No Evidence of Linkage Between the Very–Low-Density Lipoprotein Receptor Gene and Fasting Serum Insulin or Homeostasis Model Assessment Insulin Resistance Index: The National Heart, Lung, and Blood Institute Family Heart Study

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A major gene effect on the fasting insulin level and insulin resistance has been suggested in previous studies. Several candidate genes for insulin resistance in rare syndromes have been proposed. However, there has been limited success in finding genes for common forms of insulin resistance. There is accumulating evidence of a relationship between insulin resistance and a disturbance of free fatty acid (FFA) metabolism. The very–low-density lipoprotein (VLDL) receptor, which is associated with FFA metabolism, could serve as a possible candidate gene for insulin resistance. We performed linkage analyses between the VLDL receptor gene and fasting insulin and the homeostasis model assessment (HOMA) insulin resistance index (fasting insulin · fasting glucose/22.5) in 1,050 sibpairs participating in the phase II physical examination of the National Heart, Lung, and Blood Institute Family Heart Study (FHS). Data analyses were completed using the SIBPAL component of the SAGE software package (SAGE, Statistical Analysis for Genetic Epidemiology, Version 3.1; Computer program package available from the Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH, 1997). We did not find evidence for linkage of the fasting insulin or the HOMA insulin resistance index with a polymorphic marker at the VLDL locus ($P = .316$ and .402, respectively). Adjustment of fasting insulin and the HOMA insulin resistance index for the body mass index (BMI) did not change the results ($P = .319$ and .472, respectively). In conclusion, no evidence was found for a linkage between a locus controlling the fasting insulin level or HOMA insulin resistance index and a VLDL polymorphism in the present study. Additional adjustment of fasting insulin or the HOMA insulin resistance index for the BMI did not change the linkage results significantly.

INSULIN RESISTANCE, type 2 diabetes, and atherosclerotic disease are common chronic diseases in our society. Both genes and environmental factors are involved in the genesis of these traits. A moderate to high genetic influence on insulin resistance indices, such as fasting insulin and the homeostasis model assessment (HOMA) insulin resistance index, has been observed.[1-4] Moreover, a major gene effect on fasting insulin has also been reported by some studies,[5-7] but not all.[8] Several candidate genes for rare syndromes involving insulin resistance have been proposed[9]; however, despite intensive efforts, the success rate in finding genes for common forms of insulin resistance has been disappointing.

The very–low-density lipoprotein (VLDL) receptor is a member of the low-density lipoprotein (LDL) receptor family.[10,11] The chromosomal location of the structural gene for the VLDL receptor is 9p24.[12] Like the LDL receptor, the VLDL receptor binds apolipoprotein E–containing lipoproteins, but unlike the LDL receptor, it does not bind LDL. Another important difference between the VLDL receptor and LDL receptor is that the LDL receptor is expressed abundantly in the liver and adrenal gland, while the VLDL receptor is expressed mainly in skeletal muscle, adipose tissue, and the heart. Its tissue distribution and ligand specificity have led to the hypothesis that the VLDL receptor plays a role in the delivery of triglyceride-rich lipoproteins to peripheral tissue.[13] However, unlike the LDL receptor, the functions of the VLDL receptor in the human are far from clear. Because VLDL receptor mRNA is abundant in tissues that are the site of active fatty acid metabolism and insulin-mediated glucose disposal[10] and fatty acid variation is involved in the regulation of insulin resistance,[14-16] we were prompted to investigate the association between the VLDL receptor and fasting insulin resistance.

In the present investigation, we used quantitative trait locus (QTL) sibpair linkage methodologies and data from the National Heart, Lung, and Blood Institute Family Heart Study (FHS)[17] to test for the presence of a linkage between insulin resistance (fasting insulin and the HOMA insulin resistance index) and a VLDL receptor polymorphic locus. Since the body mass index (BMI) is a major correlate of insulin resistance, we compared the linkage results for insulin resistance before and after adjustment for the BMI.

SUBJECTS AND METHODS

Subjects

The FHS is an investigation of the genetic and nongenetic determinants of coronary heart disease (CHD), preclinical atherosclerosis, and cardiovascular risk factors.[17] The study subjects were recruited from 4 population-based cohorts: 2 of the 4 Atherosclerosis Risk in Communities Study communities (cohorts from Forsyth County, NC, and the northwest suburbs of Minneapolis, MN), the Utah Health Family Tree

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Study in Salt Lake City, and the Framingham Heart Study. "Probands" aged 45 to 69 years were selected based on a "high-risk" family risk score that relates the observed number of CHD events within a family to the number expected based on age- and sex-specific incidence rates derived from the Framingham Study.[18] Adult family members aged 25 and older were contacted by mail and asked to complete a medical history questionnaire; 5,975 individuals were recruited from 541 randomly selected families and 610 families with elevated CHD risk scores to undergo physical examinations. Eligible family members included the proband and his or her parents, siblings, spouses, and children. The high-risk families had a mean of 2.8 relatives with CHD events.

The data for the current investigation were collected during phase II, the clinical examination of the FHS. The sample selected for genotyping consists of (1) sibships in which at least 2 full siblings had confirmed CHD (n = 243 sibs); (2) sibships in which at least 1 sibling had carotid artery intima media thickness above the 90th percentile of the study distribution (n = 231 sibs); (3) sibships with at least 2 full siblings at or above the age/sex-specific 80th percentile of the Individual Risk Score (a score derived for each individual using a sex-specific proportional hazards model to predict the age of onset of CHD based on carotid artery intima media thickness, lipids, BMI, blood pressure, hypertension, diabetes, and a CHD family history score; n = 1,090 sibs); and (4) randomly selected, unrelated subjects (n = 185). The unrelated subjects were used only in the calculation of allele frequencies, and did not contribute to the statistical genetic analysis. Since many of these participants belonged in more than 1 category, the number of siblings with VLDL genotypes was 1,292. Among the subjects, 94 with diabetes or diabetes medication were excluded from the statistical genetic analysis. This resulted in a total number of siblings of 1,198. Because a family of 2 siblings can contribute 1 sibpair, 3 siblings can contribute 3, 4 siblings can contribute 6, 5 siblings can contribute 10, and so on, the available number of sibpairs for the present study is 1,050. The main characteristics of the study population are described in Table 1.

The study was approved by an institutional review committee at each site, and all subjects provided written informed consent.

VLDL receptor genotyping. Genotyping for the VLDL receptor is based on polymerase chain reaction amplification of the subjects' genomic DNA. Primers for the trinucleotide repeat (CGG) marker are located in the 5′ untranslated region of the VLDL receptor gene.[19] The allele frequency distribution for the VLDL receptor gene is summarized in Table 2. The heterozygosity index for the VLDL receptor gene is 0.668 in the FHS sample.

Insulin, glucose, and HOMA insulin resistance index. Serum insulin was determined by radioimmunoassay (Coat-A-Count; Diagnostic Products, Los Angeles, CA). Serum glucose levels were measured using an enzymatic (glucose oxidase) method (Kodak EKTACHEM 700 Analyzer; Eastman Kodak, Rochester, NY). The HOMA insulin resistance index was calculated as (fasting insulin · fasting glucose)/22.5.[20]

Table 1. Characteristics of the Study Population

Characteristic	No. of Subjects	Mean ± SD
Age (yr)	1,198	57.4 ± 11.1
BMI (kg/m²)	1,186	28.8 ± 5.8
HDL cholesterol (mg/dL)	1,194	50.5 ± 15.6
Triglycerides (mg/dL)	1,194	161.6 ± 108.4
Fasting glucose (mg/dL)	1,196	98.6 ± 19.0
Fasting insulin (μU/mL)	1,195	11.8 ± 8.3
HOMA insulin resistance index	1,195	53.9 ± 46.1

NOTE. HOMA insulin resistance index = (fasting insulin · fasting glucose)/22.5.

Table 2. Allele Frequency Distribution of the VLDL Receptor Gene

Allele	Frequency
193	0.003
196	0.367
199	0.003
202	0.008
205	0.246
208	0.359
211	0.003
214	0.010
217	0.003

Blood lipids. Total triglyceride levels were measured using enzymatic methods. High-density lipoprotein (HDL) cholesterol levels were measured after dextran-magnesium precipitation.

Anthropometric measures. All anthropometric measurements were made with the participants wearing a scrub suit or an examination gown without shoes. Standing height, rounded down to the nearest centimeter, was measured using a wall-mounted vertical metal ruler. Body weight was recorded to the nearest pound using a balance scale. The BMI was calculated as (weight in kilograms)/(height in meters)^2.

Statistical Analysis

A logarithmic transformation was made for insulin and the HOMA insulin resistance index before genetic analyses because of the extreme skewness in the distribution of these measures.

Age adjustment. Adjustments for the effects of age on insulin and the HOMA insulin resistance index were performed separately by sex using a stepwise multiple regression procedure. Age, age^2, and age^3 were included in the regression model. The significance level for retaining the terms in the stepwise regression analysis was .15. Standardized residuals from the sex-specific age-adjusted regression models were used in the linkage analysis.

Linkage analysis. The methodology of the sibpair test of linkage was used to test for evidence of linkage between a genetic locus controlling a quantitative trait of interest (fasting insulin and the HOMA insulin resistance index in the present study) and a specific polymorphic marker locus (the VLDL receptor locus in the present study).[21] Sibpair linkage analyses were performed with the SIBPAL program (SAGE, Statistical Analysis for Genetic Epidemiology, Version 3.1; Computer program package available from the Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH, 1997).

Assuming no genetic heterogeneity, a true linkage result, from either a LOD score method or a QTL method, will hold in different families. In the present study, we used a QTL sibpair linkage approach. The marker locus is a trinucleotide repeat polymorphic VLDL receptor locus, and the quantitative traits are fasting insulin and the HOMA insulin resistance index. All sibpairs are tested simultaneously for linkage in 1 regression analysis for each trait (fasting insulin or the HOMA insulin resistance index) and the VLDL receptor marker locus. The squared

Table 3. Linkage Analysis Between Fasting Insulin or the HOMA Insulin Resistance Index and the VLDL Receptor

Genotype	Phenotype	No. of Pairs	Regression Slope	
			Slope (SE)	*P*
VLDL receptor	ln(insulin)	1,050	−0.48	.32
	ln(IR)	1,050	−0.25	.40
	ln(insulin) adjusted for BMI	1,027	−0.46	.32
	ln(IR) adjusted for BMI	1,027	−0.07	.47

NOTE. All phenotypes were age-adjusted.

Abbreviations: IR, HOMA insulin resistance index. ln, natural logarithm.

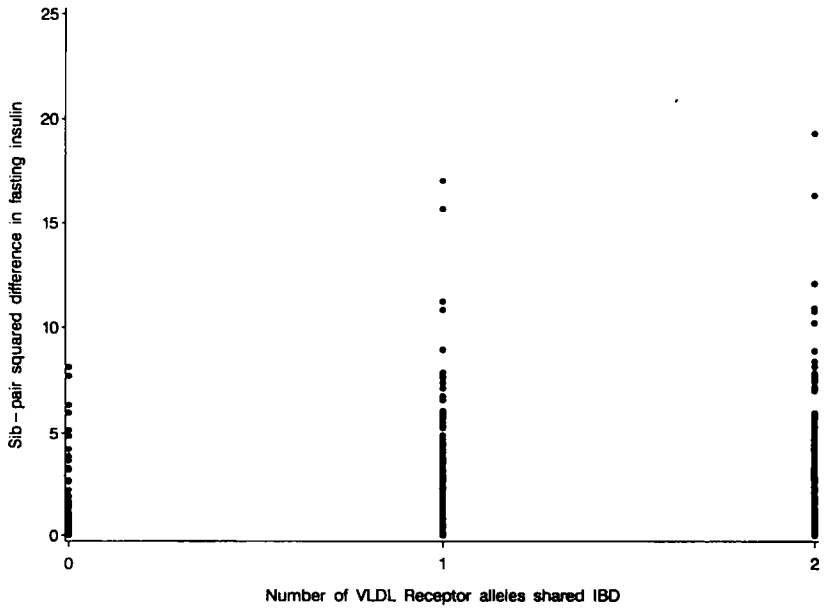

Fig 1. Association between sibpair squared difference in age- and sex-adjusted fasting insulin and the number of VLDL receptor alleles shared IBD.

difference in the trait is regressed on the estimated proportion of a VLDL receptor marker locus that a sibpair shares identical by descent (IBD). Assuming a codominant effect of the alleles, the squared difference in the quantitative trait should be smallest in sibpairs sharing 2 alleles IBD, intermediate in sibpairs sharing 1 allele IBD, and largest in sibpairs sharing no alleles IBD. In our sample, 44.5%, 44.2%, and 11.3% of the sibpairs shared 2, 1, and no alleles IBD, respectively. An overall estimate of excess allele-sharing applies only to affected/unaffected analysis and not to a QTL analysis as presented here. A common regression slope is estimated for all sibpairs and is the statistic which, when significantly negative, is interpreted as evidence for linkage. Since the BMI is a major correlate of insulin resistance, further linkage analyses were performed with the BMI adjusted for fasting insulin and the HOMA insulin resistance index; ie, residuals from regression models with the BMI treated as an independent variable were used in linkage analyses.

RESULTS

We tested for linkage between the VLDL receptor locus and the quantitative traits of fasting insulin and the HOMA insulin resistance index using a QTL sibpair method (Table 3). We did not find evidence for linkage between a locus on chromosome 9 that controls the fasting insulin level and the polymorphic marker at the VLDL receptor locus ($P = .32$). A linkage between a locus on chromosome 9 for the HOMA insulin resistance index and the polymorphic marker at the VLDL receptor locus also is not supported ($P = .40$). After adjustment for the BMI, the linkage between the VLDL receptor locus and the locus for the fasting insulin or HOMA insulin resistance index also was not supported ($P = .32$ and .47, respectively; Table 3), suggesting that the BMI does not affect the linkage

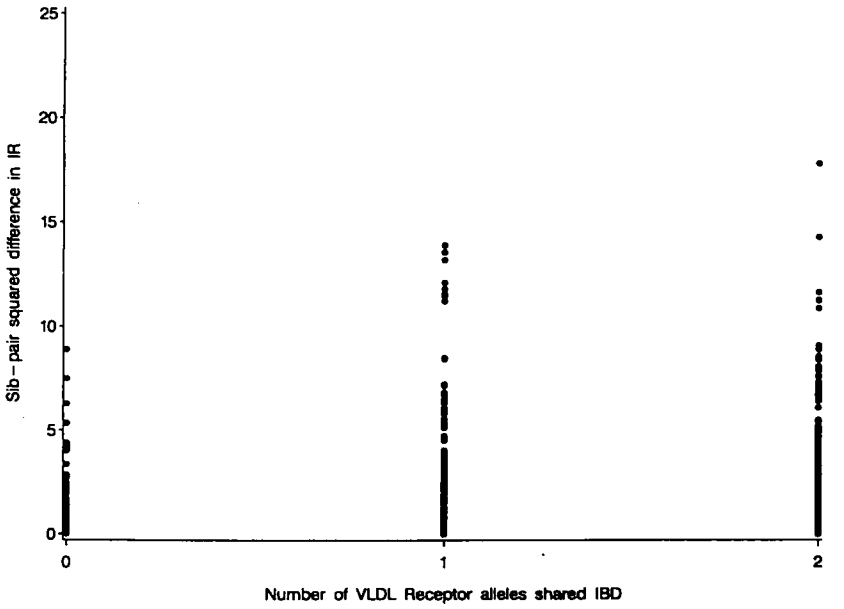

Fig 2. Association between sibpair squared difference in the age- and sex-adjusted HOMA insulin resistance index (IR) and the number of VLDL receptor alleles shared IBD.

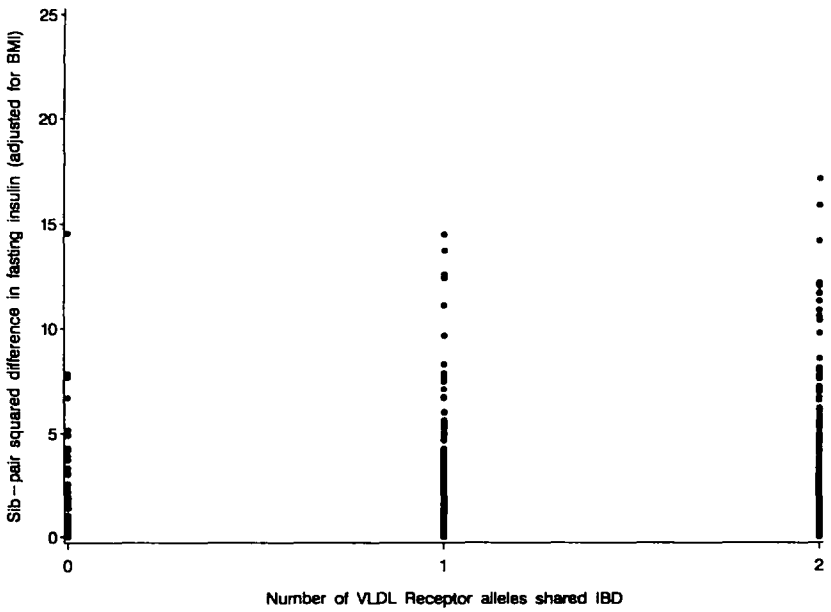

Fig 3. Association between sibpair squared difference in age-, sex-, and BMI-adjusted fasting insulin and the number of VLDL receptor alleles shared IBD.

between insulin resistance and the VLDL receptor locus. Figures 1 and 2 show the associations between the number of VLDL receptor alleles shared IBD and the sibpair squared differences in fasting insulin and HOMA insulin resistance without adjustment for the BMI. Figures 3 and 4 show these associations after adjustment for the BMI.

DISCUSSION

In the present study, we did not find evidence for a linkage between fasting insulin or the HOMA insulin resistance index and the VLDL receptor. Further adjustments of fasting insulin and the HOMA insulin resistance index for the BMI did not significantly alter the linkage results.

Although several genes for rare forms of insulin resistance have been proposed,[9] the search for genes for common forms of insulin resistance has been unsuccessful. Since the VLDL receptor has been reported to contribute to a variation in free fatty acid (FFA) metabolism, and FFA metabolism is associated with glucose metabolism and insulin resistance, the linkage between a locus for insulin resistance and the VLDL receptor locus was examined in the present study. However, within the FHS, we failed to find any evidence for linkage. There may be 3 reasons that we did not find a linkage. First, there may not be an insulin resistance–related trait locus linked to the VLDL receptor. Second, the locus may have a minor effect that could not be detected in the present study. Since a major gene effect on

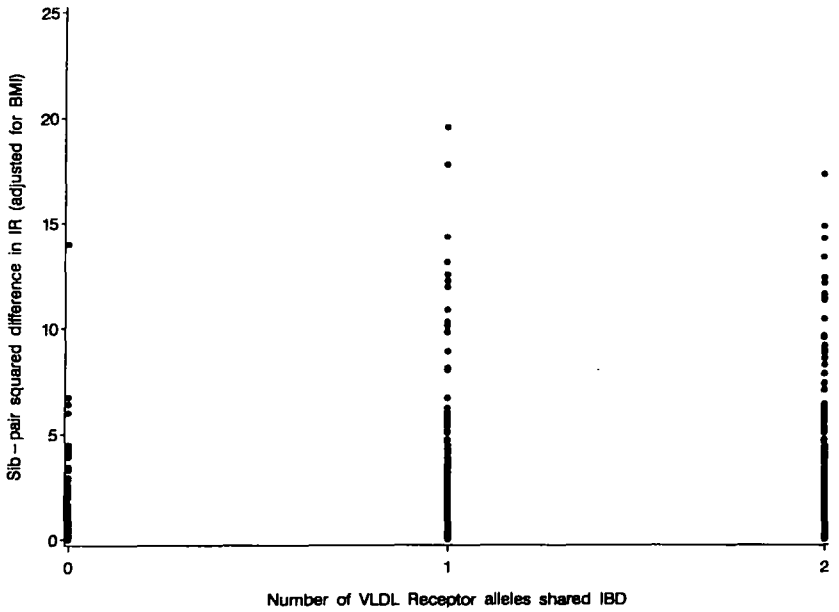

Fig 4. Association between sibpair squared difference in the age-, sex-, and BMI-adjusted HOMA insulin resistance index (IR) and the number of VLDL receptor alleles shared IBD.

fasting insulin was found in some studies, but not all,[5-8] it is likely that a few to several genes with moderate effects (oligogenic effects) on insulin resistance are present in the general population. Third, there might be tissue-specific gene expression, a locus for insulin resistance in specific tissues that may be linked to the VLDL receptor locus but does not affect plasma insulin levels.

With respect to covariate effects, although adjustment for the BMI did not influence the evidence for linkage between insulin resistance and the VLDL receptor locus, it does not preclude that the BMI would affect the linkage between a locus for insulin resistance and other potential candidate loci. Since the BMI and other obesity phenotypes are strongly correlated (partially genetic) with insulin resistance, it is interesting to assess the covariate effects of obesity phenotypes on genetic analyses of insulin resistance, or vice versa.

It should be noted that both methodological limitations and phenotypic and genetic heterogeneity might limit our ability to detect a gene of moderate effects. Our high-risk families were selected based on several criteria. Multiple criteria were used for selection to increase statistical power. Another reason for the use of multiple selection criteria is that CHD, carotid artery intima media thickness, and high cardiovascular risk scores, in general, share a common pathophysiologic and genetic background as well. Nevertheless, we performed genetic analyses for these 3 groups separately and did not find a significant difference across the groups. In addition, insulin resistance is unlikely monogenic and is most likely apparent only when present in combination with unfavorable environmental factors.[22] Therefore, possible gene-gene and gene-environment interactions may also confound the linkage results. It should also be noted that sibpairs sharing 2 alleles IBD were found at a high proportion in our study population, which indicates that a high percentage of the sibpairs are full and possibly affected sibpairs. However, given the fact that our study population was selected based on high cardiovascular risk, it is not unexpected to have a high percentage of sibpairs sharing 2 alleles IBD. Although no significant linkage signal was found between the VLDL receptor gene and insulin resistance in the present study, further investigation of the relationship between the VLDL receptor gene and other cardiovascular risk factors is warranted.

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