The Ser^{447}–Ter mutation of the lipoprotein lipase gene relates to variability of serum lipid and lipoprotein levels in monozygotic twins[1]

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Abstract Studies on monozygotic twins support a role for genetic determinants of plasma lipid, lipoprotein, and apolipoprotein levels. Gene variants of the enzyme lipoprotein lipase have been shown to associate with dyslipidemia and coronary artery disease. We assessed the gene–environment interaction by investigating the relationship between the lipoprotein lipase gene and plasma lipid, lipoprotein, and apolipoprotein variability and levels among 54 male monozygotic twin pairs (aged 18–28 years). The Ser^{447}–Ter mutation (C→G transversion) was associated with significantly smaller within-pair differences in plasma high density lipoprotein-cholesterol (CG [n = 10] vs. CC [n = 44], 3.7 ± 5.3 mg/dl vs. 6.4 ± 5.2 mg/dl, $P < 0.03$) and total cholesterol (CG [n = 10] vs. CC [n = 44], 7.9 ± 9.4 mg/dl vs. 15.8 ± 12.7 mg/dl, $P < 0.05$), indicating attenuated variability in response to environmental stimuli. This observation of a restrictive variability gene effect further supports a role for the lipoprotein lipase gene in the genetic regulation of lipids and lipoproteins and suggests that the Ser^{447}–Ter mutation exerts multiple effects. This study also raises the possibility of a genetically determined responsiveness to dyslipidemia therapies.—**Thorn, J. A., E. W. A. Needham, R. K. Mattu, J. Stocks, and D. J. Galton.** The Ser^{447}–Ter mutation of the lipoprotein lipase gene relates to variability of serum lipid and lipoprotein levels in monozygotic twins. *J. Lipid Res.* 1998. **39:** 437–441.

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The plasma levels of lipids and lipoproteins are determined by the interaction between genetic and environmental factors. Evidence for this is derived from studies in populations, pedigrees (1), and twins (monozygotic (MZ) and dizygotic (DZ)) (2–4), although the mechanisms remain unclear.

At least two types of genetic effects have been postulated in determination of plasma lipid, lipoprotein, and apolipoprotein levels: those that directly determine levels ("level genes") and those that affect the response to environmental factors ("variability genes")(5). These terms are not exclusive as some genes may function in both capacities. Population and case-control studies have been used to assess the effects of level genes. MZ twin studies offer an unique opportunity to evaluate variability genes in humans, because MZ twins are genetically identical and therefore all within-pair lipid/lipoprotein differences should result from environmental influences, such as diet and life-style. Restrictive variability genes limit the differences provoked by environmental stimuli, whereas permissive variability genes produce the converse (6). This may be analyzed by investigating multiple sets of MZ twins and observing the relationship between genotypes and variance of within-pair differences (5, 6). Previous twin studies showed non-homogeneous distribution of within-pair differences of plasma lipids, lipoproteins, and apolipoproteins (apo) (7). This important observation in the presence of environmental stimuli, which may function as continuous variables, suggests that there is a major genetic determinant of the variabilities of these phenotypes. This has led to further studies of MZ twins that show significant associations between variants at multiple genetic loci and within-pair differences in high density lipoprotein-cholesterol (HDL-C), total and low density lipoprotein (LDL) cholesterol, triglycer-

Abbreviations: apo, apolipoprotein; MZ, monozygotic; DZ, dizygotic; HDL-C, high density lipoprotein-cholesterol; LDL, low density lipoprotein; LPL, lipoprotein lipase; CAD, coronary artery disease.

[1]This manuscript is dedicated to the memory of Dr. Drago Reichl who passed away during the course of the work presented here.

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ides, and apoB (5, 6). Recently, we demonstrated an association between an apoA-II gene variant and reduced within-pair differences in plasma apoA-II levels (8).

Lipoprotein lipase (LPL) is a key enzyme in lipid and lipoprotein metabolism. It affects the clearance of triglyceride-rich lipoproteins from the circulation (9, 10) and facilitates apolipoprotein and phospholipid exchange between very low density lipoprotein (VLDL) and HDL (11), and LDL generation from VLDL clearance (l2, 13).

The human LPL gene is localized to the short arm of chromosome 8 (14) and has genotypes that associate with dyslipidemia (15–19) and coronary artery disease (CAD) (16, 17). The most common coding sequence mutation described in this gene, the Ser^{447}–Ter, involves a **C→G** transversion at nucleotide 1595 in exon 9 (20). This would convert the serine 447 codon (TCA) to a premature termination codon (TGA) to produce a truncated enzyme lacking the two carboxyl terminal amino acids (Ser–Gly). Recent case-control studies designed to detect deleterious effects of this mutation have not demonstrated any such effects on either dyslipidemia(17, 19–21) or CAD (17, 19). On the contrary, in these studies, the mutation appears at lower frequencies among dyslipidemic (17, 19, 21) and CAD (17) subjects, and recently we reported that this mutation related to protective lipid/lipoprotein profiles and a 27% reduced risk of CAD (R. K. Mattu, unpublished results).

In the present study, we examined the Ser^{447}–Ter mutation, *Hind-III* (intron 8) polymorphism, and *Pvu-II* (intron 6) polymorphism for associations with within-pair differences of plasma lipids, lipoproteins, and apolipoproteins in MZ twins.

METHODS

Subjects

The subjects have been described elsewhere (22). Briefly, healthy white male MZ twin pairs (n = 54) aged 18 to 28 years were recruited from the twin register of the University of Birmingham. Twin zygosity was established by identity of six blood systems and confirmed by matching of all DNA polymorphisms at a minimum of four loci. Blood typing was carried out by standard hematalogic techniques at the Department of Haematology and Blood Transfusion, Queen Elizabeth II Hospital, Birmingham, UK. No subjects had secondary hyperlipidemia and 47 of the twin pairs lived in a common household.

Plasma lipid and lipoprotein determinations

Plasma cholesterol and triglycerides were measured by fully enzymatic methods (Boehringer-Mannheim). HDL cholesterol and triglycerides were analyzed by the heparin–$MnCl_2$ precipitation method. HDL_3 cholesterol and triglyceride were determined by further precipitation with dextran sulfate. ApoA-I and A-II were measured by rocket immunoelectrophoresis and apoB by immunonephelometry.

DNA analysis

DNA was isolated from 10 ml of frozen cells from each individual in the study, as previously described (17). *Hind-III* and *Pvu-II* RFLP genotypes were determined by Southern hybridization with a ^{32}P-labeled LPL cDNA clone, as detailed elsewhere (16). Samples for which no genotypes were obtained with Southern analysis were typed by restriction digestion of PCR-amplified genomic regions encompassing one or other RFLP site (17). Genotypes of the Ser^{447}–Ter polymorphism were also determined by PCR analysis. A modified downstream primer was used to produce a mutant PCR product that introduced a *Hinf-I* cutting site in the presence of the G but not the C allele (21).

Data analysis

We tested the absolute values of within-pair differences (δ) in lipid, lipoprotein, and apolipoprotein levels of all the twin pairs for heterogeneity using the method of Fisher as applied by Martin, Rowell, and Whitfield (7). In this test a positive value indicates non-homogeneity; where $\{\Sigma[(\text{mean of } \delta)^2]$ minus $\Sigma[\text{mean of } (\delta^2)]\}$ represents the final value. The relationships of within-pair differences with genotype were analyzed using the Mann-Whitney U-test. Similar analyses were undertaken to assess the mean twin pair biochemical levels with respect to genotypes. All statistical tests were undertaken using the SPSS/PC+ statistical package.

RESULTS

The distributions of within-pair differences (δ) of lipid, lipoprotein, and apolipoprotein levels in the MZ twins were non-homogeneous (data not shown).

The *Hind-III* and *Pvu-II* polymorphisms are bi-allelic, with the presence of restriction sites denoted by the H2 and P2 alleles, respectively. Presence of the Ser^{447}–Ter mutation is denoted by the G allele yielding the genotypes CC, CG, and GG. No subjects with the GG (0%), 10 with the CG (19%), and 44 with the CC (81%) geno-

type were observed in our study group (**Table 1**). These genotype frequencies were not significantly different from those reported previously of 2.4% (GG), 15.4% (CG), and 82.2% (CC) in a white Caucasian Welsh population (17) and 1.3% (GG), 19.2% (CG), and 79.5% (CC) in a group of healthy white male Caucasians from London (21).

The mean within-pair differences of HDL-C (CG vs. CC, 3.7 ± 5.3 mg/dl vs. 6.4 ± 5.2 mg/dl, $P < 0.03$) were significantly lower in twin pairs with the Ser^{447}–Ter mutation (Table 1). This association was also evident for total cholesterol (CG vs. CC, 7.9 ± 9.4 mg/dl vs. 15.8 ± 12.7 mg/dl, $P < 0.05$) and reflected in triglyceride levels (CG vs CC, 15.7 ± 19.0 mg/dl vs 32.6 ± 41.4 mg/dl, $P < 0.09$). No similar significant associations were observed with *Hind-III* or *Pvu-II* genotypes.

The mean lipid, lipoprotein, and apolipoprotein levels for twin pairs were not significantly different between twin pairs in relation to genotypes, apart from the mean total cholesterol levels being significantly lower in those twins carrying at least one H1 allele (H1H1 + H1H2 vs. H2H2, 150.8 ± 27.3 m/dl vs. 173.6 ± 34.0 mg/dl, $P < 0.01$, Mann-Whitney test), data not shown.

DISCUSSION

Our observation of non-homogeneity in the distributions of the within-pair differences of plasma lipids, lipoproteins, and apolipoproteins supports a genetic determination of the variability of these phenotypes. We demonstrate that the lipoprotein lipase gene may be such a determinant.

In this study, the Ser^{447}–Ter mutation appears to exert a restrictive variability gene effect, as twins possessing this mutation have smaller differences in HDL-C, total cholesterol, and triglyceride levels, thus suggesting that individuals without this mutation are more susceptible to fluctuations in their plasma lipid and lipoprotein levels in response to environmental influences.

Previous studies have shown that the LPL gene is implicated in dyslipidemia and coronary artery disease (15–24)(R. K. Mattu et al., unpublished results). In case-control and large population studies we demonstrated that the Ser^{447}–Ter mutation may have an important protective effect upon dyslipidemia and CAD (17, 21) (R. K. Mattu et al., unpublished results). It appeared to influence lipid, lipoprotein, and apolipoprotein levels by exerting a "level gene" effect, while also exhibiting a gene–dose effect. In the present study we did not observe a significant level gene effect but a trend towards beneficial profiles (data not shown). This was not unexpected as the study design and power were not appropriate to demonstrate such an effect. However, this study demonstrates a restrictive "variability gene" effect and it appears that this is not a reflection of the "level gene" effects of the mutation. How the Ser^{447}–Ter mutation may attenuate the environmental impact upon plasma lipids and lipoproteins remains to be determined.

It remains unclear how truncation of the two carboxy terminal amino acids of LPL leads to the biochemical changes observed in this and other studies, as this region is distant from the catalytic domains of the en-

TABLE 1. Within-pair differences in lipoprotein levels with different LPL gene variants

	Ser^{447}–Ter		*Hind-III*		*Pvu-II*	
	CC (n = 44)	CG (n = 10)	H1H1 + H1H2 (n = 18)	H2H2 (n = 31)	P1P1 + P1P2 (n = 37)	P2P2 (n = 10)
	mg/dl		*mg/dl*		*mg/dl*	
TC	15.8 ± 12.7	7.9 ± 9.4[a]	15.1 ± 15.7	15.2 ± 13.5	16.3 ± 15.4	11.1 ± 9.3
Tg	32.6 ± 41.4	15.7 ± 19.0[b]	34.8 ± 52.2	26.4 ± 31.8	33.3 ± 44.5	23.4 ± 19.4
HDL-C	6.4 ± 5.2	3.7 ± 5.3[c]	5.6 ± 5.5	5.7 ± 5.0	6.0 ± 5.3	4.2 ± 3.9
HDL-Tg	2.5 ± 3.2	2.7 ± 2.1	2.8 ± 3.5	2.5 ± 2.9	3.0 ± 3.3	1.4 ± 1.6
HDL_3-C	4.6 ± 3.6	4.1 ± 3.9	4.6 ± 3.8	4.2 ± 3.4	4.9 ± 3.8	2.9 ± 2.4
HDL_3-Tg	2.0 ± 3.2	2.4 ± 1.9	2.3 ± 3.2	2.1 ± 3.1	2.4 ± 3.3	1.5 ± 2.2
ApoA-I	5.7 ± 7.7	6.5 ± 6.7	6.2 ± 6.6	5.5 ± 7.8	6.1 ± 7.4	4.2 ± 6.3
ApoA-II	2.9 ± 3.4	3.6 ± 4.6	4.1 ± 4.7	2.1 ± 2.5	3.5 ± 3.9	1.1 ± 1.2
ApoB	5.9 ± 4.9	8.1 ± 5.2	6.0 ±4.3	6.2 ± 5.1	6.4 ± 5.1	6.6 ± 4.6

Values are given as mean ± SD. TC, total cholesterol; Tg, total triglyceride; HDL, high density lipoprotein; apo, apolipoprotein. All within-pair differences were analyzed with respect to genotype using the Mann-Whitney *U*-test.

[a] CC versus CG, $P < 0.05$.

[b] CC versus CG, $P < 0.09$.

[c] CC versus CG, $P < 0.03$.

zyme. Interestingly, mutations affecting the carboxy region have been reported to alter in vitro LPL specific activity (25–27). However, the in vitro specific activity data that have been reported after transfection studies involving the Ser^{447}–Ter mutation are inconsistent (25, 28–31), and probably reflect methodological differences.

The precise functions of the carboxy terminus are also uncertain, but proposed roles include interaction with lipid substrates (30, 32), endothelial binding of the enzyme (33), facilitation of binding of chylomicrons to the low density lipoprotein receptor-related protein (34), and changes in specific activity, possibly effected through influences upon tertiary structure (35–38). The mechanisms through which possession of the Ser^{447}–Ter mutation may exert level gene and restrictive variability gene effects requires further investigation.

Our observations further support a role for the lipoprotein lipase gene in the genetic regulation of lipids and lipoproteins and suggest that the Ser^{447}–Ter mutation exerts multiple effects, and they also raise the possibility of a genetically determined responsiveness to dyslipidemia therapies.

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