Genetic Relationship Between Measures of HDL Phenotypes and Insulin Concentrations

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Abstract We used data from the San Antonio Family Heart Study to determine the HDL correlates of the insulin resistance syndrome (IRS), as reflected by insulin concentrations in nondiabetic subjects. We measured insulin concentrations both in the fasting state and 2 hours after a glucose challenge (2-hour insulin) and we assessed seven aspects of HDL phenotype, including size and concentration of both lipid and protein components. Measurements were obtained from 1202 nondiabetic members of 42 families. Initial quantitative genetic analyses revealed that a substantial portion of phenotypic variation in the nine variables was due to genes (heritabilities, h^2, ranged from 0.32 to 0.47). We then conducted a series of bivariate genetic analyses, which indicated that there were significant additive genetic correlations (ie, pleiotropy) between the two measures of insulin and five of seven HDL measures tested, including concentrations of HDL cholesterol (fasting insulin only) and triglyceride, and HDL size distributions of apoAI, apoAII, and cholesterol; concentrations of apoAI and apoAII were not genetically related to either insulin measure. Increased insulin levels were associated with relatively smaller HDL phenotypes, and considering a similar association with small, dense LDLs, this finding suggests a common effect of insulin resistance on particle size distributions for these lipoproteins. Thus, these results suggest the existence of genes that pleiotropically influence variation in both HDLs and insulin levels and therefore contribute to the clustering of proatherogenic traits in the IRS. (***Arterioscler Thromb Vasc Biol.* 1997;17:3414-3419.**)

Key Words • HDL • gradient gel electrophoresis • diabetes • insulin

Syndrome X,[1] or the IRS,[2] is associated with a variety of metabolic abnormalities, including hypertension, impaired glucose tolerance, and dyslipidemia, particularly hypertriglyceridemia and low levels of HDLs. Although a number of components of the IRS remain controversial, such as inclusion of hypertension,[3] the strong association between insulin resistance and hyperinsulinemia is well established. The familial clustering of these traits suggests defects in one or more genes may contribute to IRS, although the precise nature of such genes and their actions are not well understood.

Many studies have shown hyperinsulinemia to be associated with high triglyceride and low HDL cholesterol levels,[2,4-12] mainly HDL_2.[8,11] Although insulin levels are not associated with the absolute concentrations of LDL cholesterol,[13] hyperinsulinemia is associated with a relative increase in small, dense LDL particles (LDL subclass B),[8,14-17] which in turn are associated with an increased risk of cardiovascular disease independent of LDL cholesterol concentrations.[18-20] Furthermore, hyperinsulinemia is associated with an increase in apo B and a decrease in apoAI concentrations.[11] This atherogenic lipoprotein profile is also associated with insulin resistance.[21-23] It is noteworthy that several of these studies reporting significant relationships between insulin and lipoprotein phenotypes were prospective.[2,11,24] For example, baseline insulin concentrations predicted the development of hypertriglyceridemia, high apoB, and low apoAI.[11] In addition, elevated insulin concentrations predicted subsequent lowering of the HDL cholesterol/apoAI ratio. Thus, hyperinsulinemia is associated prospectively with proatherogenic changes in HDL composition, as well as changes in absolute concentrations.

There is ample evidence that genes influence variation in many of the traits associated with IRS, including insulin concentration,[25,26] insulin resistance,[27,28] and HDL phenotypes.[29-31] In a previous study, we found that genes account for a significant proportion of the variability in fasting and 2-hour insulin concentrations.[32] Also in the study, genes accounted for one third to one half of variation in seven measures of HDL concentrations (ie, h^2 ranged from 0.34 to 0.46 for apoAI, apoAII, apoE, lipoprotein AI, and cholesterol in HDL, HDL_{1+2}, and HDL_3)[32] and for a similar proportion of variance in HDL size distributions of apoAI, apoAII, and cholesterol.[33] For at least some of these traits (eg, postchallenge insulin[25,26] and fasting concentrations of HDL-C[34] and apoAI[35]), segregation analyses have revealed the existence of individual genes with relatively large effects. Recently, we have demonstrated that significant amounts of variation in fasting insulin and HDL cholesterol are controlled by shared genes (ie, pleiotropy), suggesting they may be responsible in part for the clustering of proatherogenic traits in IRS.[36] In this report, we extend these studies by considering the relationship(s) between insulin and a broad selection of HDL phenotypes that include measures of HDL size and concentration.

Methods

Study Participants and Samples

The San Antonio Family Heart Study is a study of risk factors for cardiovascular disease in Mexican-American families.[32] All first-, second-, and third-degree relatives of randomly ascertained probands were invited to participate. At a clinic

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Selected Abbreviations and Acronyms

apo = apolipoprotein
CETP = cholesteryl ester transfer protein
HDL-C = HDL cholesterol
IRS = insulin resistance syndrome
TG = triglyceride

visit, blood samples were obtained after an overnight fast and again 2 hours after administration of a 75-g glucose load (Orangedex, Custom Laboratories). Plasma samples were prepared by low-speed centrifugation and stored in plastic tubing segments[37] or in freezer vials at −80°C. Weight, height, and circumferences of waist and hip also were measured during the clinic visit; body mass index was calculated as weight divided by height squared (kg/m^2), and waist-hip ratio was calculated as waist circumference divided by hip circumference. Procedures were approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, and all subjects gave written informed consent.

Biochemical Measurements

Plasma glucose (mmol/L) was measured by using an Abbott V/P Analyzer, and insulin (pmol/L) was measured by using a commercial radioimmunoassay kit (Diagnostic). Diabetic subjects were defined, using World Health Organization criteria, as individuals satisfying at least one of the following conditions: fasting glucose concentrations ≥7.8 mmol/L (140 mg/dL), glucose concentrations ≥11.1 mmol/L (200 mg/dL) 2 hours after administration of a 75-g glucose load, or currently taking medications for diabetes. Cholesterol and TG concentrations were assayed enzymatically in frozen plasma samples using a Gilford SBA-300 clinical chemistry analyzer with commercial reagents supplied by Boehringer-Mannheim Diagnostics and Stanbio, respectively. Interassay coefficients of variation for control products in these assays were 2.1% for cholesterol and 6.2% for TG.

HDL Measurements

Concentrations of HDL-TG and HDL-C were measured as above in plasma samples after precipitation of β-lipoproteins by use of dextran sulfate.[38] The interassay coefficients of variation for control products in these assays were 6.2% for HDL-C and 10.8% for HDL-TG. Apolipoprotein concentrations were determined by a commercial laboratory (Medical Research Laboratories, Highland Heights, Ky). ApoAI concentrations were determined by nephelometry,[39,40] and apoAII concentrations were determined using competitive immunoassays.[41] The interassay coefficients of variation for control products in these assays were 3.5% for apoAI and 4.4% for apoAII.

Size distributions of HDL particles were determined by electrophoresis of plasma in nondenaturing 3% to 31% polyacrylamide gradient gels, which were made in the laboratory as described.[42] After electrophoretic separation, the proteins were transferred to nitrocellulose paper and detected by binding with sheep anti-apoAI or sheep anti-apoAII (both from Boehringer-Mannheim) as previously described.[43,44] These antibodies were in turn bound by donkey anti-sheep IgG (Chemicon International, Inc), which was radioiodinated by using the chloramine T method,[45] and distributions were measured by densitometry of autoradiograms using an LKB Ultroscan laser densitometer with GSXL software (Pharmacia). The distribution of cholesteryl esters among HDLs was detected by use of staining with Sudan black B and densitometry as described.[46,47] HDL absorbance profiles were decomposed by a curve-fitting procedure as suggested;[48] we developed in house a program to automatically fit curves representing the HDL subclasses.[44] However, to summarize the data for statistical analyses, we summed all absorbances for HDL_2 and HDL_1 and divided by the total HDL absorbance to generate a variable that expressed the proportion of each analyte occurring on HDL particles larger than HDL_3 (ie, larger than 8.8 nm diameter); these variables are termed large HDL-C, large HDL-apoAI, and large HDL-apoAII.

Statistical Genetic Analyses

A total of 1431 individuals were enrolled in the San Antonio Family Heart Study, but we excluded from analyses 212 diabetic subjects and 17 nondiabetic subjects who were taking antilipid medications. Thus, there were data on most or all phenotypes for 1202 nondiabetic individuals in 42 families. The pedigrees were multigenerational and contained a rich diversity of pairwise relationships for genetic analyses, including 2024 pairs of first-degree relatives, 2438 pairs of second-degree relatives, 3525 pairs of third-degree relatives, 3082 pairs of fourth-degree relatives, and 1141 pairs of fifth-degree relatives. To improve assumptions of normality, insulin concentrations were transformed to their natural logarithms before analyses. The transformed variables gave distributions not significantly different from normal (see the Figure). The other measures were analyzed without transformation. We used the PEDSYS package of programs[49] to manage the phenotype data and pedigree information.

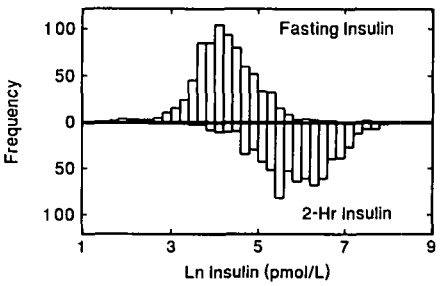
The statistical genetic analyses were based on the principle of partitioning the total phenotypic variance of a trait into the variance due to the effects of genes and residual (environmental) factors. Under this framework, the heritability (h^2) may be defined as that proportion of the total phenotypic variance that is attributable to the additive effects of genes.[50] Maximum-likelihood methods were used to estimate simultaneously the effects of age, age^2, and sex and the residual heritability in each phenotype.

We tested the general hypothesis that a common set of genes influences both insulin concentrations and HDL phenotypes by obtaining the genetic and environmental correlations between each pair of traits from the genetic and environmental variance-covariance matrices. The variance-covariance matrices were obtained by modeling the joint distributions of the phenotypes as a function of their population means, the covariates and their regression coefficients, the additive genetic values, and random environmental deviations, based on the kinship coefficients among individuals.[36] The genetic and environmental correlations obtained from these matrices represent estimates of the effects of shared genes, or pleiotropy, and of shared environmental factors, respectively, on the phenotypic covariance for each pair of traits. The significance of the correlations was determined by comparing the likelihood of an unrestricted model in which the correlation was estimated with the likelihood of a submodel in which the correlation was fixed at zero. The fraction of the total genetic variance between insulin and HDL phenotypes that is explained by the additive effects of genes in common is obtained by squaring the genetic correlation, ρ_G; similarly, the fraction of the total environmental (nongenetic) covariance between two traits is obtained by squaring the environmental correlation, ρ_E. All statistical genetic analyses were conducted by using our modified version of the pedigree analysis program, PAP V3.0.[34,36,51]

Results

Study Population

There were 1202 nondiabetic individuals who were not taking antilipid medications and who had data



Frequency histogram for ln fasting insulin (above the line) and 2-hour insulin (below the line) concentrations

TABLE 1. Characteristics of the Study Population

Parameter	Females		Males		Sex Effect, P*
	n	Mean (SD)	n	Mean (SD)	
Age, y	707	36.6 (15.1)	495	36.2 (16.3)	.683
Body mass index, k/m^2	701	29.3 (6.8)	488	27.7 (5.9)	<.001
Waist-hip ratio	698	0.86 (0.10)	487	0.93 (0.08)	<.001
Fasting glucose, mmol/L	702	4.74 (0.57)	490	4.91 (0.66)	<.001
2-h glucose, mmol/L	685	5.81 (1.75)	473	5.28 (1.79)	<.001
Fasting insulin, pmol/L†	691	64 (42,99)	479	59 (38,99)	.079
2-h insulin, pmol/L†	667	376 (224,659)	460	259 (119,481)	<.001
HDL-C, mmol/L	670	1.36 (0.32)	466	1.23 (0.33)	<.001
Non–HDL-C, mmol/L	670	3.47 (0.93)	466	3.60 (1.01)	.026
Triglycerides, mmol/L†	670	1.27 (0.91,1.76)	465	1.43 (0.97,1.97)	.001

*Effect of sex was tested using analysis of variance; insulin and triglyceride concentrations were log transformed prior to this test.

†Values are medians and in parentheses are the lower and upper quartiles for these nonnormally distributed traits.

available for these analyses. Table 1 gives some of the characteristics of this group of Mexican Americans. Approximately 64% of the participants were women, and the average age was 36.4 years. The population tended to be obese, with mean body mass index of 28.6 kg/m^2. As expected, the sexes differed in several measures of diabetes and lipoprotein phenotypes.

Mean levels of insulin and each HDL phenotype are shown in Table 2 according to age and sex. In women, but not men, the HDL concentration measures tended to increase with age. Levels of 2-hour insulin and HDL concentrations (except for apoAII) were greater in women than in men, and HDL particle sizes tended to be correspondingly larger in women. Insulin values, particularly 2-hour insulin, were higher in females and increased with age in both sexes.

Univariate Quantitative Genetic Analyses of Insulin and HDL Phenotype Measures

We performed quantitative genetic analyses for each of the measures of insulin and HDL. As suggested in Table 2, we found significant effects of age and sex for each trait. Also presented in Table 2 are heritabilities of the insulin and HDL measures. In general, the heritabilities were high, ranging from 32% for 2-hour insulin concentrations to 47% for HDL-C, and all were significantly greater than zero.

Bivariate Quantitative Genetic Analyses of Measures of HDL Phenotypes and Insulin Concentrations

Bivariate quantitative genetic analyses were used to estimate the genetic and environmental correlations of insulin concentrations with measures of HDL. The genetic, environmental, and total phenotypic correlations of fasting and 2-hour insulin with each measure of HDL concentration or size are given in Table 3. The genetic correlations between fasting insulin and two measures of HDL concentration (HDL-C and HDL-TG) were high ($\rho_G = -0.334$ and 0.299, respectively) and significant ($P=.002$ and $P=.015$, respectively), indicating that genes in common explained 9% to 11% of the genetic variance in these pairs of traits. Furthermore, genes in common explained 16% to 26% of genetic variance between fasting insulin and the various measures of HDL size phenotype (each correlation significant at $P<.001$). Similarly, genes in common explained 9% to 24% of genetic variance between 2-hour insulin and the different measures of HDL size (each correlation significant at $P<.02$). However, among the HDL concentration measures, only HDL-TG had a significant genetic correlation with 2-hour insulin ($\rho_G=.408$; $P=.004$), explaining approximately 16% of the genetic variance in these two traits. In no case was the environmental or nongenetic correlation, ρ_E, as strong as the corresponding genetic

TABLE 2. Mean Levels, According to Sex and Age Group, and Heritabilities (h^2) for Measures of Insulin and HDL Phenotype*

Trait	n	Females			Males			h^2
		16-29 y	30-49 y	50+ y	16-29 y	30-49 y	50+ y	
ln fasting insulin, pmol/L	1170	4.13 (0.70)	4.16 (0.66)	4.33 (0.72)	4.07 (0.79)	4.14 (0.74)	4.11 (0.85)	0.42±0.06
ln 2-h insulin, pmol/L	1127	5.79 (0.81)	5.94 (0.80)	6.23 (0.80)	5.29 (0.94)	5.47 (1.11)	5.76 (0.97)	0.32±0.06
HDL-C, mmol/L	1136	1.34 (0.29)	1.36 (0.33)	1.39 (0.33)	1.24 (0.31)	1.27 (0.36)	1.15 (0.32)	0.47±0.05
HDL-TG, mmol/L	1135	0.24 (0.07)	0.27 (0.08)	0.30 (0.07)	0.21 (0.06)	0.25 (0.08)	0.24 (0.06)	0.39±0.02
ApoAI, g/L	1028	1.43 (0.23)	1.50 (0.25)	1.57 (0.26)	1.36 (0.23)	1.46 (0.28)	1.34 (0.22)	0.42±0.07
ApoAII, g/L	1030	0.56 (0.10)	0.58 (0.10)	0.57 (0.11)	0.58 (0.11)	0.60 (0.13)	0.53 (0.08)	0.42±0.07
Large HDL-C, %	1052	51.8 (10.6)	50.5 (10.7)	51.7 (11.9)	45.5 (10.9)	44.5 (12.1)	46.8 (11.6)	0.43±0.03
Large HDL-apoAI, %	1111	45.8 (7.1)	44.4 (7.3)	43.8 (7.1)	40.4 (7.0)	39.5 (8.4)	40.1 (8.0)	0.43±0.02
Large HDL-apoAII, %	1108	43.9 (7.1)	42.2 (6.8)	42.4 (7.7)	39.6 (7.3)	38.7 (8.0)	39.2 (8.0)	0.33±0.02

*Means for males and females are given for three age groups; SD are in parentheses. The proportions of individuals in each sex-age category were as follows (data given for fasting insulin): 23% were women <30, 26% were women 30 to 49, 10% were women ≥50, 18% were men <30, 14% were men 30 to 49, and 8% were men ≥50. Heritabilities, h^2, are given as mean±SEM.

TABLE 3. Genetic (ρ_G), Environmental (ρ_E), and Phenotypic (ρ_P) Correlations (±SEM) Between Fasting and 2-h Insulin Levels and HDL Phenotype Measures

HDL Measures	n	ρ_G	ρ_E	ρ_P
Fasting insulin levels				
HDL concentration				
HDL-TG	1115	+0.299±0.118	+0.069±0.060	+0.160±0.035
HDL-C	1116	−0.334±0.102	−0.124±0.069	−0.217±0.041
ApoAI	1010	−0.216±0.121	−0.074±0.079	−0.136±0.046
ApoAII	1012	−0.131±0.127	+0.020±0.077	−0.044±0.047
HDL size				
Large HDL-apoAI	1092	−0.398±0.101	−0.165±0.063	−0.267±0.037
Large HDL-apoAII	1089	−0.514±0.111	+0.031±0.006	−0.180±0.038
Large HDL-C	1032	−0.456±0.096	−0.091±0.052	−0.253±0.047
2-h insulin levels				
HDL concentration				
HDL-TG	1074	+0.408±0.137	+0.106±0.064	+0.206±0.045
HDL-C	1075	−0.180±0.120	−0.150±0.066	−0.158±0.022
ApoAI	973	−0.076±0.143	−0.104±0.073	−0.092±0.048
ApoAII	975	+0.022±0.146	+0.026±0.072	+0.024±0.049
HDL size				
Large HDL-apoAI	1051	−0.334±0.126	−0.171±0.066	−0.228±0.044
Large HDL-apoAII	1048	−0.438±0.134	−0.036±0.065	−0.169±0.045
Large HDL-C	995	−0.490±0.119	−0.096±0.070	−0.241±0.045

correlation, suggesting that the phenotypic correlations between insulin and HDL were primarily due to the pleiotropic actions of shared genes.

Discussion

We selected insulin concentration as a measure to represent insulin resistance; several studies have shown that serum insulin concentrations in nondiabetic individuals are strongly associated with measures of insulin resistance, in both the fasting and postchallenge states.[52–54] Clinical abnormalities associated with IRS, including diabetes, obesity, and dyslipidemia, may reflect a common metabolic environment.[1,2,55] We have explored this issue by investigating whether shared genes may explain the clustering of one category of IRS-related traits, dyslipidemia, with insulin concentrations. In a previous study, we demonstrated significant genetic correlations between insulin concentrations and one measure of HDL phenotype, HDL-C.[36] In the present study, we have attempted to extend the earlier report by evaluating several different measures of HDL phenotype. These HDL phenotypes probably represent different aspects of HDL metabolism. Several lines of evidence suggest the existence of different (independent) sets of genes that influence some of these different HDL phenotypes. For example, measures of apoAI concentration and particle size distribution are substantially independent of each other,[44] although they each are under strong genetic control.[32,33,44] Also, we have conducted principal components analyses on the genetic correlations among seven different measures of HDL phenotype[33,56] and find evidence for at least three independent gene sets explaining covariance among the traits (A.G. Comuzzie and D.L. Rainwater, 1997, unpublished observations). Thus, the various HDL phenotype measures contain information about different sets of genes that influence different aspects of HDL metabolism, only some of which may be responsible for the clustering of HDL among IRS-related traits.

We found modest but significant genetic correlations for several measures of HDL with insulin concentrations. These genetic correlations, which represent the additive effects of genes, suggest that one or more genes exert pleiotropic effects on both HDL and insulin variation. HDL size variables were the strongest correlates of insulin among the HDL measures, explaining 3% to 7% of total phenotypic covariance but 11% to 26% of the genetic variance. The negative correlations suggest that increasing resistance to insulin is associated with relatively smaller HDL particles. These data extend previous reports of significant effects of insulin and diabetes on HDL size phenotypes.[44,57,58] Together with a similarly negative correlation with LDL particle size,[8,14–17,58] these data suggest the possibility that in fact a general lipoprotein size phenotype, reflected in measures of LDL and HDL particle size distributions, is associated with IRS. If so, then we predict that shared genes are responsible for the correlated variation in LDL and HDL size phenotypes because both are strongly genetic traits. The primary source of this relationship could be increased free fatty acid transport in IRS, which leads to hypertriglyceridemia and corresponding modifications of lipoprotein composition and metabolism.[59] In any event, we suggest that IRS (and diabetes) is associated with a small, dense lipoprotein phenotype, at least with respect to the two major classes of cholesterol-containing lipoproteins.

HDL-C concentrations were also negatively correlated with insulin. Coupled with the fact that the two major proteins of HDLs, apoAI and apoAII, were not at all correlated with insulin, the negative correlation with the major HDL lipid component, HDL-C, may also reflect the association of smaller HDL particles with insulin resistance. Further studies will be necessary to determine whether these correlations reflect a single common metabolic pathway or several. HDL-TG also was significantly correlated with insulin. Because the correlation was positive, it is unlikely that HDL-TG is reporting directly on particle size. However, HDL-TG is

positively correlated with total TG (r^2=.34 in this study), and the correlation of insulin with HDL-TG could simply reflect variation in total plasma TG, a lipid measure also known to be associated with IRS.[5,12,22] Alternatively, HDL-TG concentrations could indicate important compositional variation in HDL particles that in turn influences their metabolism.[59] These are not exclusive hypotheses, and the present data do not distinguish them.

CETP facilitates the coordinated exchange of triglycerides and cholesteryl esters among HDLs and β-lipoproteins. The decrease of HDL-C and increase of HDL-TG with increases of insulin suggest an alteration of CETP activity. Insulin appears to influence CETP function, although the reported effects are not consistent across studies.[60,61] This likely is due in part to the difficulties of extrapolating in vitro CETP activity or mass assay measurements to in vivo function across individuals. Nevertheless, the present data are consistent with an opposite effect of insulin resistance on TG and cholesterol in HDL, possibly mediated by CETP.

A number of environmental factors can potentially influence HDL and/or insulin phenotypes. However, the significant genetic correlations we report here should, by definition, be unaffected by such factors. When we excluded 71 subjects who were taking medications that might influence lipid measures (postmenopausal women undergoing estrogen replacement therapy and subjects taking medications for hypertension) from the analyses, we found virtually identical genetic correlations as were found for the entire group (B.D.M., 1997, unpublished observations). Similarly, alcohol, which has a significant effect on HDL but not insulin measures in this population,[32] did not appreciably alter the genetic or environmental correlations when included as a covariate (B.D.M., 1997, unpublished observations).

In previous studies of this same population, we have detected the existence of a single locus with relatively large effects that influences variation in serum levels of 2-hour insulin[26] and another that influences plasma levels of HDL-C.[34] Both loci were detected by using complex segregation analysis, although their chromosomal locations have yet to be identified. The major locus for 2-hour insulin concentrations accounts for 31% of the total phenotypic variation and also has a modest effect (P=.02) on fasting insulin concentrations.[26,36] The major locus for HDL-C, detected after adjustment for triglyceride and apoAI concentrations, explains 55% of the residual variation in men and 17% in women.[34] Because these loci exert major effects on trait variation, we speculated that one (or both) of them might be one of the pleiotropic genes responsible for the relationships between insulin and HDL found in this study. Accordingly, we used bivariate segregation analyses[36] to determine whether the major locus for 2-hour insulin influences any of the HDL measures and whether the major locus for HDL-C influences the insulin measures. We found that neither major locus is one of the genes responsible for the genetic correlations between insulin and HDL shown in Table 3, and therefore, we conclude that neither is a significant contributor to the IRS.[36,62]

Taken together, the results of this study lead to several important conclusions regarding insulin and HDL phenotypes: (1) The clustering of these phenotypes associated with IRS is explained in part by genes that exert pleiotropic effects; (2) like LDL particle size, average HDL particle size is also correlated with insulin resistance; and (3) the previously detected major loci for 2-hour insulin and HDL-C concentrations are not among the genes responsible for the aggregation of proatherogenic traits associated with IRS.

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