Interactions Among the Glucocorticoid Receptor, Lipoprotein Lipase, and Adrenergic Receptor Genes and Plasma Insulin and Lipid Levels in the Quebec Family Study

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The aim of the study was to investigate the possible interactions among the glucocorticoid receptor (GRL), lipoprotein lipase (LPL), and adrenergic receptor (ADR) genes on plasma insulin and lipid levels. The study was cross-sectional and based on 742 individuals from phase 2 of the Quebec Family Study (QFS) cohort. Gene markers were identified by Southern blot analysis or polymerase chain reaction (PCR). Plasma glucose and insulin in the fasted state and during an oral glucose tolerance test (OGTT) were determined and insulin and glucose areas were computed. Triglyceride (TG) and cholesterol concentrations in plasma and lipoprotein fractions were determined enzymatically. The results show that GRL and LPL variants had independent effects on plasma high-density lipoprotein cholesterol (HDL-C) and two β2-ADR variants were related to total cholesterol concentrations. The α2-ADR gene *Dra*I polymorphism was the only variant that had an independent effect on the plasma insulin area. Gene-gene interaction effects were found between GRL and α2-ADR genes for low-density lipoprotein cholesterol ([LDL-C] $P = .013$) and between GRL and LPL genes for HDL-C ($P = .045$). Higher-order interaction effects involving GRL, LPL, and ADR markers were observed for the plasma insulin area ($P = .001$ to $.025$) but not the glucose area. After correction for multiple tests, the findings remained essentially unchanged for the insulin area but became nonsignificant for the lipid phenotypes. In conclusion, multiple interactions among GRL, LPL, and ADR gene markers contribute to insulin metabolism and perhaps to lipid levels, while no significant effect is found for each gene separately. The LPL locus appears to determine the pattern of interactions with ADR and GRL loci. These results suggest that gene-gene interaction effects could play a role in the etiology of risk factors for common chronic diseases.

HIGH FREE FATTY ACID (FFA) levels have been shown to contribute to systemic hyperinsulinemia.[1-4] Adipose tissue, especially an enlarged abdominal or visceral fat depot, is associated with enhanced lipolysis and perhaps a greater flux of FFAs to the liver, which may predispose to insulin resistance.[1-4] However, the role of abdominal visceral fat in insulin resistance remains controversial.[5] Factors that control the storage (primarily lipoprotein lipase [LPL] activity)[6] and mobilization of lipids from adipose tissue could be important candidate regulators of insulin sensitivity. The notion that glucocorticoids, via specific receptors (GRL),[7] could be among such factors is supported by the observation that they stimulate LPL activity and are also markedly involved in the regulation of lipolysis.[8] Another important receptor family is the adrenergic receptor (ADR) system, as it can stimulate (β1, β2, and β3)[9] or inhibit (α2)[10] lipolysis. Thus, the GRL, LPL, and ADR genes are reasonable candidate genes for insulin resistance and related morbidities.

As several genes probably influence insulin metabolism, it is likely that gene-gene interaction effects need to be considered at the molecular level.[11] For example, the GRL, LPL, and ADR genes may have effects on insulin and lipoprotein metabolism that are not observed when the genes are considered individually. We therefore investigated the interactions among polymorphisms in the GRL, LPL, and ADR genes on insulin and lipoprotein metabolism in the Quebec Family Study (QFS) cohort.

SUBJECTS AND METHODS

The QFS cohort has been previously described.[12] Briefly, a total of 1,628 individuals from French-Canadian families living in and around Quebec City were recruited through the media during the years 1978 to 1981 (phase 1). DNA was available on 742 subjects from 194 families participating in phase 2 of the QFS, and they were included in the present study. The study has been approved by the Medical Ethics Committee of Laval University, and the analysis of the present data received approval from the Institutional Review Board of the Pennington Biomedical Research Center.

Phenotype Measurements

Body density obtained by underwater weighing[13] was converted to percent body fat using the equation of Siri,[14] with pulmonary residual volume measured with the helium dilution method.[15] Fat mass was obtained from percent body fat and body weight. Resting blood pressure was measured in the morning after a 12-hour fast with the subjects free of caffeine and tobacco products.

Blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA after a 12-hour overnight fast. The plasma was separated immediately after blood collection by centrifugation at 3,000 rpm for 10 minutes at 4°C for the measurement of plasma lipid and lipoprotein levels. Triglyceride (TG) and cholesterol

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concentrations in plasma and lipoprotein fractions were determined enzymatically on a Technicon RA-500 automated analyzer (Bayer, Tarrytown, NY). Plasma very–low-density lipoproteins ($d < 1.006$ g/mL) were isolated by ultracentrifugation (50,000 rpm) in a Beckman 50.3 Ti rotor (Beckman, Palo Alto, CA) as reported previously.[16] High-density lipoprotein (HDL) particles were isolated from the bottom fraction (>1.006 g/mL) after precipitation of low-density lipoprotein (LDL) with heparin and $MnCl_2$.[17] The TG and cholesterol content of the infranatant fraction was determined before and after the precipitation step to assess LDL and HDL composition.

A 75-g oral glucose tolerance test (OGTT) was performed in the morning after a 12-hour fast. The plasma insulin level was measured by radioimmunoassay,[18] and plasma glucose was determined by an enzymatic method.[19] Plasma insulin was determined at 15-minute intervals during the first hour following glucose ingestion and every 30 minutes for the subsequent 2 hours. The total areas under the curve for insulin and glucose during the OGTT were computed from the plasma levels using the trapezoidal method as previously described.[20]

DNA Analysis

Genomic DNA was isolated from lymphoblastoid cell cultures[21] by digestion with proteinase K and extraction with phenol chloroform.

Polymerase chain reaction analysis of β2- and β3-ADR markers. The β2- and β3-ADR polymorphisms and the polymerase chain reaction (PCR) technique by which they were analyzed were described previously.[22]

PCR analysis of LPL markers. The replacement of a thymine (T) with a guanine (G) base in intron 8 of the LPL gene abolishes the *Hin*dIII restriction site.[23] The primer sequence for the *Hin*dIII polymorphism was derived from published data.[24] An S447X mutation in exon 9 of the LPL gene caused by a C-G transversion results in a premature termination codon.[25] This leads to a truncated protein lacking the two carboxyl-terminal amino acids (Ser-Gly). The modified primers covering this site and creating an S447X restriction site in the presence of the G allele were reported previously.[26]

Each 20-μL reaction for PCR analysis of the *Hin*dIII polymorphism contained 100 ng genomic DNA, 0.3 μmol/L of each primer, 0.2 mmol/L of each dNTP, and 1.0 U Taq DNA polymerase in a standard buffer, and 10% dimethyl sulfoxide. The reactions were incubated at 95°C for 3 minutes, 60°C for 2 minutes, and 72°C for 2 minutes followed by 35 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 60 seconds and 1 cycle at 72°C for 10 minutes using a thermal cycler.

In the PCR studies of the S447X polymorphism, a volume of 20 μL containing 250 ng DNA, 0.45 μmol/L of each primer, 0.3 mmol/L of each dNTP, 1.12 U Taq polymerase, 1.87 mmol/L $MgCl_2$, and standard buffer plus Q-solution was used.

The PCR was started at 94°C for 3 minutes, 53°C for 1 minute, and 72°C for 1 minute followed by 40 cycles at 94°C for 30 seconds, 53°C for 30 seconds, and 72°C for 45 seconds and 1 cycle at 72°C for 10 minutes. The digestion procedures were performed by adding 10 U *Hin*dIII or *Hin*fI enzymes (New England Biolabs, Beverly, MA) for 18 hours at 37°C. The fragments were separated on 1.5% and 3.0% agarose gels for *Hin*dIII and S447X polymorphisms, respectively, and visualized under UV light after staining with ethidium bromide.

Southern blot analysis. All of the samples of the α2-ADR *Dra*I, GRL *Bcl*I, and LPL *Bam*HI polymorphisms, as well as about half of the samples of the LPL *Hin*dIII markers, were identified by the Southern blot technique. The human α2-ADR genomic probe was obtained from the American Type Culture Collection (Rockville, MD). The human GRL genomic DNA[27] was provided by R. Evans and the human LPL cDNA clone[28] by R. Lawn. Five micrograms of genomic DNA was digested for 18 to 20 hours with 30 U *Dra*I for α2-ADR, *Bam*HI plus *Hin*dIII for LPL, and *Bcl*I for GRL according to conditions recommended by the manufacturer. Southern blot analysis was performed as described previously.[29]

Statistical Analysis

All analyses were performed with the SAS statistical analysis package (SAS Institute, Cary, NC). Associations between the gene markers and phenotypes were tested using the analysis of covariance procedure. Plasma lipid and lipoprotein concentrations and the plasma insulin area were adjusted for total fat mass, age, and sex. Because of the skewed distributions, insulin values and areas are logarithmically (common) transformed and the 95% confidence interval (CI) is presented. Gene-gene interactions were tested with the general linear model procedure by including the main gene effects, interaction terms, and covariates in the same model. *P* values were adjusted for multiple tests using the modified Bonferroni test.[30]

RESULTS

The independent effects of each polymorphism, their interactions on the insulin area and plasma lipids, and their contribution to the phenotype variance are shown in Table 1.

Independent Effects of Each Polymorphism

The α2-ADR gene *Dra*I polymorphism was the only variant with an independent effect on the plasma insulin area. The insulin area under the curve during the OGTT was lowest (47.30×10^3 pmol/L · min, 95% CI = 38.90 to 58.50, *P* for trend = .004) in 6.3-kilobase (kb) homozygotes compared with homozygotes for the 6.7-kb allele (67.70×10^3 pmol/L · min, 95% CI = 64.90 to 70.50, *P* = .001 *v* 6.3/6.3-kb genotype) or the heterozygotes (66.30×10^3 pmol/L · min, 95% CI = 62.3 to 72.0, *P* = .002 *v* 6.3/6.3-kb genotype).

Moreover, the LPL S447X, *Hin*dIII, and *Bam*HI and GRL *Bcl*I variants had significant effects on the plasma HDL-C concentration. Both β2-ADR variants were associated with total cholesterol levels (Tables 1 and 2).

Gene-Gene Interactions and Plasma Insulin Concentration

Between GRL and LPL genes. Interaction effects between the GRL *Bcl*I and LPL S447X, *Bam*HI, and *Hin*dIII markers (*P* for interaction = .002 to .003) influenced the mean size of the OGTT insulin area (Table 3). Noncarriers of both the 4.5-kb allele of GRL *Bcl*I and the Ter allele of LPL S447X markers had a lower mean insulin area than carriers of the 4.5-kb allele that were noncarriers of the Ter allele (group I) or noncarriers of the 4.5-kb allele that carried the Ter allele. In addition, 4.5-kb allele and Ter allele carriers had a lower mean insulin area than group I. The 4.5-kb allele noncarriers of the GRL *Bcl*I that were 33-kb allele carriers of the LPL *Bam*HI or noncarriers of the G allele of the LPL *Hin*dIII polymorphisms had the lowest mean OGTT insulin area (Table 3).

Interactions similar to those for the OGTT insulin area were also found for the fasting plasma insulin concentration, although the interaction between GRL and LPL S447X markers did not reach statistical significance (Table 4).

Between GRL, LPL, and ADR genes. Significant interactions were observed for the mean OGTT insulin areas among the GRL, LPL, and α2-ADR genes (*P* for interaction = .001; Fig 1), GRL, LPL, and β2-ADR genes (*P* for interaction = .025; Fig 2), and GRL, LPL, and β3-ADR genes (*P* for

Table 1. Independent and Interaction Effects of Each Polymorphism on Insulin Area and Plasma Lipids

Polymorphism	Insulin Area	R^2	Total Cholesterol	R^2	HDL-C	R^2	LDL-C	R^2
Independent effects								
α2-ADR *Dra*I	Yes	.016	No	—	No	—	No	—
β2-ADR Gln27Glu	No	—	Yes	.010	No	—	No	—
β2-ADR Arg16Gly	No	—	Yes	.009	No	—	No	—
β3-ADR Trp64Arg	No	—	No	—	No	—	No	—
LPL S447X	No	—	No	—	Yes	.006	No	—
LPL *Hin*dIII	No	—	No	—	Yes	.015	No	—
LPL *Bam*HI	No	—	No	—	Yes	.009	No	—
GRL *Bcl*I	No	—	No	—	Yes	.007	No	—
Interaction effects								
GRL × α2-ADR	No	—	No	—	No	—	Yes	.008
GRL × LPL S447X	Yes	.014	No	—	Yes	.005	No	—
GRL × LPL *Bam*HI	Yes	.014	No	—	No	—	No	—
GRL × LPL *Hin*dIII	Yes	.013	No	—	No	—	No	—
GRL × LPL S447X × α2-ADR	Yes	.027	No	—	No	—	No	—
GRL × LPL S447X × β2-ADR Gln27Glu	Yes	.016	No	—	No	—	No	—
GRL × LPL S447X × β3-ADR	Yes	.021	No	—	No	—	No	—

NOTE. No significant results were observed for plasma TG. Yes, significant association; No, nonsignificant association.

interaction = .005; Fig 3). The OGTT glucose areas did not exhibit any genotype interaction effects. However, the interaction between GRL, LPL, and α2-ADR genes influenced the plasma fasting insulin level (P for interaction = .009) but not glucose. All P values for the interaction effects on plasma OGTT insulin areas remained significant after correction for the presence of multiple tests.

Table 2. Independent Effects of the GRL, LPL, and β2-ADR Polymorphisms on Plasma Lipids

Polymorphism	Group	Total Cholesterol	HDL-C	Total TG
GRL/*Bcl*I				
4.5/4.5 kb (n = 83)	I	5.04 ± 0.10	1.24 ± 0.03	1.35 ± 0.16
4.5/2.3 kb (n = 302)	II	5.02 ± 0.05	1.19 ± 0.02*	1.68 ± 0.08
2.3/2.3 kb (n = 229)	III	5.00 ± 0.10	1.25 ± 0.02	1.43 ± 0.10
P for trend		NS	.046	NS
LPL/S447X				
S447S (n = 478)	I	5.01 ± 0.04	1.20 ± 0.01	1.57 ± 0.07
S447X + X447X (n = 133)	II	5.03 ± 0.08	1.27 ± 0.02	1.45 ± 0.13
P for trend		NS	.026	NS
β2-ADR/Arg16Gly				
Arg16Arg (n = 69)	I	4.81 ± 0.11	1.18 ± 0.03	1.48 ± 0.18
Arg16Gly (n = 279)	II	4.98 ± 0.05	1.22 ± 0.02	1.48 ± 0.09
Gly16Gly (n = 264)	III	5.12 ± 0.06†	1.22 ± 0.02	1.64 ± 0.09
P for trend		.027	NS	NS
β2-ADR/Gln27Glu				
Glu27Glu (n = 110)	I	5.12 ± 0.09	1.26 ± 0.03	1.39 ± 0.14
Gln27Glu (n = 286)	II	5.10 ± 0.05	1.23 ± 0.02	1.65 ± 0.09
Gln27Gln (n = 217)	III	4.88 ± 0.06‡	1.19 ± 0.02	1.48 ± 0.10
P for trend		.016	NS	NS

NOTE. Values are the mean ± SEM (adjusted for age, sex, and fat mass).

Abbreviation: NS, nonsignificant.

*P = .020, II *v* III.

†P = .013, III *v* I.

‡P = .030, III *v* I; P = .009, III *v* II.

Table 3. Interactions Between the GRL and LPL Genes for the Insulin Area Under the Curve During the OGTT

Polymorphism	Group	Insulin Area ($\times 10^3$ pmol/L · min)	95% CI	P
GRL *Bcl*I and LPL S447X				
4.5-kb$^+$ and X447$^-$ (n = 237)	I	69.2	64.6-72.4	.001*v* II
4.5-kb$^-$ and X447$^-$ (n = 143)	II	57.5	53.7-63.1	—
4.5-kb$^+$ and X447$^+$ (n = 72)	III	58.9	52.5-66.1	.015*v* I
4.5-kb$^-$ and X447$^+$ (n = 38)	IV	69.2	60.3-81.3	.039*v* II
P for interaction		.002		
GRL *Bcl*I and LPL *Bam*HI				
4.5-kb$^+$ and 33-kb$^+$ (n = 132)	I	69.2	63.1-74.1	.001*v* II
4.5-kb$^-$ and 33-kb$^+$ (n = 89)	II	54.9	50.1-60.3	—
4.5-kb$^+$ and 33-kb$^-$ (n = 178)	III	64.6	60.3-69.2	.008*v* II
4.5-kb$^-$ and 33-kb$^-$ (n = 91)	IV	67.6	61.7-74.1	.004*v* II
P for interaction		.004		
GRL *Bcl*I and LPL *Hin*dIII				
4.5-kb$^+$ and G$^+$ (n = 154)	I	66.1	60.3-70.8	.002*v*IV
4.5-kb$^-$ and G$^+$ (n = 81)	II	69.2	63.1-77.6	.001*v*IV
4.5-kb$^+$ and G$^-$ (n = 158)	III	67.6	61.7-72.4	.001*v*IV
4.5-kb$^-$ and G$^-$ (n = 98)	IV	53.7	49.0-58.9	—
P for interaction		.003		

NOTE. Values are the mean (logarithmically back-transformed) adjusted for age, sex, and fat mass.

Table 4. Interactions Between the GRL and LPL Genes for Fasting Plasma Insulin

Polymorphism	Group	Plasma Insulin (pmol/L)	95% CI	*P*
GRL *Bcl*I and LPL S447X				
4.5-kb$^+$ and X447$^-$ (n = 247)	I	57.5	53.7-63.1	NS
4.5-kb$^-$ and X447$^-$ (n = 145)	II	45.7	41.7-51.3	NS
4.5-kb$^+$ and X447$^+$ (n = 75)	III	53.7	45.7-61.7	NS
4.5-kb$^-$ and X447$^+$ (n = 40)	IV	52.5	42.7-64.6	NS
P for interaction		NS		
GRL *Bcl*I and LPL *Bam*HI				
4.5-kb$^+$ and 33-kb$^+$ (n = 140)	I	58.9	53.7-66.1	.001 *v* II
4.5-kb$^-$ and 33-kb$^+$ (n = 90)	II	43.6	38.0-50.1	—
4.5-kb$^+$ and 33-kb$^-$ (n = 183)	III	54.9	50.1-60.3	.004 *v* II
4.5-kb$^-$ and 33-kb$^-$ (n = 94)	IV	52.5	45.7-60.3	.049 *v* II
P for interaction		.031		
GRL *Bcl*I and LPL *Hin*dIII				
4.5-kb$^+$ and G$^+$ (n = 158)	I	54.9	50.1-61.7	.007 *v* IV
4.5-kb$^-$ and G$^+$ (n = 84)	II	52.5	45.7-61.7	.051 *v* IV
4.5-kb$^+$ and G$^-$ (n = 167)	III	58.9	53.7-64.6	.001 *v* IV
4.5-kb$^-$ and G$^-$ (n = 99)	IV	43.6	38.9-50.1	—
P for interaction		.036		

NOTE. Values are the mean (logarithmically back-transformed) adjusted for age, sex, and fat mass.

Abbreviation: NS, nonsignificant.

Gene-Gene Interactions and Plasma Lipids and Lipoproteins

Between GRL and α2-ADR genes. Significant interactions between the GRL *Bcl*I and α2-ADR *Dra*I markers were observed on LDL-C (*P* for interaction = .013). Carriers of the 4.5-kb allele of GRL *Bcl*I and the 6.3-kb allele of α2-ADR *Dra*I polymorphisms had the highest LDL-C (Table 5).

Between GRL and LPL genes. A significant interaction effect was found between the GRL and LPL genes for HDL-C (*P* = .045; Table 5). HDL-C was lowest in joint carriers of the 4.5-kb allele of the GRL *Bcl*I variant and noncarriers of the Ter allele of the LPL S447X variant. However, after adjustment for multiple tests, the *P* values for interactions on plasma lipid phenotypes became nonsignificant.

Contribution of gene-gene interactions to the phenotype variance. The GRL and LPL interactions explained about 1.4% of the variation in the OGTT insulin area, while the GRL, LPL, and ADR interactions accounted for up to 2.7% of the variation in the same phenotype (Table 1). About 0.8% of the total variation in LDL-C and 0.5% of the total variation in HDL-C levels were explained by the interactions for GRL plus α2-ADR and GRL plus LPL markers, respectively.

DISCUSSION

Interaction effects on plasma lipids were observed between the GRL and α2-ADR and GRL and LPL gene markers on LDL-C and HDL-C, respectively. Since these analyses were all performed on data adjusted for fat mass, the results provide strong support for the concept that the interactions are independent of the overall level of fatness. However, it can also be speculated that the interactions may affect plasma cholesterol levels indirectly by influencing the rate of lipolysis, which is known to contribute to an increase in VLDL secretion and in the transfer of VLDL-TG to LDL.[31] The influence on HDL-C could perhaps be best explained by the change in the activity of LPL.

The interactions among the GRL, LPL, and α2-ADR genes were characterized by an association between the 6.3-kb allele of the α2-ADR *Dra*I polymorphism and high plasma insulin in the 4.5-kb carriers of GRL *Bcl*I plus noncarriers of the X447 allele at the LPL locus. In contrast, for other genotype combinations, the 6.3-kb allele carrier status was associated with lower insulin levels compared with the 6.3-kb allele noncarriers. Although the exact mechanisms remain to be determined,

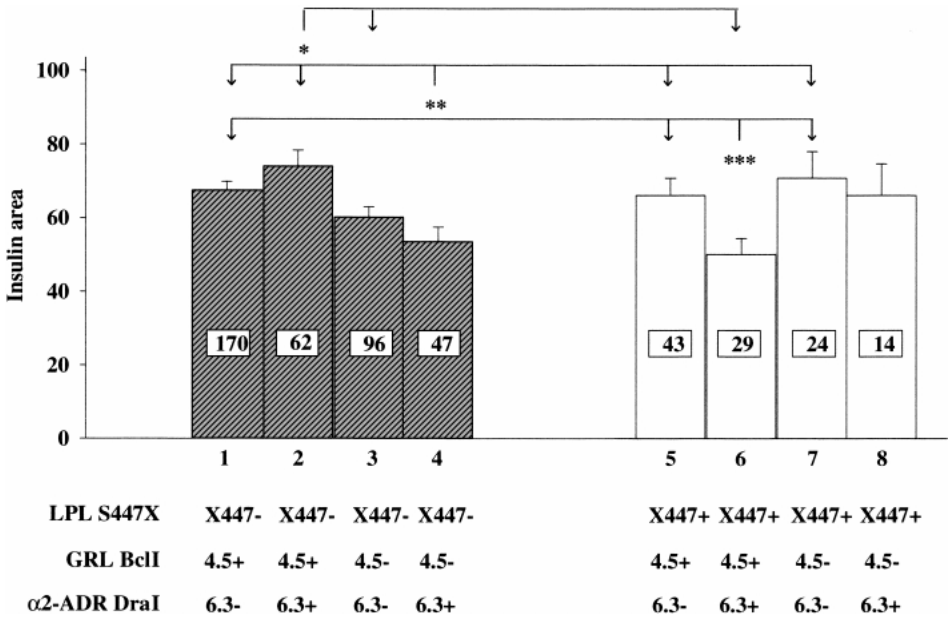

Fig 1. Mean insulin area (10^3 pmol/L · min) under the curve during the OGTT in relation to the LPL, GRL *Bcl*I, and α2-ADR *Dra*I genotypes (mean ± SEM). LPL S447X genotype S447S = X447−, and genotypes S447X and X447X = X447+. The number of subjects is shown in the columns. For the LPL × GRL × α2 ADR marker interaction, *P* = .001. **P* = .007 for 2 *v* 3, *P* < .001 for 2 *v* 6. ***P* = .003 for 4 *v* 1, *P* < .001 for 4 *v* 2, *P* = .033 for 4 *v* 5, *P* = .014 for 4 *v* 7. ****P* = .002 for 6 *v* 1, *P* = .013 for 6 *v* 5, *P* = .006 for 6 *v* 7.

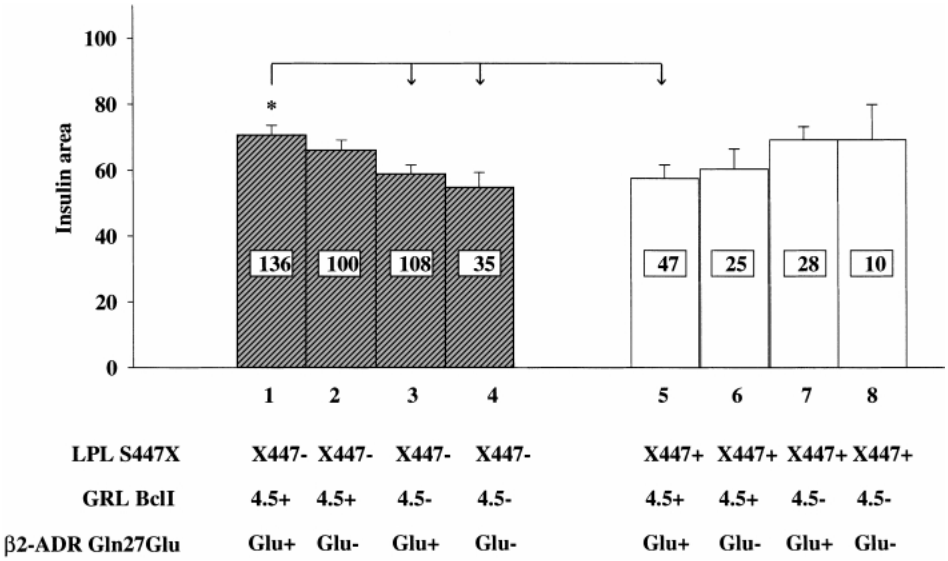

Fig 2. Mean insulin area (10^3 pmol/L · min) under the curve during the OGTT in relation to the LPL, GRL *Bcl*I, and β2-ADR genotypes (mean ± SEM). For the LPL × GRL × β2-ADR marker interaction, *P* = .025. **P* = .002 for 1 *v* 3, *P* = .003 for 1 *v* 4, *P* = .009 for 1 *v* 5.

the interactions among the three loci may influence the rates of lipolysis and FFA release from adipose tissue.

In other studies, the Glu allele of the β2-ADR Gln27Glu variant[32] and the 4.5-kb allele of the GRL *Bcl*I variant[33] were associated with higher plasma insulin, while the GRL *Bcl*I variant was shown to be related to a higher amount of abdominal visceral fat.[34] On the other hand, the X447 allele carriers of the LPL S447X polymorphism have been reported to have higher postheparin LPL activity compared with noncarriers.[35] In the current study, the combined carrier status of β2-ADR Glu and GRL 4.5-kb allele was associated with the highest insulin concentrations. However, this was found only in subjects who were noncarriers of the X447 allele at the LPL S447X polymorphism. Taken together, these studies suggest that subjects who have a lower level of postheparin LPL activity, perhaps as a result of their genotype at LPL S447X, and who also happen to be Glu allele and 4.5-kb allele carriers have the highest risk to develop hyperinsulinemia. Are these subjects genetically more sensitive to the effects of factors known to decrease insulin sensitivity, such as elevated glucocorticoids? This needs to be investigated further.

It has been suggested that the Trp64Arg variant of the β3-ADR gene may increase the susceptibility to insulin resistance, although the association is weak.[36] The data of the present study show that the interactions between the GRL, LPL, and β3-ADR loci modulate the association between the Arg allele and the OGTT insulin area. The effect of the Arg allele seems to be deleterious only in X447 allele carriers, and

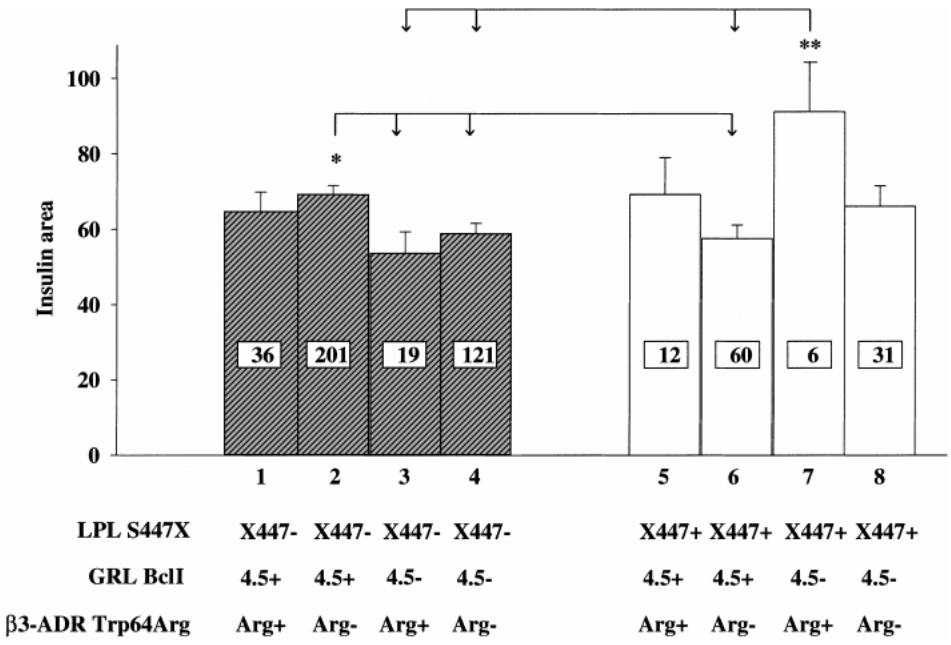

Fig 3. Mean insulin area (10^3 pmol/L · min) under the curve during the OGTT in relation to the LPL, GRL *Bcl*I, and β3-ADR Trp64Arg genotypes (mean ± SEM). For the LPL × GRL × β3-ADR marker interaction, *P* = .005. **P* = .025 for 2 *v* 3, *P* = .002 for 2 *v* 4, *P* = .004 for 2 *v* 6. ***P* = .020 for 7 *v* 3, *P* = .029 for 7 *v* 4, *P* = .024 for 7 *v* 6.

Table 5. Plasma Lipids in Relation to the Genotype at GRL Plus ADR or LPL Genes

Polymorphism		No.	Group	Total Cholesterol	LDL-C	HDL-C	Total TG
GRL *Bcl*I 4.5-kb allele	α2-ADR *Dra*I 6.3-kb allele						
+	−	268	I	5.00 ± 0.06	3.08 ± 0.05	1.21 ± 0.02	1.64 ± 0.09
−	−	153	II	5.04 ± 0.07	3.17 ± 0.06	1.23 ± 0.02	1.46 ± 0.12
+	+	110	III	5.12 ± 0.09	3.27 ± 0.07*	1.18 ± 0.03	1.55 ± 0.14
−	+	76	IV	4.90 ± 0.11	3.01 ± 0.09	1.28 ± 0.03	1.36 ± 0.17
P for interaction				NS	.013	NS	NS
GRL *Bcl*I 4.5-kb allele	LPL S447X X447 allele						
+	−	293	I	5.00 ± 0.05	3.11 ± 0.04	1.18 ± 0.02†	1.67 ± 0.09
−	−	182	II	5.02 ± 0.07	3.14 ± 0.06	1.25 ± 0.02	1.41 ± 0.11
+	+	86	III	5.11 ± 0.10	3.22 ± 0.08	1.28 ± 0.03	1.42 ± 0.16
−	+	46	IV	4.88 ± 0.14	2.98 ± 0.11	1.24 ± 0.04	1.49 ± 0.22
P for interaction				NS	NS	.045	NS

NOTE. Values are the mean ± SEM. +, carriers; −, noncarriers.
*$P = .029$ for III *v* I, $P = .026$ for III *v* IV.
†$P = .004$ for I *v* II, $P = .003$ for I *v* III.

even more so in noncarriers of the 4.5-kb allele of the GRL *Bcl*I variant. However, the situation in noncarriers of the LPL X447 allele is the opposite, with noncarriers of the Arg allele having higher insulin areas. Whatever the mechanisms, these interaction effects among GRL, LPL, and β3-ADR loci may help to explain why the findings on the Trp64Arg polymorphism have been so inconsistent in other studies. Thus, when LPL Ter allele carriers and noncarriers are pooled together, the effect of the Trp64Arg polymorphism on the insulin area disappears. Since there was no difference in OGTT glucose areas between any of the genotype combinations, one could speculate that the interaction effects among GRL, LPL, and ADR genes on insulin metabolism impact insulin sensitivity rather than insulin secretion. The fact that interactions between GRL and LPL and between GRL, LPL, and α2-ADR genes also influenced fasting plasma insulin supports this hypothesis.

What are the potential functional links between the variants and metabolic abnormalities reported here? First, the GRL *Bcl*I variant is not located in a coding region and its functional role is uncertain.[34] However, 4.5-kb allele carriers of the GRL *Bcl*I polymorphism have been reported to have higher cortisol values.[37] This supports the concept that GRL function either centrally or peripherally may be affected by the GRL polymorphism or perhaps by another mutation in linkage disequilibrium with it. Second, the LPL S447X mutation changes the amino acid sequence of LPL and was shown to have an effect on postheparin LPL activity[35]; moreover, it is in linkage disequilibrium with the *Bam*HI and *Hin*dIII polymorphisms.[24] The S447X mutation may also alter TG uptake into adipocytes, and this could play a role in insulin resistance.[38] The functional significance of the α2-ADR *Dra*I variant is not yet known, although the mutation does not seem to be within a coding region of the gene.[39] Equally, the importance of the β3-ADR Trp64Arg mutation on the phenotypes of the study is still debated.[36] The β2-ADR Gln27Glu and Arg16Gly markers are associated with altered β2-ADR function in recombinant cells.[40,41] The β2-ADR Arg16Gly variant has been associated with an increase in agonist sensitivity in women, although such an effect has not been reported for the Gln27Glu variant, which is in tight linkage disequilibrium with the former.[22,32] Therefore, in the presence of the joint occurrence of the LPL S447 and GRL 4.5-kb variants, an increased storage of TG in adipocytes and an elevated hypothalamic-pituitary-adrenal axis activity, as evidenced by higher cortisol levels, could progressively lead to higher insulin levels and insulin resistance.

In conclusion, the GRL and LPL variants had independent effects on plasma HDL-C and both β2-ADR variants had independent effects on total cholesterol concentrations, whereas the α2-ADR gene *Dra*I polymorphism was the only variant that had an independent effect on the OGTT plasma insulin area. Multiple interactions among the GRL, LPL, and ADR loci affecting the variation in OGTT insulin response and lipid levels independently of the overall level of fatness were also observed. These interaction effects seem to be more significant for the insulin phenotype, since the findings remained essentially unchanged for the OGTT insulin area after correction for multiple tests. The LPL locus seems to determine the extent of the interaction effects between the GRL and ADR loci. Thus, these data support the hypothesis that gene-gene interactions influence plasma insulin and lipid levels. However, these interaction effects account for only a relatively small proportion of the variance in the phenotypes.

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