Association of Lipoprotein Lipase Gene Variation with the Physiological Components of the Insulin-Resistance Syndrome in the Population of the San Luis Valley, Colorado

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OBJECTIVE — To cross-sectionally evaluate the presence of clustering of the insulin-resistance syndrome components. Tests were conducted for association of the *Hind*III restriction site polymorphism at the lipoprotein lipase locus with clustering of the physiological components of the insulin resistance syndrome.

RESEARCH DESIGN AND METHODS — DNA samples of 370 normoglycemic Hispanics and 520 normoglycemic non-Hispanic whites from the San Luis Valley, Colorado, were amplified by the polymerase chain reaction. Lipids and glucose were determined by the standard procedures. Cross-tabulation and χ^2 analysis were used.

RESULTS — The insulin-resistance syndrome components (elevated fasting insulin, reduced high-density lipoprotein cholesterol, and elevated triglycerides) appeared together in individuals of this population sample more often than expected by chance. Individuals in the population with the (+/+) lipoprotein lipase-*Hind*III restriction of fragment-length polymorphism genotype were more likely to have elevated fasting insulin and triglycerides and a reduced high-density lipoprotein-cholesterol level than subjects with the (+/−) genotype (odds ratio = 2.3, 95% confidence interval 1.38–3.98).

CONCLUSIONS — As expected from the physiological function of lipoprotein lipase, the primary association of lipoprotein lipase genotypes is with triglyceride and high-density lipoprotein-cholesterol levels. This appears to be the first reported genetic association with the insulin-resistance syndrome and may reflect genotype specific differences in the regulation of lipoprotein lipase by insulin.

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TG, triglyceride; HDL, high-density lipoprotein; NIDDM, non-insulin-dependent diabetes mellitus; CAD, coronary artery disease; IRS, insulin-resistance syndrome; LPL, lipoprotein lipase; RFLP, restriction fragment length polymorphism; SLVDS, San Luis Valley Diabetes Study; WHO, World Health Organization; BP, blood pressure; dBP, diastolic blood pressure; BMI, body mass index; PCR, polymerase chain reaction; bp, base pair; df, degrees of freedom; CI, confidence interval.

Sustained hyperglycemia, dyslipidemia (e.g., high levels of TGs and low levels of HDL cholesterol in plasma), and hyperinsulinemia tend to cluster in obese individuals and are traits shared by patients with NIDDM, hypertension, and CAD. Syndrome X (1) and the IRS (2) are collective terms currently used to refer to these co-occurring disorders. The term IRS makes explicit the widely supported hypothesis that an insufficient insulin-mediated uptake of glucose (insulin resistance) and a compensating increase in insulin secretion leading to hyperinsulinemia is the central feature of this syndrome (3–4). However, the existence of this syndrome has been questioned (5–6), and in some cases its predictions have not been met. For instance, in some populations with a high frequency of NIDDM no concomitant increases in CAD have been found (7–8). Moreover, severe insulin resistance may exist without hypertension (9).

A definite test of the IRS hypothesis would be a prospective study in which the development of insulin resistance was shown to precede the development and clustering of the elements of the syndrome. A caveat to such a study is the relative uncertainty of the current direct measurements of insulin resistance, especially at its early stages, and the difficulty in conducting such measurements on a population scale.

An alternative approach to the study of insulin resistance in populations is to rely on indirect measurements of insulin resistance, such as an elevated circulating insulin without a β-cell defect (10–11). In fact, several cross-sectional studies have shown that elevated levels of fasting insulin relate to the presence of the IRS elements (12–16). In a prospective study, Haffner et al. (17) showed that increased levels of fasting insulin correlate positively with the incidence of clusters of IRS-related disorders in both Mexican Americans and non-Hispanic whites, regardless of sex. The

possible contribution of genetics to the IRS has not been widely explored, despite the fact that some individual elements of this syndrome show a strong genetic component, namely obesity and diabetes.

In several studies, variation at the LPL gene has been found in association with elevated plasma levels of TGs and fasting insulin and with low plasma levels of HDL cholesterol (18–21). LPL plays a key role in plasma lipid metabolism: it hydrolyzes TGs, providing the tissues with free fatty acids for oxidation or storage, and affects the maturation of circulating lipoprotein particles. LPL is synthesized in many tissues, but the primary sites of synthesis are in muscle and adipose tissue. Tissue-specific regulation of LPL synthesis and activity is mediated by insulin, perhaps by other hormones, and by nutritional status (22). Because IRS involves abnormalities in lipid metabolism, in circulating insulin levels, and increased deposition of adipose tissue, the LPL gene (chromosome $8P^{22}$) could be an important candidate gene in determining the expression of the IRS phenotype.

Herein, we cross-sectionally evaluate the clustering of elevated fasting insulin levels, elevated plasma TGs, and reduced HDL-cholesterol levels in normoglycemic individuals. Then, we test a genetic hypothesis by examining the association between the presence of this cluster and *Hind*III RFLP at the LPL locus in the population of the San Luis Valley, Colorado.

RESEARCH DESIGN AND METHODS

Sampling

The subjects of this study are a subset of the SLVDS. The SLVDS was designed to examine risk factors for NIDDM using a case-control approach in Hispanic and Anglo populations from the San Luis Valley in southern Colorado (23). The Anglo population immigrated to the valley from various northern European countries. Much of the Hispanic population has lived in this area since the time of Spanish and Mexican land grants in the 1800s. Today, little immigration to the area has taken place, especially from Mexico, in contrast with conditions in Texas and southern California. During the 150 years since the settlement of this valley, little Amerindian-Hispanic admixture has occurred. According to serological data, the proportion of Amerindian genes is 3% among the Anglos and 19% among the Hispanics. Sampling in the SLVDS has been detailed elsewhere (23). These analyses are based on 1099 unrelated normoglycemic control subjects that are representative of the population of the San Luis Valley, of whom 890 had DNA samples available. Control subjects were selected in a two-stage procedure. First, ~205 of all residents in the San Luis Valley area were given a home interview. Second, a random selection of interviewed subjects (stratified by age, sex, ethnic group, and county) was conducted. The subjects had never been told by a physician that they had diabetes, and their normal glucose tolerance was confirmed by a 75-g oral glucose tolerance test, following the WHO criteria. Ethnicity was self-reported.

Measures

Lipids and glucose were determined in the Clinical Research Center, University Hospital, Denver, Colorado, from blood drawn after an overnight (>8–12 h) fast. BP was measured 3 times after the subject was in a supine position for at least 5 min; the average of the second and third readings was considered the subject's BP. BMI was used as a measure of obesity (24).

DNA analysis

DNA was extracted from lymphocytes as described by Miller et al. (25). DNA samples of 520 non-Hispanic whites and 370 Hispanics were amplified by the PCR in a Perkin-Elmer Cetus DNA Thermal Cycler (Norwalk, CT). The primers were derived from sequences from exons 8 and 9 of the LPL gene, flanking a *Hind*III restriction site located in intron 8 (the forward primer was 5′TTTAGGCCTGA AGTTTCCAC–3′; the reverse primer was 5′CTCCCTAGAACAGAAGATC–3′) (26). The amplified fragment was 1.3-kb long.

The 50-μl reaction mixture contained 1 × PCR buffer (10 mM Tris, pH8.3, 50 mM KCl, 1.5 mM MgCl2), dNTPs at 200 μM, 0.3 μM each primer, 0.5 μg genomic DNA, and 1.25 U of Taq DNA polymerase. The amplification of the region flanking the *Hind*III site was conducted for 33 cycles at 95°C for 1 min, at 60°C for 2 min, and at 72°C for 2 min. Amplified products were digested with *Hind*III, and the resulting fragments were separated on 2% agarose gels. When the restriction site is present (+ allele) the digestion will yield two fragments of 600 and 700 bp.

Statistical analyses

To examine the association of the RFLP at the LPL locus with the physiological IRS components, we divided our sample by tertiles of TGs, HDL cholesterol, and fasting insulin. We then created a group with all those individuals in the upper tertiles of the distributions of TGs and fasting insulin, those in the lower tertile of HDL cholesterol (the syndrome group), and the rest of the sample defined as the nonsyndrome group. The tertile analysis selects individuals with extreme values in the phenotypic distributions as the syndrome group in the normoglycemic individuals. The tertile analysis selects individuals who have already expressed the IRS components and those at the highest risk of expressing the IRS phenotype. We conducted the analysis among normoglycemic individuals to avoid the confounding effects of unknown diabetes duration and undocumented control. Cross-tabulation and χ^2 analysis were used to test the genotype-group association.

RESULTS— Table 1 shows the means ± SE of fasting TGs, fasting insu-

Table 1—*Mean levels of TG, FINS, HDL cholesterol, BMI, and dBP in the syndrome and the nonsyndrome groups drawn from the San Luis Valley, Colorado, normoglycemic sample*

	Syndrome		Nonsyndrome	
	n	Mean ± SE	*n*	Mean ± SE
BMI (kg/m^2)	90	28.4 ± 0.4	799	25.3 ± 0.1
FINS (μIU/ml)	91	21.3 ± 0.9	799	11.1 ± 0.2
TG (mg/dl)	91	262.2 ± 17.2	799	131.5 ± 2.1
HDL cholesterol (mg/dl)	91	33.5 ± 0.5	799	52.5 ± 0.5
dBP (mmHg)	91	76.7 ± 0.9	799	74.6 ± 0.3

lin, HDL cholesterol, BMI, and dBP in the syndrome group, which is defined as individuals in the upper tertile of TG and fasting insulin but in the lower tertile of HDL cholesterol. The nonsyndrome group is defined as individuals in the lower two tertiles of TG and fasting insulin and in the upper two tertiles of HDL cholesterol. As expected, the mean levels of TG and fasting insulin are higher, and the mean levels of HDL cholesterol are lower in the syndrome group. Note that the mean BMI and dBP are also elevated in this group as expected (1).

Table 2 shows the LPL-*Hind*III genotypes in the syndrome and nonsyndrome groups defined by levels of TG, fasting insulin, and HDL cholesterol as before. Clustering of these three physiological components of the IRS is evident from the total number of individuals in each group. In a total sample of 890 individuals, we would expect to observe 33 individuals in the syndrome group, if the three components of the syndrome were independent. In this population-based sample of 890 normoglycemic individuals, 91 have the combination of elevated TG and fasting insulin and reduced HDL cholesterol. The LPL-*Hind*III genotype frequency of this group was compared with the LPL-*Hind*III genotype frequency of the rest of the sample (the nonsyndrome group). Table 2 shows the distribution of LPL genotypes in these two groups. The genotype-group association is highly significant (χ^2 = 12.20, 2 df, P = 0.002): the (+/+) genotype frequency is 57% in the nonsyndrome group and 76% in the syndrome group. Normoglycemic individuals with the (+/+) genotype are 2.3 times (95% CI 1.38–3.98) more likely to be in the syndrome group than individuals with the (+/−) genotype.

CONCLUSIONS— The goal of this study was to test whether genotype defined by the *Hind*III polymorphism at the LPL locus was associated with the occurrence of three physiological features of the IRS in individuals. In the normoglycemic population of the San Luis Valley, the combination of elevated fasting insulin and TG levels and reduced HDL cholesterol occurred more frequently than expected if these three metabolic conditions were independent. The frequency of the LPL-*Hind*III (+) site determined by the presence of a restriction site polymorphism in intron 8 of the LPL gene was significantly greater in the syndrome group (0.86) than in the total population (0.76; P = 0.007) or in the nonsyndrome group (0.75; P = 0.002). Thus, the clustering in an individual of elevated fasting insulin and TGs and reduced HDL cholesterol is significantly associated with the presence of the LPL-*Hind*III (+) allele in this population.

The IRS was originally defined to include hypertension, obesity, and NIDDM. Clinically defined NIDDM is associated with a variety of altered metabolic parameters but the specific etiological relationship between NIDDM and changes in lipid levels and BP is not known. Initial analyses did not show any association of the LPL polymorphism with either hypertension or obesity. A large normoglycemic cohort from the general population of the San Luis Valley was examined to determine whether extreme values of the quantitative traits related to the clinically defined end points of the IRS tended to occur together in a normoglycemic sample. Table 1 indicates a difference between the syndrome and nonsyndrome groups in fasting insulin, TGs, HDL cholesterol, BMI, and BP. The association between the LPL-*Hind*III genotype and biochemical endpoints related to the IRS suggests that the genotype at the LPL locus plays a role in determining an individual's susceptibility to develop the IRS. The association of various components of the IRS in the general population has been noted by others (17,27), but association with gen-

Table 2—*Distribution of LPL-HindIII genotypes in the syndrome and the nonsyndrome groups drawn from the San Luis Valley, Colorado, normoglycemic population*

	LPL genotypes			
	−/−	+/−	+/+	
	obs (exp)	obs (exp)	obs (exp)	Total
Syndrome	3 (5.6)	19 (31.9)	69 (53.4)	91
Nonsyndrome	52 (49.4)	293 (280.1)	454 (469.5)	799

For genotype-group association, χ^2 = 12.20; P = 0.002; odds ratio for (+/+) with syndrome vs. (+/−) = 2.3; 95% CI 1.38–2.98. Obs, observed; exp, expected.

otype at a specific genetic locus has not been reported previously.

Any explanation for the association between the LPL genotype and the IRS must be speculative at this time. The LPL-*Hind*III polymorphism occurs in intron 8 of the LPL gene, and no functional significance can be assigned to the nucleotide substitution leading to this polymorphism. Thus the effect noted for the *Hind*III polymorphism must be caused by its nonrandom association with a functional mutation elsewhere in the gene. Elevated levels of circulating insulin are characteristic of insulin resistance, and insulin is known to regulate LPL activity. Although the dominant site of regulation is not known, studies of cultured adipocytes suggest that insulin regulates LPL activity at the mRNA level (22,28) and/or by posttranslational mechanisms (29). We speculate that the (+) allele is associated with an LPL functional allele that is less sensitive to insulin regulation or leads to expression of a reduced LPL activity allele. This would explain the elevated TG levels observed in IRS. The reduced HDL-cholesterol levels would arise from reduced remodeling of circulating TG-rich lipoprotein particles by the *Hind*III (+) associated allele. This finding is consistent with the observation of lower HDL-cholesterol levels in individuals with heritable forms of lipoprotein lipase deficiency. The alternative possibility that the (+) allele is associated with increased LPL function, leading to increased storage of TG in fat cells, a decrease in adipocyte insulin sensitivity, and an increase in insulin secretion cannot be ruled out without molecular studies to identify the associated functional mutation.

In conclusion, the results obtained from this cross-sectional study support a genetic basis for the IRS in this population sample from the San Luis Valley and suggest that the expression of this complex phenotype is affected by genetic variation at the LPL locus. Replication in other populations is needed to confirm or refute theses observations.

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