Genome-Wide Linkage Analysis of Serum Adiponectin in the Pima Indian Population

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Adiponectin is a circulating protein secreted by adipocytes and is thought to have insulin-sensitizing effects. We present genetic analysis of adiponectin levels in 517 Pima Indians without diabetes (from 162 families, 750 sib-pairs). Adiponectin concentrations were heritable, with 39% of the variance of age- and sex-adjusted adiponectin potentially accounted for by additive genetic influences in this population. In genome-wide linkage analyses, suggestive linkage (logarithm of odds [LOD] = 3.0) of adiponectin adjusted for age and sex was found on chromosome 9p at 18 cM. Linkage was also present after inclusion of adiponectin concentrations of siblings with type 2 diabetes not treated pharmacologically (total siblings 582, 182 families, 860 sib-pairs: LOD = 3.5). Tentative evidence of linkage was also found on chromosomes 2 (LOD = 1.7 at 89 cM), 3 (LOD = 1.9 at 124 cM), and 10 (LOD = 1.7 at 70 cM), offering some support to findings of a previous genome-wide scan of adiponectin. Our data suggest that quantitative trait loci on chromosomes 2, 3, 9, and 10 may influence circulating adiponectin concentrations in the Pima population. ***Diabetes*** **52:2419–2425, 2003**

Adipose tissue expresses a variety of secretory proteins of potential importance to metabolic and vascular disease. Recently, adiponectin, a 244–amino acid adipocyte-derived protein, was described (1), which, despite being solely derived from adipose tissue in humans, is paradoxically reduced in obesity (2). Both type 2 diabetes and insulin resistance (in nondiabetic subjects) are associated with decreased adiponectin concentrations (3,4), whereas weight reduction (5) or administration of thiazolidinediones (6) increase adiponectin concentrations. Further, administration of adiponectin increases insulin sensitivity in animal models (7,8). These observations suggest that adiponectin may be an important mediator of insulin sensitivity.

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IBD, identical by descent; IGT, impaired glucose tolerance; ISI, insulin sensitivity index; LOD, logarithm of odds; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

The Pima Indians of Arizona suffer from a particularly high prevalence of type 2 diabetes (9). Obesity is both highly prevalent in the Pima population and is a powerful risk factor for the development of type 2 diabetes (10). Recently, we have shown that higher adiponectin concentrations are protective against future development of type 2 diabetes in BMI-matched Pima case and control subjects (11).

Recent analyses in a predominantly northern European population suggested that variation in plasma adiponectin levels had a strong genetic component (h^2 = 46%) and that adiponectin concentrations were significantly linked (logarithm of odds [LOD] = 4.1) to a quantitative trait locus on chromosome 5p (12). To assess the genetic basis of this phenotype in the Pima Indians, we carried out a genome-wide linkage analysis of adiponectin concentrations in this population.

RESEARCH DESIGN AND METHODS

Subjects and phenotypes. The subjects of this report are participants in a previously reported genomic scan for loci linked to type 2 diabetes and obesity (13) and are all participants in the National Institutes of Health survey of health in the Gila River Indian Community (9). All members of the community >5 years of age are invited to a biennial examination that includes a 75-g oral glucose tolerance test (OGTT). Diabetes is diagnosed by World Health Organization 1985 criteria or if a diagnosis is made in the course of clinical care (14). Height and weight are measured, with the subject wearing light clothing and no shoes, for calculation of BMI (kg/m^2).

A sample of 1,338 individuals (332 nuclear families and 112 extended pedigrees) were selected for the original genome-wide scan (13). Criteria for inclusion were availability of DNA and membership in a nuclear family informative for diabetes or its metabolic correlates. For this report, individuals were selected who *1*) had taken part in the genome-wide scan and *2*) had a stored frozen serum sample available from biennial examination obtained after 1 January 1977, when the subject was >16 years of age. For the primary analysis, samples were selected from examinations in which the subject was not diabetic (by OGTT and medical history). Where available, additional samples were obtained at examinations after diagnosis of diabetes but where subjects were not receiving medication with either oral hypoglycemic agents or insulin. Additional analyses that include these individuals along with nondiabetic participants are presented separately. Finally, initial analysis of this cohort indicated that adiponectin concentrations were significantly higher in the presence of abnormal renal function. For this reason, subjects with serum creatinine ≥1.2 mg/dl or urinary albumin:creatinine ratio ≥30 mg:g (the level indicative of the presence of abnormal protein excretion in this population) were excluded from all analyses.

Adiponectin was measured in stored serum samples from the 2-h time point of the OGTT. A pilot study indicated no significant differences in adiponectin concentration in serum samples taken fasting or 2 h after an OGTT (2-h 96% of fasting: absolute difference 0.26 ± 0.3 μg/ml, paired t test P = 0.44, unpublished data in 12 subjects). Adiponectin was measured using an enzyme-linked immunosorbent assay using an adiponectin-specific antibody, as described previously (15). Intra- and interassay coefficients of variation were 3.3 and 7.4%, respectively.

Insulin was measured by modification of the radioimmunoassay method of

Yalow and Berson (between 1973 and 1986) and by Autopak Insulin RIA (Concept 4; ICN Biomedicals, Horsham, PA) since 1987. To allow use of all available measures, a corrected insulin was calculated by linear regression as previously described (16).

Measures of adiponectin were available from 695 subjects without diabetes and 188 subjects with diabetes not treated pharmacologically. For linkage analysis, log-transformed adiponectin was adjusted for age and sex (model 1) or age, sex, and BMI (linear and quadratic terms [model 2]) separately in the diabetic and nondiabetic groups using general linear models. This resulted in a trait that was approximately normally distributed (Shapiro-Wilk statistic = 0.99 for adjusted traits for both model 1 and model 2). The variance components technique assumes multivariate normality, and deviations from this potentially lead to inflation of the type 1 error rate (17). Analysis of the trait after transformation to normalize the distribution completely did not substantially alter results (data not shown). Linkage analysis was carried out *1*) in families with two or more siblings without diabetes when samples were obtained for adiponectin (517 individuals from 162 families, 750 sib-pairs) and *2*) after inclusion of siblings with type 2 diabetes not treated pharmacologically (582 total siblings, 182 families, 860 sib-pairs, with 45 individuals with diabetes and 20 additional siblings without diabetes included).

Genome-wide linkage analyses. Genotypic data used were identical to those in previous reports (13,18). In brief, 503 autosomal microsatellite markers were typed in the laboratory of J. Weber at the Marshfield Medical Research Foundation (19). An additional 13 markers were typed at Glaxo-Wellcome. The median rate of agreement between duplicate samples was 97%, and no marker had an agreement rate <90%. All markers were inspected for Mendelian errors, and genetic distances between markers were determined (from the Pima data) as previously described (13).

Linkage analysis was conducted in sibships using the variance-components method of Amos (20). In brief, a linear mixed model is fitted estimating the trait mean (μ) and three components of variance. Using the standard biometric model (21), variance is partitioned into *1*) an additive "monogenic" component linked to the region of interest (σ^2_M), *2*) a "polygenic" component that incorporates overall familial effects (σ^2_G), and *3*) an "environmental" component that incorporates effects unique to the individual (σ^2_E). Under the assumption of no recombination between the trait and marker loci, the phenotypic variance-covariance matrix (Ω) for individuals in a pedigree is as follows:

$$\Omega = \Phi\sigma^2_G + \Pi\sigma^2_M + I\,\sigma^2_E$$

where Φ is a matrix of the expected proportion of alleles shared identical by descent (IBD), Π is a matrix of the proportions of alleles actually shared IBD for a particular marker (as estimated on the basis of genotypic data), and I is an identity matrix. The parameters of these models were estimated, under the assumption that the distribution of the trait was multivariate normal, by maximizing the likelihood over all sibships using a Newton-Raphson algorithm (using the "PROC MIXED" function of SAS; SAS Institute, Cary, NC). The null hypothesis of no linkage was assessed by comparing the full model to one in which σ^2_M was constrained to equal 0, and the models were compared using a likelihood ratio test (22). The LOD score for variance component analysis was calculated by dividing the likelihood ratio test for linkage by $2*\log_e[10]$. Finally, in models with σ^2_M constrained to 0, estimates of σ^2_G and σ^2_E were used to assess polygenic influences on adiponectin, and heritability (h^2) was taken as the contribution of σ^2_G to the total variance (21).

IBD of individual markers was calculated using the estimation developed by Curtis and Sham (23). Multipoint estimates of IBD were obtained using the method of Fulker et al. (24).

For the purposes of presentation, we have reported only regions where LOD scores in multipoint analysis exceeded 1.44 (point-wise $P = 0.005$) in either BMI-adjusted or unadjusted models.

All protocols were approved by the Tribal Council of the Gila River Indian Community and by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases, and all subjects provided written informed consent before participation.

RESULTS

Demographic information. In subjects without diabetes and normal renal function, adiponectin concentrations were negatively related to BMI (Spearman $r = -0.22$, $P < 0.0001$) and fasting insulin ($r = -0.28$, $P < 0.0001$, available in 445 subjects) and positively related to age ($r = 0.15$, $P = 0.0001$) but unrelated to serum creatinine ($r = 0.05$, $P = 0.17$). In a multivariate analysis, adiponectin concentrations were positively related to age ($P < 0.0001$) and negatively related to BMI ($P < 0.0001$), but not significantly related to storage time ($P = 0.29$). Adiponectin concentrations were similar in males (median 5.1 μg/dl [interquartile range 3.7–7.2]) and females (5.0 μg/dl [3.8–6.7]). In models also including terms for BMI, age, and length of sample storage, no significant effect of sex was found ($P = 0.51$).

TABLE 1
Baseline characteristics

	No diabetes	Diabetes
n (M/F)	237/300	15/30
Age (years)	33.5 ± 10.0	41.7 ± 10.6
Height (m)	1.65 ± 0.08	1.63 ± 0.08
Weight (kg)	98.7 ± 25.3	94.1 ± 22.9
BMI (kg/m^2)	36.1 ± 8.4	35.3 ± 8.0
Creatinine (mg/dl)	0.75 ± 0.13	0.69 ± 0.13
Duration of diabetes (years)	—	7.0 (0–12.8)
Storage (years)	10.2 (4.5–17.9)	7.5 (3.3–12.2)
Adiponectin (μg/ml)	4.9 (3.7–6.8)	5.6 (4.2–8.6)

Data are means ± SD or median (interquartile range). Characteristics of 582 individuals (from 182 families) included in linkage analysis who were either nondiabetic or had diabetes treated by diet alone at the time adiponectin was measured.

Adiponectin concentrations were also highly related to HDL cholesterol concentrations (Spearman $r = 0.42$, $P < 0.0001$, $n = 238$) and triglycerides ($r = -0.37$, $P < 0.0001$, $n = 228$) but not to total cholesterol ($r = 0.02$, $P = 0.5$, $n = 238$).

Adiponectin was lower in subjects with impaired glucose tolerance (IGT) (4.6 μg/dl [3.6–6.2], $n = 222$) than in those with normal glucose tolerance (NGT) (5.3 μg/dl [3.9–7.3], $n = 473$) even after adjustment for age, BMI, and sex ($P < 0.0001$ for IGT vs. NGT). By contrast, subjects with diabetes had adiponectin concentrations similar to the NGT group (5.6 μg/dl [4.2–7.4], $n = 188$). Baseline characteristics of the subgroup of subjects included in the linkage analysis are shown in Table 1.

Linkage analysis. Linkage analysis was first restricted to families in which adiponectin had been measured in two or more siblings without diabetes at the time of measure-

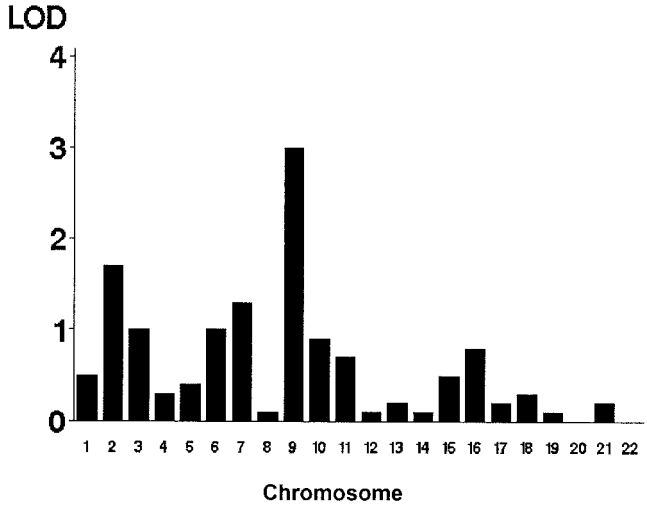

FIG. 1. Multipoint linkage of age- and sex-adjusted adiponectin concentrations. Linkage analysis in 517 individuals from 162 families (750 sib-pairs). Maximum LOD scores by variance components are shown on the *y*-axis for each chromosome.

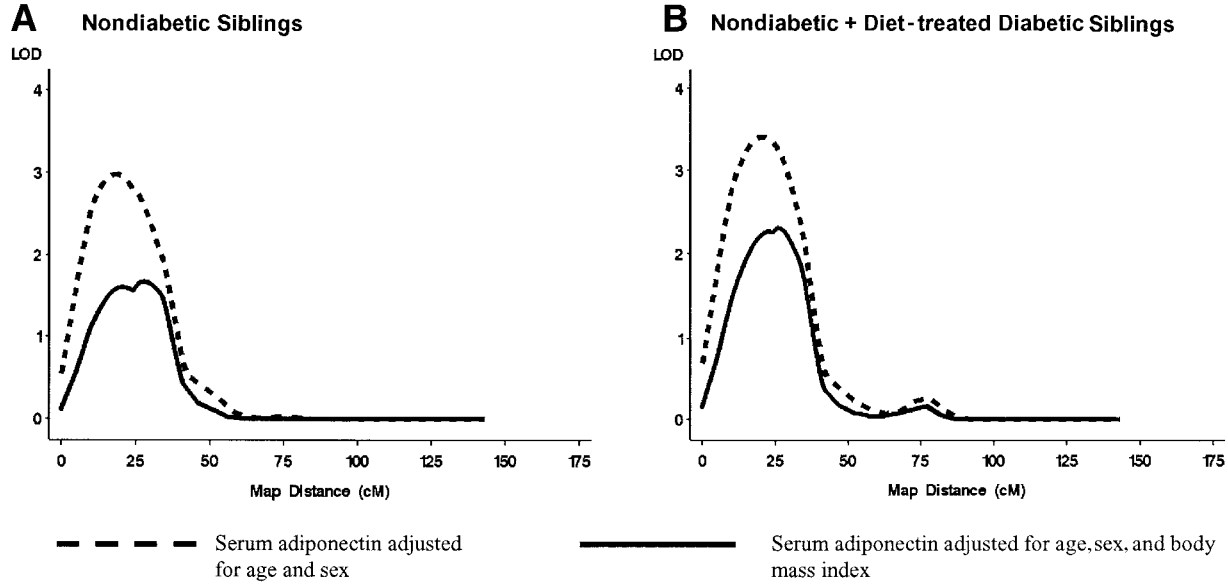

FIG. 2. Multipoint linkage of age- and sex-adjusted adiponectin concentrations, chromosome 9. Linkage analysis is shown in 517 individuals without diabetes from 162 families (750 sib-pairs) (*A*) and 582 individuals without diabetes or with diabetes not treated pharmacologically from 182 families (860 sib-pairs) (*B*).

ment (517 individuals from 162 families, 750 sib-pairs). Suggestive linkage (LOD = 3.0) of adiponectin adjusted for age and sex was found on chromosome 9 (Fig. 1), with peak of linkage at 18 cM (Fig. 2) and the LOD-1 support interval between 7 and 33 cM. All four markers across this region showed linkage with LOD >1.0 in a single-point analysis—D9S168: 10.0 cM, LOD = 2.2; D9S925: 24.0 cM, LOD = 1.1; D9S741: 33.3 cM, LOD = 1.3; D9S1121: 34.6 cM, LOD = 1.9. Further adjustment of the trait for BMI resulted in a reduction in the magnitude of the evidence for linkage (LOD = 1.7; Fig. 2). Inclusion of siblings with adiponectin measurements after diagnosis of diabetes (but who were not treated with oral hypoglycemic agents or insulin) allowed linkage analysis in 582 individuals (182 families, 860 sib-pairs; and 45 with diabetes at the time of examination and 537 without diabetes). Results were similar; in particular, evidence for linkage (LOD = 3.5) of age- and sex-adjusted adiponectin was still present in this larger analysis (Table 2). Once again, all four markers across the region showed linkage in single-point analyses—D9S168: LOD = 2.3; D9S925: LOD = 1.6; D9S741: LOD = 1.8; D11S1121: LOD = 2.1.

No substantial evidence for linkage was observed on chromosomes 5 or 14 (Fig. 1). On chromosome 3, a peak of linkage of adiponectin was seen at 124 cM (LOD = 1.0) (Fig. 3), which increased after inclusion of diabetic siblings (LOD = 1.9; Fig. 3). Two other results are worth noting. We observed a minor peak on chromosome 10 at 70 cM (LOD = 1.7), close to a previously reported linkage peak of adiponectin (Table 2) and a further peak of linkage on chromosome 2 (LOD = 1.7), the position of which was ~40 cM distant from that of previous reports (this peak lay at 89 cM and the reported peak at 50 cM [12]).

DISCUSSION

Adiponectin is produced exclusively by adipocytes and bears important cross-sectional relationships to measures of insulin resistance (4). Linkage analysis of adiponectin in this and other populations (12) furthers the investigation of the genetic control of adiponectin and potentially other related phenotypes (most importantly insulin sensitivity and type 2 diabetes).

Relationships of adiponectin to other phenotypes in this study are largely consistent with other series. As previously reported by others, we found that adiponectin is

TABLE 2
Linkage of adiponectin

Chromosome	Peak of LOD score (cM)	Flanking markers (position)	Siblings without diabetes		Siblings without diabetes or with diabetes not treated pharmacologically	
			Model 1	Model 2	Model 1	Model 2
2	89	D2S1777 (88 cM), D2S428 (93 cM)	1.7	1.6	0.9	1.6
3	124	D3S4018 (121 cM), D3S1769 (129 cM)	1.0	0.8	1.9	1.8
9	18	D9S168 (10 cM), D9S925 (24 cM)	3.0	1.7	3.5	1.7
10	70	D10S1426 (64 cM), D10S1220 (75 cM)	0.9	1.0	1.7	1.0

Linkage results of adiponectin adjusted by linear regression for age and sex (model 1) or age, sex, and BMI (model 2) in subjects without diabetes (517 individuals from 162 families, 750 sib-pairs) or with inclusion of 45 siblings with diet-treated type 2 diabetes (total siblings 582, 182 families, 860 sib-pairs).

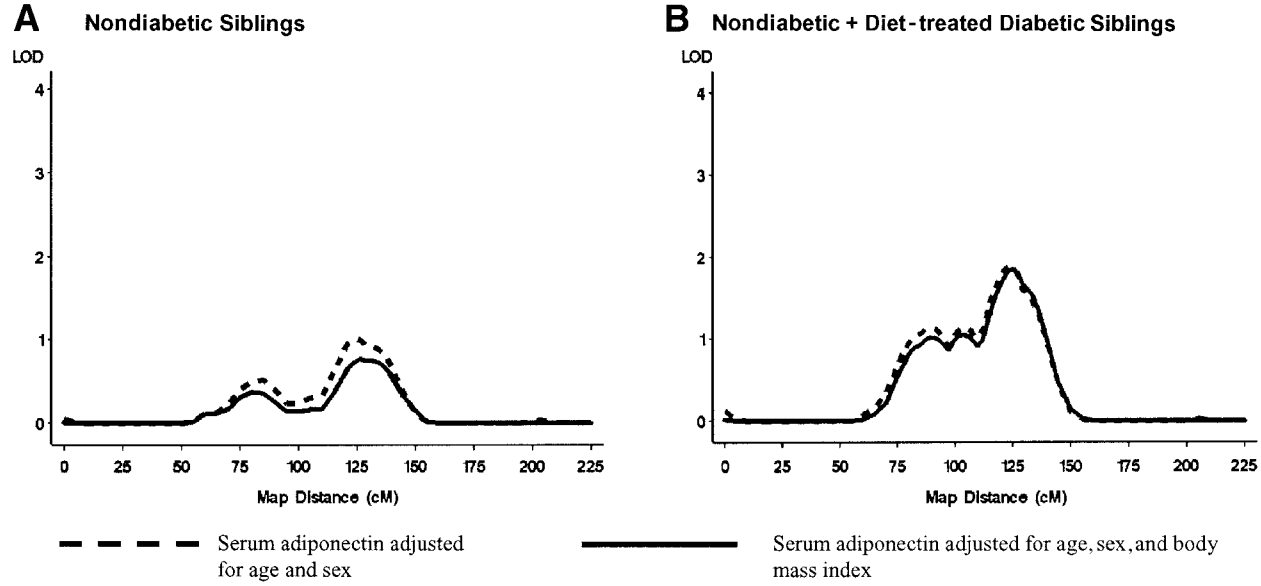

FIG. 3. Multipoint linkage of age- and sex-adjusted adiponectin concentrations, chromosome 3. Linkage analysis is shown in 517 individuals with diabetes from 162 families (750 sib-pairs) (*A*) and 582 individuals without diabetes or with diabetes not treated pharmacologically from 182 families (860 sib-pairs) (*B*).

inversely associated with measures of adiposity (15) and fasting insulin concentrations (4). In most previous populations, a sex difference in adiponectin has been observed: women have higher adiponectin concentrations than men in Japanese (3) and European (25) populations. In contrast, in this report and in a previous report with fewer Pima volunteers (4), no sex difference was found. The reason for this is presently not clear. Whereas this population has a higher prevalence of obesity than previous populations studied, no sex difference is detected, even in Pimas with BMIs <25 kg/m^2 (data not shown). It is also notable that HDL cholesterol, with which adiponectin is highly correlated in this and other populations (12), also shows little sex difference in the Pimas (26) in contrast to other populations (27).

We find that adiponectin concentrations are positively correlated with age, even after adjustment for BMI. This result has also been recently reported in another large series, although the reasons for this are not known (28). It is an intriguing finding, however, because sensitivity to insulin declines with age (28). Given the direct relationship of adiponectin to insulin sensitivity, the increase in adiponectin with age is unexpected, and it suggests that the relationship of adiponectin to insulin action may vary with age. Two previous reports, including one from the Pima population, have suggested that adiponectin concentrations are lower in the presence of IGT (4) and type 2 diabetes than in those with NGT (3,4), whereas adiponectin concentrations are higher in the presence of type 1 diabetes (29). Our results for IGT are in keeping with this; however, we find that adiponectin concentrations in subjects with diabetes were similar to those with NGT.

Knowledge of the physiological and molecular control of adiponectin expression remains incomplete. The adiponectin gene (*APM1*) promoter region contains consensus sequences for both peroxisome proliferator–activated receptor-α and glucocorticoid receptor (12,30). In vivo, adiponectin is upregulated by peroxisome proliferator–activated receptor-γ agonists (6), whereas in cell culture, it is downregulated by glucocorticoids and tumor necrosis factor-α (31,32). A number of previous studies have examined the association of polymorphisms of the adiponectin gene with circulating concentrations of the protein and metabolic disease. Rare missense mutations (frequency $<1\%$ in control subjects without diabetes) in exon 3 of the adiponectin gene have been associated with reductions in circulating adiponectin (R112C, I164T) (30,33) and with type 2 diabetes (I164T) (34). In addition, more common single nucleotide polymorphisms in coding (silent mutation 45T → G) and intronic (276G → T) regions have been associated with the risk of type 2 diabetes (35) and features of the metabolic syndrome (25) including increased adiposity (25,36). Notably however, neither relationships with circulating adiponectin (25,35) nor the particular alleles conveying risk of disease (25,35,36) are consistent. This finding suggests that more important, potentially causal, polymorphisms have yet to be described (25). The adiponectin gene lies at ~200 cM on chromosome 3 and is therefore a somewhat distant candidate for the linkage of adiponectin we observed on chromosome 3.

One previous study has examined linkage of adiponectin to quantitative trait loci. They reported significant linkage on chromosome 5 and a further peak of suggestive linkage on chromosome 14 (12). Further, they reported peaks on chromosomes 2, 3, 10, and 17, with the peak on chromosome 3 being of particular interest because it lays at 201 cM, close to the site of the adiponectin gene (12). There are relatively few coincident peaks of linkage in the two studies. The major peak that we found on chromosome 9 was not seen in the previous analysis (12). On chromosome 10, Comuzzie et al. (12) reported an LOD peak of 1.9 at 67 cM, whereas we detected a maximum LOD of 1.7 with age- and sex-adjusted adiponectin at 70 cM. Linkage is found in both studies on chromosomes 2 and 3 but with peaks of linkage that are relatively distant between the

two studies (chromosome 2: this study, 89 cM; the study by Comuzzie et al., 50 cM; chromosome 3: this study, 124 cM; Comuzzie et al., 201 cM) (12). We provide no corroborating evidence for linkage of adiponectin on chromosomes 5 or 14. Differences between populations in loci identified by linkage analysis may arise because of genetic heterogeneity, i.e., the set of important genes is different in each population. They may also arise simply by chance because the stochastic variability of effect estimates can be substantial for loci of moderate effects with linkage studies of typical sample size, even in the absence of genetic heterogeneity. Although the present sample size is typical for a linkage study, such studies require quite large sample sizes to detect loci with moderate genetic effects. For example, using formulae derived by Sham et al. (37), we estimated that the power to detect linkage (LOD >1.44) of the present sample (860 sib-pairs, assuming a polymorphic information content of 0.7) is 85% for a locus accounting for 39% of the variance (i.e., all the heritability in age/sex-adjusted adiponectin), 58% for a locus accounting for 30% of the variance, and 23% for a locus accounting for 20% of the variance. Thus, loci that are detectable by linkage studies of this size are likely to be those with major effects on the trait.

By far the largest peak of linkage we observed is on chromosome 9, suggesting that genetic polymorphisms in this region may be contributing to variation in adiponectin concentrations in the Pima population. The estimates derived from the present analyses suggest that this locus accounts for 46% of the variation in age/sex-adjusted adiponectin levels. This estimate must be interpreted with caution, however, because the multiple testing involved in a genome-wide scan will produce an upward bias, the magnitude of which is difficult to quantify (38). The LOD-1 support interval for the chromosome 9 region comprises ~14 Mbp containing at least 130 genes. Chromosome 9p was not linked to type 2 diabetes in the previous genome-wide scan in this population (13). The previous genome-wide scan also did not give strong linkage with BMI on chromosome 9p (LOD = 0.4), although in the present families, the BMI linkage was nominally significant (LOD = 0.7). The linkage signal with adiponectin levels was only partly attenuated with adjustment for BMI. This suggests that the effect of the putative adiponectin locus on chromosome 9p is, at least in part, independent of any effect on BMI.

A number of genome-wide scans examined type 2 diabetes in other populations, and only a single report suggested linkage of chromosome 9 to age of onset of type 2 diabetes in Mexican Americans (39). Interestingly, quantitative trait loci related to insulin action have been described in this chromosomal region in the Pima population (18). Both glucose disposal during euglycemic-hyperinsulinemic clamp (LOD = 1.0, peak 10 cM) (18) and the insulin sensitivity index (ISI) (LOD = 2.0, peak 3 cM) (40) have shown modest linkage in this region of chromosome 9. This raises the possibility of pleiotropic effect—that a polymorphism of a single gene in this region might influence both adiponectin and insulin sensitivity. Bivariate linkage analyses with examination of both traits (in this case, adiponectin and ISI) allow hypothesis testing of pleiotropy versus coincident linkage (41). These analyses partition the correlation between the two traits into monogenic, polygenic, and environmental components, in addition to estimating the variance components for each trait simultaneously. Bivariate analyses in participants without diabetes in the present study did not, however, support a significant pleiotropic effect. The LOD peak of the bivariate phenotype (ISI and adiponectin) was smaller than that of adiponectin alone (LOD = 2.3 at 15 cM). Further, the monogenic genetic correlation between the two traits (a measure of the extent to which the locus influencing adiponectin overlaps with that influencing ISI) was 0.51 and was not significantly different from 0 (P = 0.0931). We also report a strong relationship of adiponectin concentrations to HDL cholesterol, as has also been seen in other populations (12). Although the basis of this relationship is, as yet, unknown, strong evidence for a quantitative trait locus for HDL cholesterol has been reported on chromosome 9p in Mexican Americans (42). Linkage of HDL cholesterol was not apparent on chromosome 9 in a similar analysis in the Pima population (43).

Regions of chromosome 3 were not linked to type 2 diabetes in the previous genome-wide scan in the Pima population (13); however, linkage was detected in a number of phenotypes relating to insulin action at around 150 cM (fasting insulin, glucose disposal during a euglycemic-hyperinsulinemic clamp at both physiological and supraphysiological insulin doses) (18). We also describe modest linkage on chromosome 10. Although this result does not fulfil criteria for suggestive linkage (44), it is of interest because it appears to corroborate the findings of Comuzzie et al. (12) and is in a region of previous linkage to BMI in another population (45). It is not in a region of linkage to BMI (13), type 2 diabetes (13), or insulin sensitivity (13,18) in the Pima population. Linkage on chromosome 2 in this study is also not coincident with linkage to BMI (13), type 2 diabetes (13), or insulin sensitivity (18,40) in previous investigations in the Pima population.

Adiponectin has putative anti-inflammatory actions (46). Interestingly, chromosome 9p is the site of the VLDL receptor, which is expressed in adipose tissue (47) and is believed to influence uptake of free fatty acids into peripheral tissues including adipose (48). VLDL may also have proinflammatory actions, increasing cytokine secretion (49) and activating nuclear transcription factor-κB, the key signaling pathway in inflammatory response (50). Adiponectin is believed to exert anti-inflammatory actions by inhibition of the same nuclear transcription factor-κB pathway (46). It is plausible then that variation in VLDL receptor might influence variation in adiponectin. Chromosome 9p is also the site of a number of interferons, although they are somewhat more distant than the peak that we have observed.

In conclusion, we present evidence that sites on chromosomes 2, 3, 9, and 10 may be influencing concentrations of adiponectin in the Pima population.

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REFERENCES

1. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF: A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 270:26746–26749, 1995
2. Hu E, Liang P, Spiegelman BM: AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 271:10697–10703, 1996
3. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y: Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 20:1595–1599, 2000
4. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA: Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 86:1930–1935, 2001
5. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM: Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab* 86:3815–3819, 2001
6. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y: PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 50:2094–2099, 2001
7. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7:941–946, 2001
8. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE: The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 7:947–953, 2001
9. Knowler WC, Pettitt DJ, Saad MF, Bennett PH: Diabetes mellitus in the Pima Indians: incidence, risk factors and pathogenesis. *Diabetes Metab Rev* 6:1–27, 1990
10. Knowler WC, Pettitt DJ, Savage PJ, Bennett PH: Diabetes incidence in Pima Indians: contributions of obesity and parental diabetes. *Am J Epidemiol* 113:144–156, 1981
11. Lindsay RS, Funahashi T, Matsuzawa Y, Hanson RL, Tanaka S, Tataranni PA, Knowler WC, Krakoff J: Adiponectin protects against development of type 2 diabetes in the Pima Indian population. *Lancet* 360:57–58, 2002
12. Comuzzie AG, Funahashi T, Sonnenberg G, Martin LJ, Jacob HJ, Black AE, Maas D, Takahashi M, Kihara S, Tanaka S, Matsuzawa Y, Blangero J, Cohen D, Kissebah A: The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. *J Clin Endocrinol Metab* 86:4321–4325, 2001
13. Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, Foroud T, Kobes S, Baier L, Burns DK, Almasy L, Blangero J, Garvey WT, Bennett PH, Knowler WC: An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. *Am J Hum Genet* 63:1124–1132, 1998
14. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group.* Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
15. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257:79–83, 1999
16. Dabelea D, Pettitt DJ, Hanson RL, Imperatore G, Bennett PH, Knowler WC: Birth weight, type 2 diabetes, and insulin resistance in Pima Indian children and young adults. *Diabetes Care* 22:944–950, 1999
17. Allison DB, Neale MC, Zannolli R, Schork NJ, Amos CI, Blangero J: Testing the robustness of the likelihood-ratio test in a variance-component quantitative-trait loci-mapping procedure. *Am J Hum Genet* 65:531–544, 1999
18. Pratley RE, Thompson DB, Prochazka M, Baier L, Mott D, Ravussin E, Sakul H, Ehm MG, Burns DK, Foroud T, Garvey WT, Hanson RL, Knowler WC, Bennett PH, Bogardus C: An autosomal genomic scan for loci linked to prediabetic phenotypes in Pima Indians. *J Clin Invest* 101:1757–1764, 1998
19. Dubovsky J, Sheffield VC, Duyk GM, Weber JL: Sets of short tandem repeat polymorphisms for efficient linkage screening of the human genome. *Hum Mol Genet* 4:449–452, 1995
20. Amos CI: Robust variance-components approach for assessing genetic linkage in pedigrees. *Am J Hum Genet* 54:535–543, 1994
21. Falconer DS, Mackay TFC: *Introduction to Quantitative Genetics.* Harlow, U.K., Longman, 2000
22. Hopper JL, Mathews JD: Extensions to multivariate normal models for pedigree analysis. *Ann Hum Genet* 46:373–383, 1982
23. Curtis D, Sham PC: Using risk calculation to implement an extended relative pair analysis. *Ann Hum Genet* 58:151–162, 1994
24. Fulker DW, Cherny SS, Cardon LR: Multipoint interval mapping of quantitative trait loci, using sib pairs. *Am J Hum Genet* 56:1224–1233, 1995
25. Menzaghi C, Ercolino T, Di Paola R, Berg AH, Warram JH, Scherer PE, Trischitta V, Doria A: A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. *Diabetes* 51:2306–2312, 2002
26. Howard BV, Knowler WC, Vasquez B, Kennedy AL, Pettitt DJ, Bennett PH: Plasma and lipoprotein cholesterol and triglyceride in the Pima Indian population: comparison of diabetics and nondiabetics. *Arteriosclerosis* 4:462–471, 1984
27. Criqui MH, Golomb BA: Epidemiologic aspects of lipid abnormalities. *Am J Med* 105:48S–57S, 1998
28. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE: Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia* 46:459–469, 2003
29. Imagawa A, Funahashi T, Nakamura T, Moriwaki M, Tanaka S, Nishizawa H, Sayama K, Uno S, Iwahashi H, Yamagata K, Miyagawa J, Matsuzawa Y: Elevated serum concentration of adipose-derived factor, adiponectin, in patients with type 1 diabetes. *Diabetes Care* 25:1665–1666, 2002
30. Takahashi M, Arita Y, Yamagata K, Matsukawa Y, Okutomi K, Horie M, Shimomura I, Hotta K, Kuriyama H, Kihara S, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y: Genomic structure and mutations in adipose-specific gene, adiponectin. *Int J Obes Relat Metab Disord* 24:861–868, 2000
31. Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R: Hormonal regulation of adiponectin gene expression in 3T3–L1 adipocytes. *Biochem Biophys Res Commun* 290:1084–1089, 2002
32. Halleux CM, Takahashi M, Delporte ML, Detry R, Funahashi T, Matsuzawa Y, Brichard SM: Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. *Biochem Biophys Res Commun* 288:1102–1107, 2001
33. Li WD, Reed DR, Lee JH, Xu W, Kilker RL, Sodam BR, Price RA: Sequence variants in the 5′ flanking region of the leptin gene are associated with obesity in women. *Ann Hum Genet* 63:227–234, 1999
34. Kondo H, Shimomura I, Matsukawa Y, Kumada M, Takahashi M, Matsuda M, Ouchi N, Kihara S, Kawamoto T, Sumitsuji S, Funahashi T, Matsuzawa Y: Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. *Diabetes* 51:2325–2328, 2002
35. Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, Yamauchi T, Otabe S, Okada T, Eto K, Kadowaki H, Hagura R, Akanuma Y, Yazaki Y, Nagai R, Taniyama M, Matsubara K, Yoda M, Nakano Y, Tomita M, Kimura S, Ito C, Froguel P, Kadowaki T: Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes* 51:536–540, 2002
36. Stumvoll M, Tschritter O, Fritsche A, Staiger H, Renn W, Weisser M, Machicao F, Haring H: Association of the T-G polymorphism in adiponectin (exon 2) with obesity and insulin sensitivity: interaction with family history of type 2 diabetes. *Diabetes* 51:37–41, 2002
37. Sham PC, Cherny SS, Purcell S, Hewitt JK: Power of linkage versus association analysis of quantitative traits, by use of variance-components models, for sibship data. *Am J Hum Genet* 66:1616–1630, 2000
38. Goring HH, Terwilliger JD, Blangero J: Large upward bias in estimation of locus-specific effects from a genomewide scan. *Am J Human Genet* 69:1357–1369, 2001
39. Duggirala R, Blangero J, Almasy L, Dyer TD, Williams KL, Leach RJ, O'Connell P, Stern MP: Linkage of type 2 diabetes mellitus and of age at onset to a genetic location on chromosome 10q in Mexican Americans. *Am J Hum Genet* 64:1127–1140, 1999
40. Hanson RL, Imperatore G, Narayan KM, Roumain J, Fagot-Campagna A, Pettitt DJ, Bennett PH, Knowler WC: Family and genetic studies of indices of insulin sensitivity and insulin secretion in Pima Indians. *Diabetes Metab Res Rev* 17:296–303, 2001
41. Almasy L, Dyer TD, Blangero J: Bivariate quantitative trait linkage analysis: pleiotropy versus co-incident linkages. *Genet Epidemiol* 14: 953–958, 1997

42. Arya R, Duggirala R, Almasy L, Rainwater DL, Mahaney MC, Cole S, Dyer TD, Williams K, Leach RJ, Hixson JE, MacCluer JW, O'Connell P, Stern MP, Blangero J: Linkage of high-density lipoprotein-cholesterol concentrations to a locus on chromosome 9p in Mexican Americans. *Nat Genet* 30:102–105, 2002
43. Imperatore G, Knowler WC, Pettitt DJ, Kobes S, Fuller JH, Bennett PH, Hanson RL: A locus influencing total serum cholesterol on chromosome 19p: results from an autosomal genomic scan of serum lipid concentrations in Pima Indians. *Arterioscler Thromb Vasc Biol* 20:2651–2656, 2000
44. Lander E, Kruglyak L: Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 11:241–247, 1995
45. Hager J, Dina C, Francke S, Dubois S, Houari M, Vatin V, Vaillant E, Lorentz N, Basdevant A, Clement K, Guy-Grand B, Froguel P: A genome-wide scan for human obesity genes reveals a major susceptibility locus on chromosome 10. *Nat Genet* 20:304–308, 1998
46. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y: Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation* 102:1296–1301, 2000
47. Webb JC, Patel DD, Jones MD, Knight BL, Soutar AK: Characterization and tissue-specific expression of the human 'very low density lipoprotein (VLDL) receptor' mRNA. *Hum Mol Genet* 3:531–537, 1994
48. Tacken PJ, Hofker MH, Havekes LM, van Dijk KW: Living up to a name: the role of the VLDL receptor in lipid metabolism. *Curr Opin Lipidol* 12:275–279, 2001
49. Sampedro MC, Motran C, Gruppi A, Kivatinitz SC: VLDL modulates the cytokine secretion profile to a proinflammatory pattern. *Biochem Biophys Res Commun* 285:393–399, 2001
50. Dichtl W, Nilsson L, Goncalves I, Ares MP, Banfi C, Calara F, Hamsten A, Eriksson P, Nilsson J: Very low-density lipoprotein activates nuclear factor-kappaB in endothelial cells. *Circ Res* 84:1085–1094, 1999