs. 66 ± 7.6 mg/dl; $P < 0.05$), and higher diastolic blood pressure (79.7 ± 1.0 vs. 76.0 ± 1.4 mmHg; $P < 0.05$). The *LIPC*-480 T allele was more common in subjects with CAC (frequency = 0.31 ± 0.05 vs. 0.14 ± 0.04; $P = 0.006$). The proportion with CAC was 44% in *LIPC*-480CC subjects, 71% in heterozygotes, and 83% in *LIPC*-480TT subjects ($P < 0.01$). *LIPC*-480 T allele frequency increased as the amount of CAC increased ($P = 0.007$). *LIPC*-480 genotype was independently associated with the CAC (odds ratio = 2.90, 95% CI 1.22–6.92, $P < 0.05$) after adjusting for duration of diabetes, age, sex, diastolic blood pressure, HDL cholesterol, and triglyceride levels. In conclusion, the *LIPC*-480C>T polymorphism was associated with subclinical CHD in type 1 diabetes. This genetic variant may identify subjects in which early intervention to prevent CHD may be appropriate. *Diabetes* 51:1208–1213, 2002

Premature coronary heart disease (CHD) is the leading cause of death in type 1 diabetic patients. By age 55 years, 35% of patients with type 1 diabetes have died of CHD (1). In type 1 diabetes, there is a significant increase in subclinical CHD, particularly in women (2). The cause(s) of this increased susceptibility to CHD in type 1 diabetes is not well understood, nor is the role of hyperglycemia itself well established. Proteinuria is one factor that is associated with an increase in CHD in type 1 diabetes, but this does not fully account for the increased risk in this population (3). Many factors that relate to CHD in the general population (i.e., high cholesterol levels, low HDL, high triglyceride levels, and hypertension) also appear to increase the risk of CHD in patients with type 1 diabetes (4–8). Furthermore, a family history of CHD is a strong predictor of CHD events in type 1 diabetic patients (9). Investigations of the genetic susceptibility to CHD in type 1 diabetes have focused on the renin-angiotensin system. The ACE insertion/deletion polymorphism has been reported to be associated with CHD in subjects with type 1 diabetes and nephropathy (10), but this has not been confirmed (11).

Coronary artery calcification (CAC) by electron-beam computed tomography (EBCT) is a marker for the presence of subclinical atherosclerosis. The CAC score has been shown to reflect the presence and extent of atherosclerosis as measured by more invasive modalities such as angiography (12–14). Risk factors for CAC appear to be similar to that of clinical CHD (6,15). Results from a meta-analysis of 16 studies ($n = 3,683$) indicated that the presence of detectable CAC is associated with a >20-fold (weighted summary odds ratio [OR] = 25.6) increase in the prevalence of coronary disease as determined by angiography (16). In type 1 diabetes, CAC is associated with prevalent CHD (6). In addition, the presence of coronary calcification predicts subsequent cardiovascular disease events (17), even in asymptomatic individuals (18–20).

The hepatic lipase gene promoter polymorphism (*LIPC*-480C>T) is a common genetic variant associated with differences in hepatic lipase (21–24), an important enzyme in lipoprotein metabolism. *LIPC*-480C>T polymorphism is associated with higher HDL levels (21,22), primarily HDL_2 (23,25,26), and less small, dense LDL (23,27). A direct role for the *LIPC*-480C>T polymorphism in vascular disease has not been clearly established (28). The purpose of this study was to determine whether there is a relation between the *LIPC*-480C>T polymorphism and subclinical coronary disease in a high-risk population of patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Study subjects. Type 1 diabetic subjects (ages 20–55 years) were referred for participation in a pilot study of coronary calcification in type 1 diabetes. All subjects were asymptomatic for coronary disease and had no history of

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AU, Agatston unit; CAC, coronary artery calcification; CHD, coronary heart disease; EBCT, electron-beam computed tomography; HU, Hounsfield unit; IDL, intermediate-density lipoprotein; *LIPC*-480C>T, hepatic lipase gene promoter polymorphism; OR, odds ratio; SNP, single nucleotide polymorphism.

coronary artery bypass or angioplasty. DNA was genotyped in 97 subjects. Results from 91 Caucasian subjects are presented in this study. All subjects provided informed consent, and the protocol was approved by the Institutional Review Board.

Laboratory methods. Blood was collected from individuals who had fasted for at least 12 h and placed in tubes containing 0.1% EDTA. The sample was centrifuged immediately at 4°C. Plasma was maintained at 4°C until assayed for lipids. White cells were stored at 4°C before DNA extraction.

Cholesterol and triglyceride levels were measured using enzymatic methods, HDL was separated using dextran sulfate, and LDL cholesterol was calculated based on the Friedewald equation (LDL = total cholesterol – HDL – triglycerides/5). If the triglyceride level was >400 mg/dl, LDL cholesterol was calculated as the difference between the cholesterol in the d <1.006 g/ml fraction minus the cholesterol in the dextran sulfate supernatant.

Genomic DNA was extracted from leukocytes after erythrocyte lysis with 13.1 mmol/l ammonium chloride and 0.9 mmol/l ammonium bicarbonate by salt deproteinization, and resuspended in 10 mmol/l Tris-HCl, 0.2 mmol/l Na_2 EDTA (pH 7.5). The *LIPC*-480 promoter site (21,29) was genotyped in the context of a larger multiplex assay, the basis of which has been previously described (30). The multiplex assay included single nucleotide polymorphisms (SNPs) in the following genes in addition to *LIPC*: *ApoAIV*, *ApoB*, *ApoCIII*, *ApoE*, *β3Ad-R*, *CETP*, *LDL-R*, *LTA*, *Lp(a)*, *LpL*, *PON1*, *PON2*, and *PPARγ*. None of these SNPs were significantly related to CAC in univariate analysis (all $P > 0.10$). CETP-*Taq*IB, genotyped separately, was related to CAC ($P = 0.02$).) In brief, biotinylated primer pools were used to simultaneously amplify multiple targets, and the amplified alleles were detected colorimetrically using sequence-specific oligonucleotide probes that had been immobilized in a linear array. *LIPC* primers defining a 145-bp product were one pair of a total of 24 primer pairs in a single reaction. The specificity of the immobilized probes was confirmed using genomic targets of known genotype, as determined by sequencing.

Measurement of CAC by EBCT. High-resolution, noncontrast, contiguous 3-mm tomographic images were acquired at a 100-ms exposure time using an Imatron C-150XLP EBCT scanner (Imatron, San Francisco, CA). Scanning started from near the lower margin of bifurcation of the main pulmonary artery and proceeded caudally until the entire epicardial system was imaged during a single held breath, typically 35–45 s in duration. Prospective electrocardiogram triggering was set at 80% of the R-R interval. The threshold for CAC was set at a density of 130 Hounsfield units (HU) in at least one pixel (an area >0.51 mm^2) (2). The region of interest was encircled within each coronary artery, and computer-driven measurement of the lesion area and maximum density were recorded according to the method described by Agatston. A score for each region is calculated by multiplying the area by density score (one for 130–199 HU, two for 200–299 HU, three for 300–399 HU, and four for >399 HU). A total CAC score in Agatston units (AU) was calculated by adding up scores for all slices (typically 35–40 images acquired) and for left main coronary artery, left anterior descending coronary artery, circumflex artery, and right coronary artery.

Statistical analysis. Allele frequency was determined by counting, and Hardy-Weinberg equilibrium was tested using χ^2 analysis. Differences in mean values of quantitative traits were determined by two-tailed Student's t test or Mann-Whitney test if the quantitative data were not normally distributed.

TABLE 1
Characteristics of study subjects (n = 91) with type 1 diabetes

Characteristic	Value
Sex (male/female)	48/43
Age (years)	38 ± 7.8
Duration of diabetes (years)	22 ± 10.2
HbA_{1c}	6.7 ± 1.17
Insulin dosage (units/kg body wt)	0.62 ± 0.187
LIPC-480 allele frequency	0.24

Data are n or means ± SE.

Triglyceride levels were transformed (natural logarithm) for statistical analysis (untransformed data are presented). Sex differences were assessed by χ^2 analysis (Yates corrected). Significance of the percent of subjects with CAC by *LIPC*-480 genotype and allele frequencies among categories of CAC were determined using Cochrane-Armitage χ^2 test for linear trend. CAC categories were defined as no detectable calcium (CAC = 0 AU; n = 40), minimal calcium (CAC >0 to 20 AU; n = 36), moderate calcium (CAC >21 to 100 AU; n = 6), and extensive calcium (CAC >100 AU, n = 9). Although the cut points of CAC = 0 AU and CAC = 100 AU are well established, there is no consensus for cut points for minimal and moderate calcium (ranging from 5 to 50 AU). Analysis of *LIPC*-480 T allele frequencies using different cut points for minimum and moderate calcium did not alter the relation between *LIPC*-480 T allele and extent of CAC.

The a priori hypothesis of a relationship between the *LIPC*-480C>T genotypes and CAC was tested using multiple logistic regression with CAC yes/no as the dependent variable. *LIPC*-480 genotype was coded as *LIPC*-480CC = 1, CT = 2, and TT = 3. Significance level was set at $\alpha = 0.05$.

RESULTS

A total of 97 subjects with type 1 diabetes provided samples for the current study; results are reported on all Caucasian subjects (n = 91) (Table 1). These subjects had a mean age of 38 years (range 23–56 years) and a duration of diabetes of 22 years. The *LIPC*-480 allele frequency was 0.24, similar to reports from other Caucasian populations (21,23).

Coronary calcification was detectable in 51 (56%) of these type 1 diabetic subjects. The distribution of CAC was highly skewed (Fig. 1); 84% of the subjects had CAC values <20 AU, and 10% of these subjects had extensive CAC, with values >100 AU.

Factors that were related to the presence of coronary calcification in type 1 diabetes are presented in Table 2.

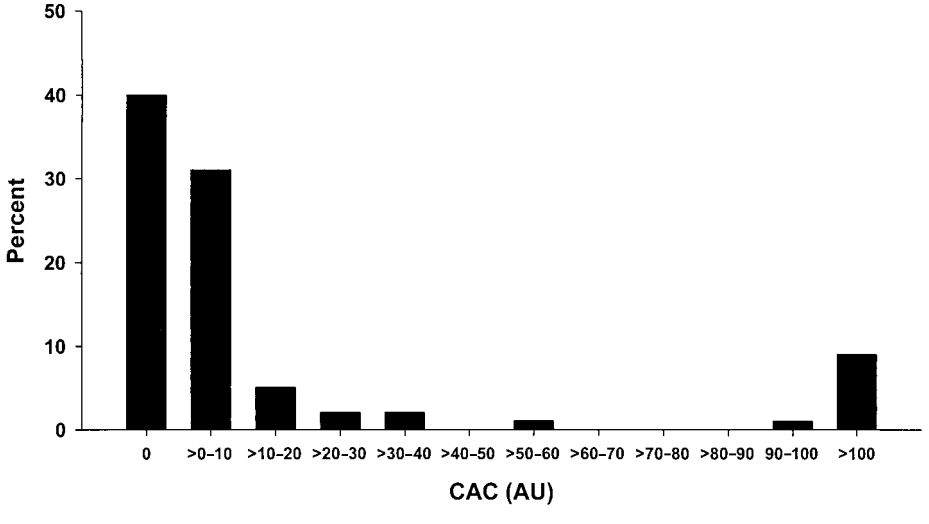

FIG. 1. Frequency distribution of CAC scores in 91 Caucasian subjects with type 1 diabetes.

TABLE 2
Differences between type 1 diabetic subjects with and without detectable CAC

Variable	Without CAC	With CAC	*P*
Sex (male/female)	16/24	31/20	0.08 (NS)
Age (years)	36.1 ± 1.5	40.0 ± 1.0	0.02
Duration of diabetes (years)	17.8 ± 1.4	26.2 ± 1.3	<0.001
HbA_{1c} (%)	6.61 ± 0.17	6.85 ± 0.19	0.47 (NS)
Insulin dosage (units · kg^{-1} body wt · day^{-1})	0.61 ± 0.03	0.63 ± 0.03	0.90 (NS)
Blood pressure (mmHg)			
Systolic	119 ± 2.6	124 ± 2.1	0.15 (NS)
Diastolic	76 ± 1.4	79.7 ± 1.0	0.04
Cholesterol (mg/dl)			
Total	178 ± 5.8	176 ± 4.9	0.65 (NS)
LDL	104 ± 4.2	106 ± 4.1	0.74 (NS)
HDL	61.0 ± 2.5	55.7 ± 2.4	0.05
Triglyceride (mg/dl)	66 ± 7.6	101 ± 17.3	0.04*
LIPC-480 allele frequency	0.14 ± 0.04	0.31 ± 0.05	0.006

Data are means ± SE. Detectable coronary artery calcification, CAC >0 AU; nondetectable coronary calcification, CAC = 0 AU. *Statistical test on the natural logarithm for triglycerides; untransformed data are presented.

Subjects with CAC >0 AU were older (40.0 ± 1.0 vs. 36.1 ± 1.3 years; $P = 0.02$) and had a significantly longer duration of diabetes (26.2 ± 1.3 vs. 17.8 ± 1.4 years; $P < 0.001$). In addition, diastolic blood pressure (79.7 ± 1.0 vs. 76.0 ± 1.4 mmHg, $P = 0.04$) and triglyceride levels (101 ± 17.3 vs. 66 ± 7.6 mg/dl; $P = 0.04$) were significantly higher, and HDL cholesterol (55.7 ± 2.4 vs. 61.0 ± 2.5 mg/dl; $P = 0.05$) was significantly lower in CAC subjects. HbA_{1c}, insulin dosage, systolic blood pressure. and LDL cholesterol were not related to the presence of CAC in this study population.

The *LIPC*-480 T allele frequency was 0.24 in the population (Table 1), and the *LIPC*-480 genotypes did not deviate from Hardy-Weinberg expectations ($P = 0.92$). The *LIPC*-480 T allele was significantly more common in subjects with coronary calcification (31 vs. 14%; $P = 0.006$) (Table 2). There was a significant difference in the proportion of subjects with CAC >0 AU by *LIPC*-480 genotype ($P = 0.02$ by Fisher's exact test). The percent of subjects with CAC >0 AU increased from 44% (24/54) among subjects with the *LIPC*-480CC genotype to 71% (22/31) in *LIPC*-480CT genotype to 83% (5/6) in homozygous *LIPC*-480TT subjects ($P < 0.007$ for Cochrane-Armitage test of linear trend) (Fig. 2). There was no significant difference in total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels or in blood pressure among *LIPC*-480 genotypes.

Subjects were categorized into four groups based on the extent of their coronary calcification (Fig. 3). The *LIPC*-480 T allele was greater within the group with higher CAC scores (test for trend, $P = 0.007$). The major increase in the *LIPC*–480 T allele frequency (a doubling from 0.14 to 0.29) was from the category with no coronary calcification to the group with CAC >0 to 20 AU. Subjects with CAC of >20 to 100 AU and >100 AU had even higher *LIPC*-480 T allele frequencies (0.31 and 0.39, respectively).

To determine the independent effect of the *LIPC*-480 allele on the presence of coronary calcification, multiple logistic regression analysis was performed (Table 3). The *LIPC*-480 genotype was significantly, independently related to the presence of CAC (OR = 2.90, 95% CI 1.22–6.92, $P < 0.05$) after accounting for the effects of age, duration of diabetes, sex, diastolic blood pressure, HDL, and triglycerides. Duration of diabetes was the only other factor that was independently related to CAC, whereas age, sex,

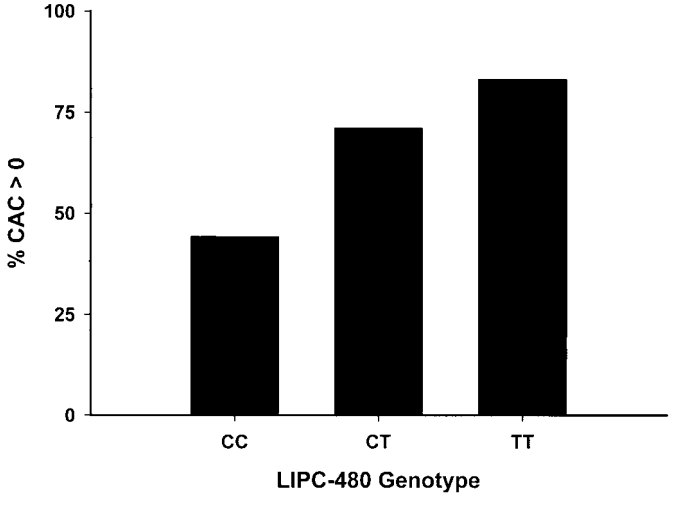

FIG. 2. Percent of subjects with CAC >0 AU by *LIPC*-480 genotype. χ^2 test for linear trend, $P < 0.01$.

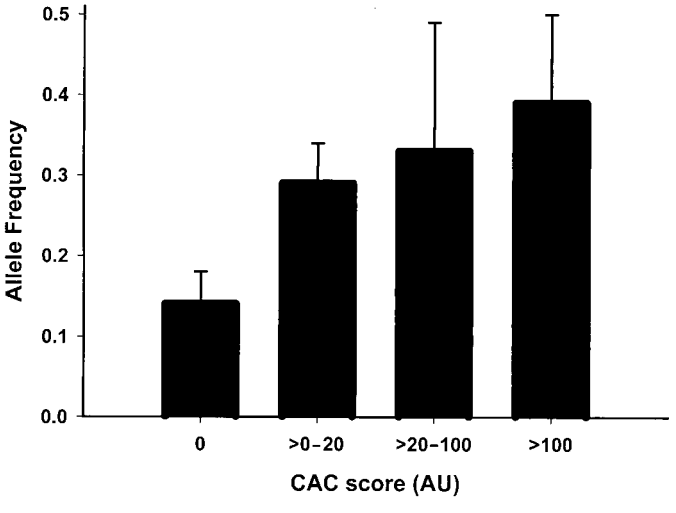

FIG. 3. *LIPC*-480 T allele frequency (± SE) by category of CAC score. χ^2 test for linear trend, $P = 0.007$.

TABLE 3
Multiple logistic regression analysis for the presence of CAC

Variable	Odds ratio	95% CI	*P*
LIPC-480 genotype	2.90	1.22–6.92	<0.05
Diabetes duration (years)	1.10	1.04–1.16	<0.01
Age (years)	1.06	0.99–1.13	0.20 (NS)
Sex	0.49	0.20–1.29	0.23 (NS)
Diastolic blood pressure	1.00	0.94–1.06	0.98 (NS)
HDL (mg/dl)	0.98	0.95–1.01	0.31 (NS)
Log triglyceride	1.89	0.83–4.31	0.21 (NS)

diastolic blood pressure, HDL, and triglycerides were not independently related to CAC.

DISCUSSION

This study is, to our knowledge, the first to show that a common polymorphism in the promoter region of the hepatic lipase gene (*LIPC*-480C>T) is associated with a greater than twofold increase in the presence of CAC among subjects with type 1 diabetes. This relation is independent of HDL cholesterol levels. In addition, there is a striking dosage-response relation. The *LIPC*-480 T allele frequency is higher as the extent of CAC is greater. These results suggest that the *LIPC*-480 T allele increases the susceptibility to subclinical coronary disease in type 1 diabetes.

It is important to note that the less-frequent allele (*LIPC*-480 T) is associated with greater CAC. This is despite the known anti-atherogenic effects of this allele on HDL_2 and LDL size. The polymorphism is associated with lower hepatic lipase (21–24) and higher HDL levels (21,22), primarily HDL_2(23,25,26). In addition, this polymorphism is associated with less small, dense LDL (23). Despite these reported favorable effects on lipoproteins, the results presented here suggest the *LIPC*-480 T allele is associated with more subclinical coronary disease, perhaps indicating alternate pro-atherogenic effects of the *LIPC* promoter variant.

One possible explanation for the pro-atherogenic effect of the *LIPC*-480 T allele may relate to nonlipolytic functions of hepatic lipase. Hepatic lipase appears to have a role in mediating remnant lipoprotein uptake (31,32). Higher hepatic lipase is associated with lower levels of intermediate-density lipoproteins (IDLs), even when the enzyme is lipolytically inactive (33). This suggests an important function for hepatic lipase in remnant clearance in addition to its function as a lipase. Therefore, the lower hepatic lipase levels associated with the decreased expression by the *LIPC* promoter variant may lead to an increase in IDL, an atherogenic lipoprotein class (34–36).

Previous data have also suggested a pro-atherogenic role for low hepatic lipase. It has been reported that the extent of coronary atherosclerosis is inversely associated with hepatic lipase levels (37), and that atherosclerotic lesion progression is associated with lower hepatic lipase (38). In high-risk subjects with familial hypercholesterolemia, hepatic lipase was inversely associated with the amount of coronary calcification (39). On the other hand, a decrease in hepatic lipase with intensive lipid-lowering therapy is associated with less angiographic lesion progression (40). The exact nature of the relation between hepatic lipase and atherosclerosis remains unclear.

Evidence is emerging that the *LIPC* promoter polymorphisms are related to coronary atherosclerosis. In the REGRESS study, the *LIPC*-480 T allele was significantly higher among coronary disease patients compared with asymptomatic control subjects (21). As pointed out by others (28), the genotype distribution at *LIPC*-480 was not in Hardy-Weinberg equilibrium in the REGRESS population. This could be explained by the marginally smaller number of *LIPC*-480CC subjects in the coronary disease population ($P = 0.06$), suggesting that the *LIPC*-480CC genotype is associated with decreased susceptibility to coronary disease. Others have not been able to demonstrate a significant difference in *LIPC* promoter allele frequencies between coronary disease patients and control subjects (24,41). Recent preliminary data presented in abstract form have indicated an increase in CHD associated with the *LIPC*-480 T allele in Caucasian (42,43) and Hispanic subjects (43). Dugi et al. (44) have shown that the extent of coronary atherosclerosis by angiography is inversely related to hepatic lipase levels and is higher in subjects with the *LIPC* promoter rare allele. A recent report indicated that the *LIPC*-480 T allele is associated with a decrease in coronary flow reserve (45), a measure of endothelial dysfunction (46). In addition, the *LIPC* promoter genotype predicts changes in coronary disease stenosis. Homozygous TT subjects have an increased progression of coronary stenosis on lipid-lowering therapy, whereas those with the CC genotype show significant regression in coronary stenosis on the same therapy (47). The current study adds to the literature suggesting an increase in CHD risk associated with the *LIPC*-480 T allele.

Despite the relatively small sample size in the current study ($n = 91$), there was a statistically significant relation between the *LIPC*-480 T allele and coronary calcification. This relation is attributable in part to the strength of the association (a greater than twofold increase in risk of coronary calcification for the presence of one *LIPC*-480 T allele) and the relatively common frequency of the *LIPC*-480 T allele (0.24). In addition, this study was performed in a population at high risk for vascular disease; in fact, >50% of these type 1 diabetic subjects had detectable coronary calcification. However, it is possible that these results are attributable to chance alone, so it will be important to verify these results with additional investigations. Support for these findings are emerging from studies of the *LIPC* promoter polymorphism in other populations (21,42–44).

It is possible that the *LIPC*-480 variant itself does not contribute to the increase in CAC, but that this marker is in linkage disequilibrium with another genetic variant that has physiological significance. This marker is known to be in complete linkage disequilibrium with three other *LIPC* promoter variants in Caucasian populations (29). Although the *LIPC*-480 T allele is associated with a decrease in hepatic lipase in vivo, it is unclear from in vitro studies which of these *LIPC* promoter variants alters gene expression (48–50). Moreover, these *LIPC* promoter variants may be in linkage disequilibrium with an as yet undiscovered polymorphism that is responsible for the decrease in hepatic lipase levels.

Patients with type 1 diabetes are at a significant increased risk for premature coronary disease. There is also a significant increase in subclinical CHD, as measured by

coronary calcification (2). This study identified a common genetic polymorphism in the hepatic lipase gene (*LIPC*-480C>T) that is associated with a greater than twofold increase in subclinical CHD in patients with type 1 diabetes. In addition, the *LIPC*-480 T allele is associated with the extent of coronary calcification in a dosage-dependent manner. Future studies will be required to determine if this polymorphism predicts progression of coronary calcification and clinical CHD events in this high-risk population.

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