Peroxisome Proliferator–Activated Receptor γ Gene Locus Is Related to Body Mass Index and Lipid Values in Healthy Nonobese Subjects

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Abstract—The peroxisome proliferator–activated receptor γ (*PPAR*γ) gene has been implicated in morbid obesity and is important to lipid and carbohydrate metabolism. However, the relevance of gene variations in healthy nonobese subjects has not been defined. We recruited monozygotic and dizygotic healthy nonobese twin subjects to test the hypothesis that the *PPAR*γ gene is important to body mass index and lipid concentrations in healthy nonobese subjects. Both linkage and association strategies were used in the same dizygotic twins. The *PPAR*γ gene locus was linked ($P<0.01$) to high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and body mass index as quantitative traits. A biallelic variant in the *PPAR*γ gene was associated with high-density lipoprotein cholesterol and body mass index ($P<0.05$). We also looked for linkage between the same variables and the retinoic X receptor gene locus. This locus was linked to total and low-density lipoprotein cholesterol as well as triglycerides. We conclude that the *PPAR*γ gene is highly relevant to lipid metabolism and body mass index, not only in the morbidly obese but also in healthy nonobese subjects. The same appears to be true for its binding partner. Sequencing these genes in twins would serve to identify gene variations contributing to body mass index and lipid concentrations in healthy nonobese subjects. **(*Arterioscler Thromb Vasc Biol*. 1999;19:2940-2944.)**

Key Words: genetics ■ *PPAR*γ ■ quantitative trait loci ■ body mass index ■ cholesterol, HDL ■ cholesterol, LDL ■ twins

Peroxisome proliferator–activated receptor γ (PPARγ) is a member of the nuclear hormone receptor superfamily that heterodimerizes with the retinoid X receptor and functions as a transcriptional regulator of genes linked to lipid metabolism and energy balance. The thiazolidine class of antidiabetic drugs and 15-deoxy-Δ12,14-prostaglandin J2 are ligands for this receptor.[1–3] PPARγ expression is highest in adipose tissue but is detectable at lower levels in other tissues (eg, kidney and liver).[4,5] Tontonoz et al[6] recently reported that PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL cholesterol. Ristow et al[7] recently reported a *PPAR*γ mutation in 4 patients with massive obesity. When expressed in fibroblasts, the mutated gene accelerated lipid uptake of the cells and caused them to differentiate into adipocytes. These observations prompted us to test the hypothesis that the *PPAR*γ locus is linked to lipid values and body mass index (BMI) in healthy nonobese dizygotic (DZ) twin subjects in terms of a quantitative trait locus (QTL). We then took advantage of a biallelic marker in the *PPAR*γ gene and were able to associate the genotypes with BMI and HDL cholesterol. We also searched for linkage between the retinoic X receptor gene locus and the same phenotypes and showed that this locus is a QTL for total and LDL cholesterol. Our data support the notion that PPARγ and its binding partner are relevant to BMI and lipid levels in healthy nonobese persons.

Methods

Study Population

We recruited 222 pairs of monozygotic (MZ; 122 pairs) and DZ (100 pairs) twins by advertisement to participate in studies involving blood pressure regulation and cardiovascular phenotypes.[8,9] We also recruited the parents of the DZ twins to permit identity by descent linkage analysis. Twin zygosity was verified with use of 5 polymerase chain reaction–amplified microsatellite markers as described in detail elsewhere.[10] The subjects were all healthy, normotensive whites recruited from various parts of Germany. The protocol was approved by the university's committee on the protection of human subjects, and written informed consent was obtained from all participants. Persons with histories of familial lipid disorders were excluded.

Phenotypic Characterization

Blood was obtained from the twins after a 12-hour fast. Total cholesterol, HDL cholesterol, and triglycerides were determined by automated methods.[11] LDL cholesterol concentrations were calcu-

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TABLE 1. Clinical Data and Serum Lipid Values

Variable	MZ	DZ
Pairs, n	122	100
Age, y	34±15	34±13
Sex (male/female), n	80/164	60/140
Height, cm	169±9	170±9
Weight, kg	67±13	71±14
BMI, kg/m^2	23±4	24±4
Total cholesterol, mg/dL*	183±39	193±42
HDL cholesterol, mg/dL*	51±14	57±17
LDL cholesterol, mg/dL*	115±34	115±32
Triglycerides, mg/dL†	87±67	100±64

Values are mean±SD.

*To convert cholesterol values into millimolar concentration (mmol/L), multiply the given value (mg/dL) by 0.01129.

†To convert triglyceride values into millimolar concentration (mmol/L), multiply the given value (mg/dL) by 0.0286.

lated by the Friedewald equation.[12] Blood was also obtained for determination of zygosity and other molecular genetic studies.

Genotyping

Microsatellite markers spanning ≈45 cM around the *PPARγ* gene on chromosome 3[13] (D3S1297, D3S1304, D3S3726, D3S3589, D3S1263, D3S3608, D3S2338, and D3S1266), as well as markers spanning 5 cM around the retinoic X receptor gene on chromosome 1 (D1S2768, D1S2844, D1S426, and D1S194), were analyzed using the PE Applied Biosystems genotyping system. A polymorphism corresponding to a silent C-to-T substitution in exon 6 of *PPARγ* was analyzed according to a published protocol.[14]

Statistical Analysis

For linkage analysis, only DZ pairs and their parents were included. Data were analyzed by using a structural equation modeling (SEM) approach[15] as implemented in the Mx statistical package.[16] This approach is based on variance-covariance matrices of sibs weighted by the probability of sharing 0, 1, or 2 alleles identical by descent. The higher power of the variance-covariance–based analysis, compared with the squared trait differences–based approach by the Haseman-Elston regression method,[17] was shown in a recent simulation study.[18] Because we used a candidate gene approach, we accepted $P<0.01$ to test for significant linkage in accordance with the criteria defined by Lander and Kruglyak.[19] To increase the power for the association analysis, mean scores of pairs of MZ twins were included together with scores of DZ pairs.[20] Statistical analysis was conducted by using ANOVA (SPSS).

Results

Table 1 shows the demographic and lipid-related variables for the twin subjects. Female sex was twice as common as male sex. Subjects were young adults of normal height, weight, and BMI. Total, HDL, and LDL cholesterol and triglyceride values were all within normal limits. Table 2 shows the results of the heritability analysis. A major genetic effect was demonstrated on all lipid parameters, although strong environmental effects were also shown. Table 3 gives the results of the structural equation modeling multipoint linkage analysis for markers spanning the *PPARγ* and retinoic X receptor gene loci and body size as well as for serum lipid concentrations. Only the peak value for each locus is given. Significant linkage was found between the *PPARγ* locus and BMI, body weight, and serum HDL and LDL cholesterol levels. For the retinoic X receptor locus, linkage was found for total and LDL cholesterol levels, as well as triglycerides. Figure 1 shows the results of the linkage analysis for *PPARγ* and HDL cholesterol. The location of the markers and the *PPARγ* gene is indicated in the figure. BMI and LDL cholesterol reached their peak significance in the same chromosomal region as HDL cholesterol. Table 4 gives the results of an association analysis using the biallelic marker in the *PPARγ* gene. Persons with the TT variant had decidedly higher HDL cholesterol values, as shown in Figure 2, and tended to have lower LDL cholesterol values and the lowest BMI compared with persons with the CC or CT variant.

We next examined within-pair differences in MZ twins, biallelic marker–concordant DZ twins, and biallelic marker–discordant DZ twins. For HDL cholesterol, MZ twins had the least pair difference, DZ concordant twins were intermediate, and DZ discordant twins had the greatest within-pair HDL cholesterol concentration difference ($P<0.01$). These results are shown in Figure 3. Similar results were obtained for LDL cholesterol (data not shown). For BMI, the results of the analysis were not significant.

TABLE 2. Genetic and Environmental Effects on Serum Lipid Values

	Genetic Effect	Nonshared Environment	c^2/DF	P	r_{MZ}	r_{DZ}
Total cholesterol	0.64	0.36	1.0/4	0.01	0.65	0.37
HDL cholesterol	0.59	0.41	0.4/4	0.01	0.63	0.30
LDL cholesterol	0.66	0.34	0.7/4	0.01	0.66	0.36
Triglycerides	0.72	0.28	0.6/4	0.01	0.72	0.44
Body weight	0.89	0.11	1.4/4	0.01	0.96	0.46
BMI	0.97	0.03	0.65/4	0.01	0.85	0.46

Fit indices for the reported models and *P* values for the genetic effect are given together with correlation coefficients for MZ and DZ.

TABLE 3. Results of Linkage Analysis

	PPARγ		RXR	
	c^2 Model Difference	P (SEM)	c^2 Model Difference	P (SEM)
Total cholesterol	1.8	0.18	4.35	0.001
HDL cholesterol	11.0	0.0009	0	NS
LDL cholesterol	7.8	0.005	6.23	0.004
Triglycerides	2.0	0.15	12.5	0.0004
Body weight	10.0	0.0015	0	NS
BMI	7.4	0.005	0	NS

Difference between SEM models with and without a QTL effect and corresponding *P* values for a QTL effect are given.

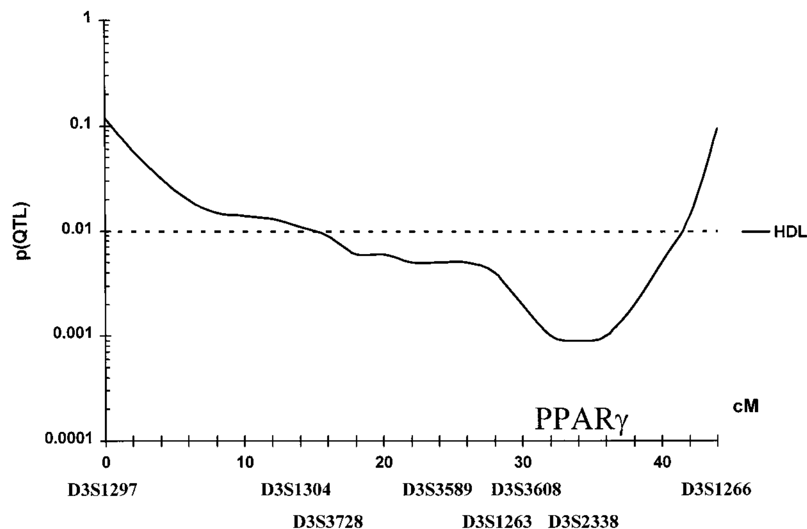

Figure 1. Results of multipoint linkage analysis for HDL cholesterol and markers at the *PPARγ* gene locus. The approximate location of the gene is indicated. The line represents the error value p for the existence of a QTL. A threshold of $P=0.01$ was set. The lowest p value over the *PPARγ* locus is indicated in Table 3.

Discussion

Because cardiovascular disease is the most common cause of death worldwide, more than half of the population can expect to develop cardiovascular disease during their lifetime.[21] Thus, identifying QTL for cardiovascular disease–relevant phenotypes in healthy nonobese persons, and subsequently demonstrating variations in the corresponding candidate genes, is important to cardiovascular disease genetics. We believe that our study exemplifies the utility of this combined linkage and association approach. MZ and DZ twins permit quantification of genetic and environmental variance. As reported earlier,[22] the body size parameters and serum lipid concentrations were all influenced by genetic variance. DZ twins, who are perfectly matched for age and who are generally exposed to very similar environmental conditions, are ideal subjects for efficient sib-pair analyses. Thus, far fewer subjects are needed to gain insight into QTL.

We showed that the *PPARγ* gene locus is a QTL for BMI, LDL, and HDL cholesterol concentrations in healthy nonobese subjects and that a biallelic polymorphism in the *PPARγ* gene is associated with BMI and lipid concentrations. The relationship between LDL cholesterol and *PPARγ*, as well as the relationship between HDL cholesterol and *PPARγ* achieved significance, whereas that for total cholesterol and *PPARγ* did not. We interpret this finding as suggesting that the effects on LDL and HDL cholesterol may be opposite in nature. Our association results would support that point of view. Furthermore, the retinoic X receptor gene locus is a QTL for LDL and total cholesterol. Our results are in concordance with data from a total genome scan conducted in 92 nuclear families using several measures of obesity.[23] In this study, a marker on chromosome 3 (D3S1286) was significantly linked to the percentage of body fat. This marker is located within the chromosomal region linked to BMI in our study. A second genomic scan conducted in Pima Indians, which pointed toward a QTL for BMI on chromosome 11, showed no significant linkage.[24] That lack of confirmation may have been due to population specifics or power restrictions. *PPARγ* has been shown to be an important disease gene for morbid obesity.[7] In a segregation analysis, evidence was found for at least 2 major loci influencing BMI.[25] Together, these loci are expected to account for 64% of the variance in BMI.

Although obesity is common, most persons do not have morbid obesity. Our data suggest that more subtle variations in *PPARγ* are important to BMI and lipid values in healthy nonobese subjects as well. We believe that these observations are particularly relevant for several reasons. Obesity is reported as the most common health problem in developed countries.[26] Low HDL cholesterol values are a recognized risk for coronary heart disease.[27] PPARγ is pivotal to a variety of serious obesity-related medical conditions, including type 2 diabetes mellitus and cardiovascular disease. Although adipose tissue has been recognized as a principal site of *PPARγ* gene expression, the gene is expressed at lower levels in many nonadipose tissues and cell types, where it

TABLE 4. Results of Association Analysis

	CC	CT	TT	*P*
Total cholesterol, mg/dL*	176.5±35.1	187.2±30.9	185.2±26.4	0.176
HDL cholesterol, mg/dL*	49.5±13.1	50.1±11.9	64.2±13.0	0.001
LDL cholesterol, mg/dL*	110.3±30.9	122.6±30.3	104.9±27.4	0.061
Triglycerides, mg/dL†	84.1±54.3	74.1±36.3	80.7±38.6	0.544
BMI, kg/m^2	23.6±3.9	25.3±5.1	22.6±2.4	0.026
Body weight, kg	68.1±12.7	69.6±14.8	66.8±14.5	0.665

*To convert cholesterol values into millimolar concentration (mmol/L), multiply the given value (mg/dL) by 0.01129.

†To convert cholesterol values into millimolar concentration (mmol/L), multiply the given value (mg/dL) by 0.0286.

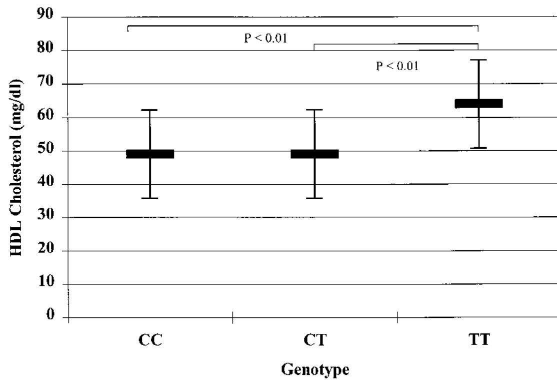

Figure 2. Results of the association analysis for the *PPARγ* polymorphism in exon 6 and HDL cholesterol. The data suggest that the T allele exerts a recessive action.

may also play an important role. Several classes of ligands have been found.[28–31] The thiazolidinediones are specific synthetic agonists for *PPARγ*. 15-deoxy-Δ12,14-prostaglandin J2 is a natural ligand. Certain polyunsaturated fatty acids, such as linoleic acid, also activate PPARγ. Nonsteroidal antiinflammatory drugs, such as ibuprofen, can activate the receptor as well. PPARγ also functions as an obligate heterodimer with the retinoic X receptor, which, among other things, is involved in triglyceride metabolism.[32]

PPARγ may actively participate in the pathogenesis of atherosclerosis. Monocytes and macrophages are pivotal to inflammation and the development of arteriosclerosis. Ricote et al[33] were able to show that PPARγ is markedly upregulated in activated macrophages. They found that PPARγ inhibits the expression of inducible nitric oxide synthase, gelatinase B, and the scavenger receptor A genes in response to synthetic ligands, probably by antagonizing the transcription factors AT-1, STAT, and NF-κB. Tontonoz et al[5] found that PPARγ is induced in human monocytes after exposure to oxidized LDL and is expressed at high levels in atherosclerotic lesions. Ligand activation of PPARγ induced monocyte differentiation and promoted the transcriptional induction of the scavenger receptor. Nagy et al[34] further elucidated this issue by showing that oxidized LDL components acted as endogenous PPARγ ligands. They demonstrated a novel signaling pathway coordinated by the macrophage scavenger receptor on the cell surface internalizing the particle and PPARγ in the nucleus, which is transcriptionally activated by its component lipids. Thus, PPARγ appears to be a key regulator of foam cell gene expression.

Our data suggest that *PPARγ* gene variants in healthy nonobese, nonhyperlipidemic subjects may have significant influence on BMI and plasma lipids. Such variants may have a great effect on the propensity to obesity, type 2 diabetes, and cardiovascular disease in later life. We can only speculate on the interrelationships among BMI, HDL cholesterol concentrations, and PPARγ. However, in epidemiological studies, a higher BMI is associated with lower HDL cholesterol concentrations,[35] consistent with our findings. Furthermore, Meirhaeghe et al[14] have described an interaction between the C-to-T substitution in the *PPARγ* gene and BMI for plasma leptin levels. They found that persons bearing at least one T allele had a lower BMI for a given leptin level, compared with CC homozygous individuals. Their results are consistent with our findings.

Deeb et al[36] recently demonstrated that the Pro12/Ala substitution in the *PPARγ* gene is associated with lower BMI and improved insulin sensitivity. The investigators were also able to show that the Pro12/Ala substitution is associated with decreased receptor activity. Yen et al[37] first reported this missense *PPARγ* mutation, which involves a C-to-G substitution at nucleotide 34. They also found an association between this mutation and type 2 diabetes mellitus in a small number of white patients. Ringel et al[38] were unable to confirm these findings in a large association study involving 522 type 1 diabetic and 503 type 2 diabetic patients, compared with 310 nondiabetic control subjects. Discrepancies in association studies are common. We believe our twin model may be more stable because we relied on both linkage and association approaches. Furthermore, our DZ sib pairs have half of their genes in common and therefore provide a much more homogeneous sample. For example, if $\approx$30 genes are responsible for obesity, the DZ twin would have concordant alleles for half of these genes. If we then examine discor-

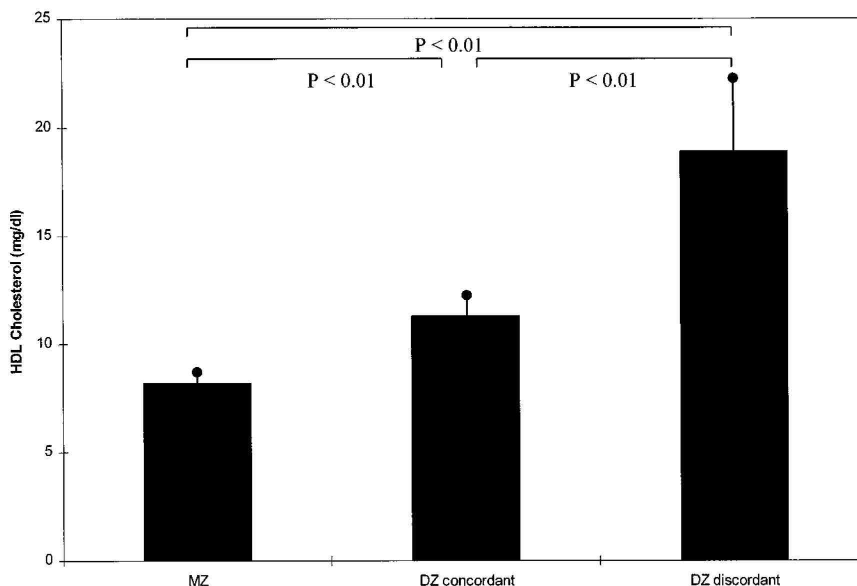

Figure 3. Within-pair differences in MZ twins and DZ twins concordant and discordant for the biallelic polymorphism in exon 6 of the *PPARγ* gene. The difference between concordant DZ and MZ twins indicates that in addition to *PPARγ*, other genes are also responsible for HDL cholesterol values.

dancy in a candidate gene, the number of confounders in our study would be decidedly less compared with that in association studies in randomly selected people.

The variant we examined is silent but apparently in linkage disequilibrium with a functional polymorphism in the *PPARγ* gene or possibly in a nearby gene. We suggest that much of the genetic variance on BMI and HDL cholesterol levels in healthy nonobese persons is attributable to the *PPARγ* gene locus. Multiplex sequencing of the *PPARγ* gene in all of our DZ twin subjects and their parents may provide insight into the functional variants involved. These findings highlight the broad, encompassing role of *PPARγ* in processes involving BMI and lipid metabolism not only in persons with disease but also in healthy, healthy nonobese persons.

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