International Journal of Obesity (2007) 31, 535–542
 0307-0565/07 $30.00
www.nature.com/ijo

ORIGINAL ARTICLE

Linkage analysis of circulating levels of adiponectin in hispanic children

ME Tejero[1], G Cai[1], HHH Göring[1], V Diego[1], SA Cole[1], CA Bacino[2], NF Butte[2] and AG Comuzzie[1]

[1]*Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX, USA and* [2]*Department of Pediatrics, USDA/ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX , USA*

Introduction: Adiponectin, a hormone produced exclusively by adipose tissue, is inversely associated with insulin resistance and proinflammatory conditions. The aim of this study was to find quantitative trait loci (QTLs) that affect circulating levels of adiponectin in Hispanic children participating in the VIVA LA FAMILIA Study by use of a systematic genome scan.
Methods: The present study included extended families with at least one overweight child between 4 and 19 years old. Overweight was defined as body mass index (BMI) 95th percentile. Fasting blood was collected from 466 children from 127 families. Adiponectin was assayed by radioimmunoassay (RIA) technique in fasting serum. A genome-wide scan on circulating levels of adiponectin as a quantitative phenotype was conducted using the variance decomposition approach.
Results: The highest logarithm of odds (LOD) score (4.2) was found on chromosome 11q23.2–11q24.2, and a second significant signal (LOD score = 3.0) was found on chromosome 8q12.1–8q21.3. In addition, a signal suggestive of linkage (LOD score = 2.5) was found between 18q21.3 and 18q22.3. After adjustment for BMI-Z score, the LOD score on chromosome 11 remained unchanged, but the signals on chromosomes 8 and 18 dropped to 1.6 and 1.7, respectively. Two other signals suggestive of linkage were found on chromosome 3 (LOD score = 2.1) and 10 (LOD score = 2.5). Although the region on chromosome 11 has been associated with obesity and diabetes-related traits in adult populations, this is the first observation of linkage in this region for adiponectin levels. Our suggestive linkages on chromosomes 10 and 3 replicate results for adiponectin seen in other populations. The influence of loci on chromosomes 18 and 8 on circulating adiponectin seemed to be mediated by BMI in the present study.
Conclusion: Our genome scan in children has identified a novel QTL and replicated QTLs in chromosomal regions previously shown to be linked with obesity and type 2 diabetes (T2D)-related phenotypes in adults. The genetic contribution of loci to adiponectin levels may vary across different populations and age groups. The strong linkage signal on chromosome 11 is most likely underlain by a gene(s) that may contribute to the high susceptibility of these Hispanic children to obesity and T2D.
International Journal of Obesity (2007) **31,** 535–542. doi:10.1038/sj.ijo.0803436; published online 8 August 2006

Keywords: adiponectin; genetics; linkage; childhood obesity; QTL; genome scan

Introduction

The hormone adiponectin is produced exclusively by adipose tissue and has insulin-sensitizing and anti-inflammatory properties. As opposed to other adipose tissue products, low circulating levels of this protein are associated with obesity, type 2 diabetes (T2D) and the metabolic syndrome.[1,2] Consistent negative correlations between adiponectin, insulin resistance and inflammatory states have been reported.[1] These latter two conditions improve after weight loss with concomitant increase of adiponectin levels.[3] Adiponectin structure is similar to tumor necrosis factor-α and seems to have a counter-regulatory activity with respect to this proinflammatory cytokine.[4] As observed in adults, obese children and adolescents have lower adiponectin levels than their normal weight counterparts, and serum adiponectin is positively correlated with insulin sensitivity and high-density lipoprotein, and negatively to fasting proinsulin and proinsulin/insulin ratio.[5] A study by Reinehr *et al.*[6] found that weight loss in children is associated with a significant increase of circulating adiponectin and a decrease of insulin resistance.

A significant heritability for adiponectin levels in Hispanic children has been previously reported by our group.[7] Heritability (h^2) of serum adiponectin was 0.93 ± 0.10

Correspondence: Dr Anthony Comuzzie, Department of Genetics, Southwest Foundation for Biomedical Research, 7620 NW Loop 410, PO Box 760549, San Antonio, TX 78245-0549, USA.
E-mail: agcom@darwin.sfbr.org
Received 27 November 2005; revised 31 May 2006; accepted 2 June 2006; published online 8 August 2006

($P = 2.4 \times 10^{-40}$). Adiponectin differed by age ($P = 0.001$), sex ($P = 0.04$) and weight ($P = 0.001$). Numerous investigations have analyzed the genetic component of obesity and T2D-related phenotypes in humans and other species.[8] Kissebah *et al*.[9] conducted linkage analyses on phenotypes of the metabolic syndrome, including body weight, hip and waist circumference, insulin and leptin levels. The multipoint linkage analyses of these phenotypes identified significant logarithm of odds (LOD) scores on chromosome 3 at 189–203 cM. Vasseur *et al*.[10] reported association between some single nucleotide polymorphisms (SNPs) and circulating levels of this adipose tissue protein. Four studies have conducted linkage analysis using adiponectin levels as a phenotype. Comuzzie *et al*.[11] identified regions on chromosomes 5 and 14, and secondary signals on chromosomes 2 and 10 in Caucasian adults. A study by Pollin *et al*.[12] in Old Order Amish found linkage of adiponectin levels to chromosome 3p27, and an investigation in Pima Indians, a population characterized by a high prevalence of obesity and diabetes, found significant linkage to chromosome 9p and suggestive evidence of linkage on chromosomes 3, 2 and 10.[13] A fourth study by Chuang *et al*.[14] found suggestive linkage of adiponectin on chromosome 15 at 39 cM for Chinese (LOD = 3.19) and on chromosome 18 at 28 cM for Japanese adults. Some of these regions have been previously reported in association with obesity and diabetes-related phenotypes.

The present study is the first linkage analysis on circulating levels of adiponectin in children. The aim of the present investigation was to find quantitative trait loci (QTLs) that affect circulating levels of adiponectin in Hispanic children participating in the VIVA LA FAMILIA Study by use of a systematic genome scan.

Materials and methods

Study design and subjects

Genetic and environmental factors influencing fasting serum adiponectin were investigated in a subsample of 466 children from the 1030 enrolled in the VIVA LA FAMILIA Study, which was designed to genetically map childhood obesity in the Hispanic population. Each family was ascertained on an overweight proband between the ages 4 and 19 years using a bivariate ascertainment scheme (i.e., 95th percentile for body mass index (BMI) and 85th percentile for fat mass. Once identified, the overweight proband and all siblings, 4–19 years of age, and their parents were invited to the Children's Nutrition Research Center for a tour and full explanation of the study. All children and their parents gave written informed consent. The protocol was approved by the Institutional Review Board for Human Subject Research for Baylor College of Medicine and Affiliated Hospitals.

The overweight proband and all siblings were then characterized for body size, and endophenotypes associated with the development of obesity. Here, we report our findings on the linkage analysis using adiponectin levels as a phenotype.

Phenotyping

Body weight was measured with a digital balance and registered to the nearest 0.1 kg. and height to the nearest 1 mm was measured with a stadiometer. Fasting serum adiponectin levels were measured by radioimmunoassay (RIA) (Linco Research Inc., St Charles, MO, USA).

Genotyping

The 760 participants (children and their parents) were genotyped in the present study. DNA was isolated from whole blood using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). To genotype each participant, we used the autosomal markers from the ABI PRISM Linkage Mapping Set-MD10 Version 2.5 (Applied Biosystems, Foster City, CA, USA). This mapping set consists of fluorescently labeled polymerase chain reaction (PCR) primers that amplify dinucleotide single tandem repeats (STRs) selected from the Genethon human linkage map.[15] The set is designed to create a map with markers spaced an average of 10 cM apart (range 2.4–24.1 cM). DNA from study participants was arrayed on 384-well PCR plates using the Robbins Hydra-96 Microdispenser (Sunnyvale, CA, USA). Each marker was amplified in a separate PCR reaction to avoid the preferential amplification that can occur in combined reactions. PCR reactions used the True Allele PCR Premix (Applied Biosystems), and amplification occurred in Applied Biosystems 9700 thermocyclers, according to the manufacturer's specifications. After PCR, the products of separate PCR reactions, for each individual, were pooled using the Robbins Hydra-384 microdispenser, and a labeled size standard was added to each pool. The pooled PCR products were loaded into an ABI PRISM 3100 Genetic Analyzer for laser-based automated genotyping. The STRs were detected and quantified by fluorescent emissions, their sizes were estimated by comparison with the labeled size standard and genotypes were scored using the Genotyper software package (Applied Biosystems).

Pedigree errors were detected using PREST (pedigree relationship statistical tests),[16] which employs likelihood-based inference statistics for genome-wide identity-by-descent (IBD) allele sharing. Pedigree errors were resolved by making changes to the existing pedigree structure that required the fewest assumptions and that were consistent with the genetic data. Using SimWalk2[17] genotypes that resulted in Mendelian inconsistencies and spurious double recombinants were blanked if not resolved by the laboratory. We used Loki[18] to compute the IBD matrix needed for our linkage analyses. All three programs, PREST, SimWalk2 and Loki, require information on the relative distances between

markers. We used the sex-averaged chromosomal maps obtained at the Marshfield Center for Medical Genetics.

Analysis

Using a variance component model, we tested the null hypothesis that the additive genetic variance owing to a QTL (σ^2_q) equals zero or absence of linkage by comparing the likelihood of this restricted model with that of a model in which σ^2_q is estimated. The difference between the two $\log_{10}$ likelihoods produces a LOD score that is the equivalent of the classical LOD score of linkage analysis. Twice the difference in $\log_e$ likelihoods of these models yields a test statistic that is asymptotically distributed as a $\frac{1}{2}$ mixture of a χ^2 variable with 1df and a point mass at zero.[19]

A genome scan was conducted in SOLAR using adiponectin levels as a phenotype and sex, age, age^2 and their interactions as covariates. Empirical LOD score adjustment was conducted by the method described by Blangero *et al*.[20] The effect of BMI-Z score was tested by including it as a covariate and repeating the genome scan. The chromosomal region investigated for positional candidate genes was defined as the 1 LOD score unit support interval. The selection of positional candidate genes was conducted using the NCBI map viewer database.

Results

The present study included a total of 466 children from 127 families, with approximately 50% each of boys and girls in the sample. Descriptive data of the cohort are shown in Table 1. Table 2 includes information on the relative pairs in this study. The average age of the children was 10.7 years. The distribution across the Tanner stages of development was as follows: for the Genital/Breast criteria 211 children corresponded to Tanner stages I, 75 to II, 88 to stage III, 47 to stage IV and 32 to Tanner stage V. For the pubic hair criteria, 220 children were classified as Tanner stage I, 64 as II, 61 as III, 76 as IV and 21 as V. The analysis of the effect of age, sex, Tanner stage and body composition on adiponectin levels in these children showed a significant difference between children classified as Tanner stage I and II–V.[7] In the present study, the effect of this variable was not significant after adjustment for sex, age and BMI-Z score. Mean fasting serum levels of adiponectin was 13.3 μg/ml in boys and 15.4 μg/ml in girls with a significant heritability of 0.93 ± 0.10 ($P = 2.4 \times 10^{-40}$).[7] Results of the linkage analysis are shown in Figure 1. The highest LOD score was observed on chromosome 11q23–24 between markers D11S925 and D11S968, with an 1 LOD support interval from 116 to 152 cM (LOD = 4.2). A second signal was identified on

Table 1 Anthropometry of the non-overweight and overweight Hispanic children

N	*Boys*, n = *234*	*Girls*, n = *238*
Age (years)	11.0 ± 3.5	10.4 ± 3.8
Weight (kg)	56.7 ± 26.6	49.0 ± 25.1
Height (m)	147.2 ± 20.5	139.0 ± 19.3
BMI (kg/m^2)	25.9 ± 7.7	24.0 ± 7.3
BMI-Z score	1.6 ± 1.02	1.3 ± 1.05

Abbreviations: BMI, body mass index. Mean ± s.d.

Table 2 Relative pairs in the analyzed cohort of Hispanic children in the VIVA LA FAMILIA Study

Relationship	N
Siblings	948
Half-siblings	137
Half-avuncular	15
First cousins	415
Half-first cousins	28
Half-siblings and half-cousins	2
Identical sibpair	2

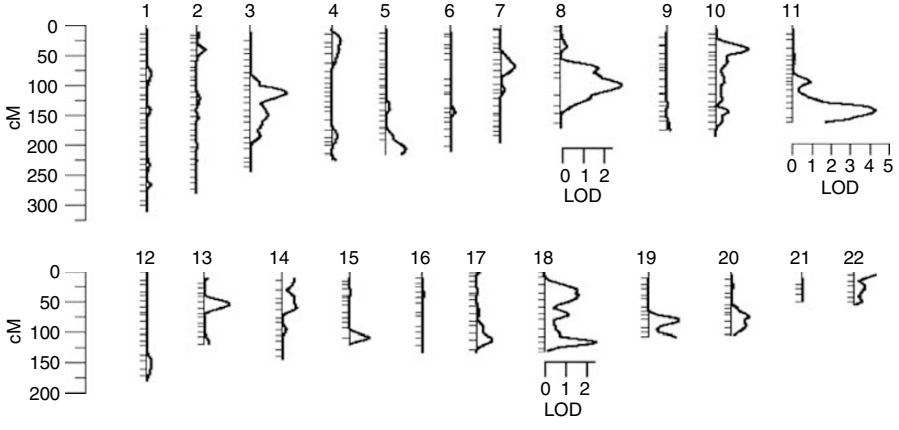

Figure 1 String plot of fasting serum adiponectin in Hispanic children using sex, age and age^2 as covariates. LOD scores were empirically adjusted using simulations.

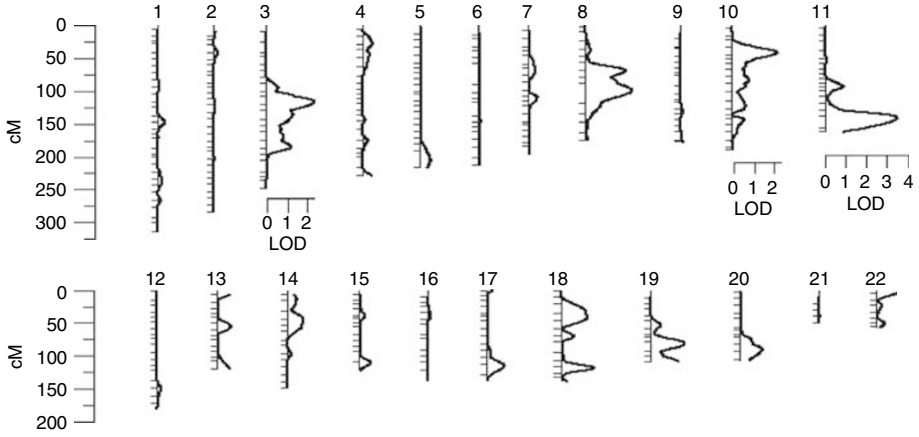

Figure 2 String plot of fasting serum adiponectin of Hispanic children using sex, age, age^2 and BMI-Z score as covariates. LOD scores were empirically adjusted using simulations.

chromosome 8, between markers D8S1771 and D8S1784 (43–112 cM, LOD = 3.0), and finally a signal suggestive of linkage was observed on the region D18S61–D18S1161 on chromosome 18 (LOD = 2.6).

After adjustment for BMI-Z score (Figure 2), the signal on chromosome 11 remained unchanged; however, the signal dropped on chromosomes 8 and 18 to LOD scores of 1.6 and 1.8, respectively. Two other regions suggestive of linkage were found on chromosomes 3 and 10. On chromosome 3, suggestive linkage was found between markers D31285 and D3S1271 (100–130 cM) with an LOD score of 2.1, and on chromosome 10 a signal was detected between D10S547 and D10S197 (29.15–52.0 cM) with a LOD score of 2.5. Figure 3 shows the marker distribution and second genome scan on chromosome 11. Other regions showing LOD scores >1.0 were found on chromosomes 5q (1.4), 19q (1.5) and 20q (1.4).

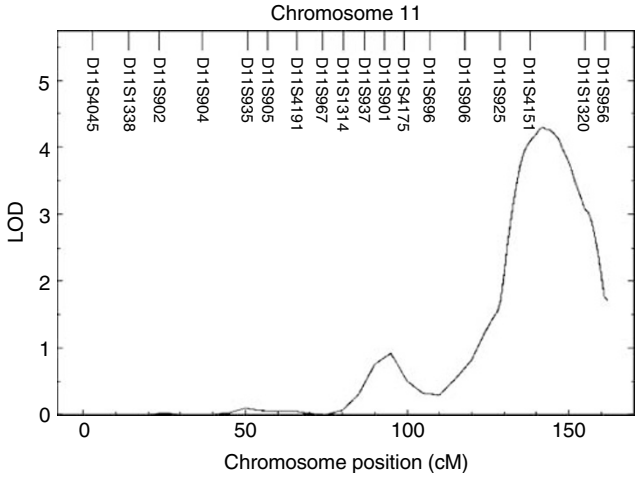

Figure 3 Markers on chromosome 11.

Discussion

Few linkage studies on childhood obesity and related phenotypes have been conducted until now. The present study represents the first genome-wide search for adiponectin levels in children. This population is at high risk for the development of obesity-related comorbidities, such as T2D. The children in this investigation showed significant metabolic abnormalities in association with excessive adiposity, as reported previously.[21] Given the high heritability of fasting serum adiponectin, and its inverse associations with insulin resistance, dyslipidemia and blood pressure in the VIVA LA FAMILIA Study,[7,21] and the high prevalence of obesity and T2D in the United States Hispanic population,[22] we sought to identify genetic loci contributing to circulating adiponectin in Hispanic children. Previous investigations have found significant linkage for adiponectin in adults (Table 3).[11–14] The present analysis identified three new regions on chromosomes 11, 8 and 18 linked to adiponectin levels in Hispanic children, and replicated two regions on chromosomes 3 and 10 reported in adults.

Our strongest linkage for fasting serum adiponectin was on chromosome 11 and its strength was not diminished after adjustment for BMI-Z score. Although this is the first identification of a linkage signal on chromosome 11 for adiponectin, this chromosomal region has been linked with obesity and diabetes-related traits (Table 4). Stein *et al*.[23] identified moderate evidence for linkage on chromosome 11 near marker D11S2008) using five components of the metabolic syndrome. Their analysis confirmed observations by other investigators who reported significant linkage of body size,[24] diabetes mellitus[25] and insulin resistance[26] to chromosome 11q. Significant linkage to chromosome 11q was found for obesity in subjects with T2D in a study of siblings by Van Tilburg *et al*.[27] After fine mapping this

Table 3 Linkage studies for circulating adiponectin levels in adults

Population	*Identified chromosomes*	*Map distance (cM)*	*LOD score*	*LOD score After adjustment for BMI*	*Reference*
White northern	5	35	4.06		Comuzzie[11]
European ancestry	14	29	3.2		
	2	50	2.7		
	10	67	1.9		
Pima Indians	3	124	1.9	1.8	Lindsay[13]
	9	18	3.5	1.7	
	2	89	0.9	1.6	
	10	70	1.7	1.0	
Hawaiian	18	28	2.4	2.23	Chuang[14]
Japanese	3	112	1.72	1.72	
	20	12	1.79	1.55	
Chinese	15	39	3.29	1.31	
Amish	3	209	2.13		Pollin[12]
	7	94	1.87		
	9	144	1.54		
	10	44	1.73		
	16	15	1.69		

Abbreviations: BMI, body mass index; LOD, logarithms of odds.

Table 4 Replications of previously reported QTLs linked to obesity and diabetes-related phenotypes

Chromosomal region	*Markers*	*Phenotype*	*Population*	*LOD score*	*Reference*
11q22–11q22.3	D11S940–D11S2000	BMI	White	2.5	Van Tilburg[27]
11q14–q24	—	—	—	—	—
11q21–q22	D11S2000	BMI	Pima Indians	2.8	Norman[45]
11q22–23	D11S2000	Body fat	Nigerian	3.3	Adeyemo[46]
11p15.5–11q23	D11S2008	Metabolic syndrome	White	$P=0.003$	Stein[23]
		BMI	White (Amish)	$P=0.0079$	Platte[47]
11q23.1	D11S2366	Body fat %	Pima Indians	2.1	Norman[48]
		Body fat %	Pima Indians	2.8	Norman[45]
11q23.3	D11S1998	BMI	Pima Indians	2.7	Lindsay[50]
11q23.3	D11S976	24 h energy expenditure	Pima Indians	2.0	Norman[45]
11q24.1	D11S4464	BMI	Pima Indians	2.7	Lindsay[50]
		BMI	Whites	2.8	Stone[39]
		BMI	Mexican-American	2.7	Arya[51]
11q24.2	D11S934	BMI	—	2.6	Stone[39]
10p12.31	D10S582	Obesity	Whites and African-American	NPL = 2.68	Dong[52]
				$P=0.0005$	Price[53]
			Whites and African–American		
10p12.2	D10S197	Obesity	White children and adolescents	2.24	Saar[38]
10p12.2	D10S197	BMI	French population	4.9	Hager[54]
10p12.1	D10S204	Obesity	—	2.5	Hinney[55]
10q21.1	D10S220	BMI-adjusted leptin	Old Order Amish	2.7	Hsueh[49]
8q24	D8S1179–	BMI	African	2.56	Palmer[56]
	D8S1128	Apnea–hypopnea index	American	1.29	
8p11.23	D8S1121	BMI	Mexican–American	3.2	Mitchell[57]
8q11.22	D8S1110	Leptin	Mexican–American	2.2	Comuzzie[40]
8q12.1	D8S1113	BMI	Mexican–American	$P=0.0013$	Gorlova[58]
8q23.1	D8S556	BMI	Whites	2.0	Chagnon[59]
8q21	D8S275	Young onset type 2 diabetes	British/Irish descents	3.0	Frayling[41]
3p11.1	D3S2465	BMI, SBP, DBP	Whites	2.59	Turner[36]
3q11.2	D3S3045	BMI $>30\,kg/m^2$	Whites	NPL = 1.88	Li[60]
18q21.32	D18S1155	Obesity	Finns	2.4	Ohman[42]
18q21.31	D18S858	BMI and blood pressure	Whites	2.6	North[61]
18q22	MC4R	Respiratory quotient	Whites	$P=0.04$	Chagnon[43]
18q22–23	D18S1371	Fasting glucose	European Americans	6.59	Li[37]

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; LOD, logarithms of odds; NPL, non-parametric linkage; QTL, quantitative trait loci; SBP, systolic blood pressure.

Table 5 Positional candidates on chromosome 11

Chromosome	*Markers*	*LOD score*	*Distance in cM*	*Candidates*
11q22–23	D11S925–D11S968	4.2	116–152	ASAM – Adipocyte-specific adhesion molecule TBRG1 – Transforming growth factor beta regulator 1 ESAM – Endothelial cell adhesion molecule CDON – Cell adhesion molecule-related/down regulated oncogenes TIRAP – Toll-interleukin-1 receptor domain/adaptor protein KCNJ1 – Potassium inwardly rectifying channel

region, they found a LOD score of 2.4 on chromosome 11q14–q24 using BMI as a phenotype.

The combined phenotype 'diabesity' had a highly significant LOD score of 5.2 at 11q23–24 in Pima Indians. This study reported 455 SNPs associated with BMI and a second region harboring a cluster of SNPs related to diabetes in this population. This group sequenced 11 physiological candidate genes in this region encoding serotonin receptor, dopamine receptor, three apolipoproteins, three zinc-finger proteins, two potassium-channel proteins and glucose-6-phosphate transferase. None of these candidates had nucleotide variants that account for the linkage signal for BMI and diabetes.[28] One of the candidates in the 11q23–24 region is the dopamine D2 receptor gene (*DRD2*). Jenkinson *et al*[29] studied polymorphisms of this gene in Pima Indians, and determined that heterozygotes at the Ser311CysDRD2 polymorphism had a higher BMI than homozygotes. The list of positional candidates proposed in the present study is included in Table 5. Adipocyte-specific adhesion molecule (ASAM) is specifically expressed in adipose tissue,[30] the transforming growth factor beta regulator 1 regulates the development and homeostasis of tissues,[31] the endothelial cell adhesion molecule is a type I transmembrane protein and is a new member of the immunoglobulin superfamily, similar to ASAM,[32] and is considered an adhesion molecule.[30] CDON (OMIM 608707) cell adhesion molecule-related/downregulated oncogenes is a cell surface receptor of the immunoglobulin (Ig)/fibronectin type III repeat family involved in myogenic differentiation.[33] Toll-interleukin1 receptor domain/adaptor protein (TIRAP) (OMIM 606252), which is a protein involved in the inflammatory response in mice.[34] Potassium inwardly rectifying channel (KCNJ1) (OMIM 600359) are important regulator of resting membrane potential and cell excitability and interacts with the phosphatidylinositol 4,5-biphosphate (PIP_2).[35] Most of these new candidates seem to be associated to the immune response.

Our genome scan replicated linkage findings in adults. The QTL on chromosome 3 for adioponectin was previously reported in Pima Indians[13] and Hawaiian Japanese adult subjects.[14] In contrast to our study, adjustment for BMI did not alter their LOD scores. Other phenotypes such as BMI and blood pressure have been linked to this chromosome 3 region.[36,37] The region localized on chromosome 10 has been linked to adiponectin levels in Pima Indians,[13] White Americans of northern European ancestry[11] and Old Order Amish.[12] In addition, linkage has been reported for other obesity-related phenotypes on this chromosomal region in numerous studies, including a previous investigation in children and adolescents.[38]

Table 6 Linkage findings for fasting serum adiponectin in hispanic children

Chromosome	*Distance (cM)*	*Flanking markers*	*LOD score Model 1*	*LOD score Model 2*
11	143	D11S925 D11S968	4.2	4.2
8	100	D8S1771 D8S1784	3.0	1.6
18	115	D18S61 D18S1161	2.6	1.8
10	44	D10S547 D10S197	1.6	2.5
3	120	D3S1285 D3S1271	1.7	2.1

Model 1: sex , age, age^2 as covariates. Model 2: sex, age, age^2 and BMI-Z score as covariates.

The region found on chromosome 8 between markers D8S1771 and D8S1784 has shown significant linkage with the phenotypes BMI[39] and leptin.[40] A study by Frayling *et al*.[41] identified a locus on chromosome 8q21 linked to early-onset T2D in a study conducted in the United Kingdom.

The region on chromosome 18 has been linked to BMI in a previous study in Caucasians of northern European background.[9] The investigation by Chuang *et al*.[14] identified significant linkage signals for adiponectin levels in Hawaiian Japanese population on chromosomes 18p and 15p (Table 6). The signals on chromosome 18 were located at 24 and 41 cM and do not overlap the region found in our study. That study reported that the LOD score on chromosome 18 remained unchanged after adjustment for age, sex and BMI, whereas the signal on chromosome 15 decreased from 3.19 to 1.31, suggesting that the contribution of this QTL may be mediated by body mass.[13] Ohman *et al*.[42] reported suggestive linkage in this region using obesity as a phenotype. This region has been linked to the respiratory quotient in Caucasians [43] and to fasting glucose.[44] The LOD score on the chromosome regions found on chromosomes 8 and 18 dropped to nonsignificant values after adjustment for BMI-Z

score, interestingly both of them have been linked to this trait in previous studies in adults.

In summary, the present investigation identified novel chromosomal regions linked with circulating adiponectin levels on chromosomes 11, 8 and 18, and suggestive of linkage on chromosomes 3 and 10. The last two replicate findings in other populations. All the chromosome regions identified in the present study have been linked to obesity and diabetes-related phenotypes in adults across different ethnicities. Further fine mapping of these regions will allow the identification of genetic polymorphisms that influence the circulating levels of adiponectin.

Acknowledgements

We thank the families who participated in this study, and to acknowledge the contributions of Mercedes Alejandro and Marilyn Navarrete for study coordination, and Sopar Seributra for nursing and Theresa Wilson, Tina Ziba, Maurice Puyau, Firoz Vohra, Anne Adolph, Roman Shypailo, JoAnn Pratt and Maryse Laurent for technical assistance, Grace Meixner and Daniel Zamarripa for genotyping and Jennifer Darling for the data analysis. This work is a publication of the US Department of Agriculture (USDA)/Agricultural Research Service (ARS) Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX, USA. This project was funded with federal funds from the NIH R01 DK59264 and from USDA/ARS under Cooperative Agreement 58-6250-51000-037. The contents of this publication do not necessarily reflect the views or policies of the USDA, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

References

1 Kershaw E, Flier J. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metabol* 2004; **89**: 2548–2556.
2 Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R *et al*. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *JAMA* 2003; **289**: 1799–1804.
3 Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC *et al*. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* 2001; **50**: 1126–1133.
4 Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J *et al*. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; **257**: 79–83.
5 Bacha F, Saad R, Gungor N, Arslanian S. Adiponectin in youth. Relationship to visceral adiposity, insulin sensitivity and β-cell function. *Diabetes Care* 2004; **27**: 547–552.
6 Reinehr T, Roth C, Menke T, Adler W. Adiponectin before and after weight loss in obese children. *J Clin Endocrinol Metab* 2004; **89**: 3790–3794.
7 Butte NF, Comuzzie AG, Cai G, Cole SA, Mehta NR, Bacino CA *et al*. Genetic and environmental factors influencing fasting serum adiponectin in Hispanic children. *J Clin Endocrinol Metabol* 2005; In press.
8 Perusse L, Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G *et al*. The human obesity gene map: the 2004 map. *Obes Res* 2005; **13**: 381–490.
9 Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG *et al*. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. *Proc Natl Acad Sci USA* 2000; **97**: 14478–14483.
10 Vasseur F, Lepretre F, Lacquemant C, Froguel P. Single nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the AMP1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. *Hum Mol Genet* 2002; **11**: 2607–2614.
11 Comuzzie AG, Funahashi T, Sonnenberg G, Martin LJ, Jacob HJ, Black AE *et al*. The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. *J Clin Endocrinol Metab* 2001; **86**: 4321–4325.
12 Pollin TI, Tanner K, O'connell JR, Ott SH, Damcott CM, Shuldiner AR *et al*. Linkage of plasma adiponectin levels to 3q27 explained by association with variation in the APM1 gene. *Diabetes* 2005; **54**: 268–274.
13 Lindsay RS, Funahashi T, Krakoff J, Matsuzawa Y, Tanaka S, Kobes S *et al*. Genome-wide linkage analysis of serum adiponectin in the Pima Indian Population. *Diabetes* 2003; **52**: 2419–2425.
14 Chuang LM, Chiu YF, Sheu WH, Hung YJ, Ho LT, Grove J *et al*. Biethnic comparisons of autosomal genomic scan for loci linked to plasma adiponectin in populations of Chinese and Japanese origin. *J Clin Endocrinol Metab* 2005; **89**: 5772–5778.
15 Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A *et al*. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 1996; **380**: 152–154.
16 Sun L, Wilder K, McPeek MS. Enhanced pedigree error detection. *Hum Hered* 2002; **54**: 99–110.
17 Sobel E, Papp JC, Lange K. Detection and integration of genotyping errors in statistical genetics. *Am J Hum Genet* 2002; **70**: 496–508.
18 Heath SM. Markov chain Monte Carlo segregation and linkage analysis for oligogenic models. *Am J Hum Genet* 1997; **61**: 748–760.
19 Hopper JL, Mathews JD. Extensions to multivariate normal models for pedigree analysis. *Am J Hum Genet* 1982; **46**: 373–383.
20 Blangero J, Williams JT, Almasy L. Robust LOD scores for variance component-based linkage analysis. *Genet Epidemiol* 2000; **29**: S8–S14.
21 Butte NF, Comuzzie AG, Cole SA, Mehta NR, Tejero ME, Bastarrachea R *et al*. Quantitative genetic analysis of the metabolic syndrome in hispanic children. *Ped Res* 2005; **58**: 1243–1248.
22 Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999–2000. *JAMA* 2002; **288**: 1728–1732.
23 Stein CM, Song Y, Elston RC, Jun G, Tiwari HK, Iyengar SK. Structural equation model-based genome scan for the metabolic syndrome. *BMC Genet* 2003; **4**: S99–S103.
24 Imperatore G, Knowler WC, Kobes S, Bennett PH, Hanson RL. Genome-wide linkage analysis of factors characterizing the metabolic syndrome among Pima Indians [abstract]. *Diabetes* 1999; **48** (Suppl): A182.
25 Ghosh S, Watanabe RM, Valle TT, Hauser ER, Magnuson VL, Langefeld CD *et al*. The Finland–United States investigation of non-insulin-dependent diabetes mellitus genetics (fusion) study. II An autosomal genome scan for genes that predispose to type 2 diabetes. *Am J Human Genet* 2000; **67**: 1174–1185.

26 Watanabe RM, Ghosh S, Langefeld CD, Valle TT, Hauser ER, Magnuson VL *et al*. The Finland–United States investigation of non-insulin-dependent diabetes mellitus genetics (fusion) study. II An autosomal genome scan for diabetes-related quantitative trait loci. *Am J Human Genet* 2000; **67**: 1186–1200.
27 Van Tilburg JH, Sandkuijl LA, Stengman EM, Pearson PL, van Haeften TW, Wijmenga C. Variance component analysis of obesity in type 2 diabetes confirms loci on chromosomes 1q and 11q. *Obes Res* 2003; **11**: 1290–1294.
28 Baier L, Kovacs P, Wiedrich C, Cray K, Schemidt A, Shen GQ *et al*. Positional cloning of an obesity/diabetes susceptibility gene(s) on chromosome 11 in Pima Indians. *Ann NY Acad Sci* 2002; **967**: 258–264.
29 Jenkinson CP, Hanson R, Cray K, Wiedrich C, Knowler WC, Bogardus C *et al*. Association of dopamine D2 receptor polymorphisms Ser311Cys and TaqIA with obesity or type 2 diabetes mellitus in Pima Indians. *Int J Obes Relat Metab Disord* 2000; **24**: 1233–1238.
30 Katoh M, Katoh M. IGSF11 gene, frequently up-regulated in intestinal-type gastric cancer, encodes adhesion molecule homologous to CXADR, FLJ22415 and ESAM. *Int J Oncol* 2003; **23**: 525–531.
31 Massague J. TGF-beta signal transduction. *Annu Rev Biochem* 1998; **67**: 753–791.
32 Hirata K, Ishida T, Penta K, Rezaee M, Yang E Wohlgemuth J, Quertermous T. Cloning of an immunoglobulin family adhesion molecule selectively expressed by endothelial cells. *J Biol Chem* 2001; **276**: 16223–16231.
33 Kang JS, Mulieri PJ, Hu Y, Taliana L, Krauss RS. BOC, an Ig superfamily member, associates with CDO to positively regulate myogenic differentiation. *EMBO J* 2002; **21**: 114–124.
34 Yamamoto M, Sato S, Hemmi H, Sanjo H, Uematsu S, Kaisho T *et al*. Essential role for TIRAP in activation of the signaling cascade shared by TLR2 and TLR4. *Nature* 2002; **420**: 324–329.
35 Lopes CM, Zhang H, Rohacs T, Jin T, Yang J, Logothetis DE. Alterations in conserved Kir channel-PIP2 interactions underlie channelopathies. *Neuron* 2002; **34**: 933–944.
36 Turner S, Kardia S, Boerwincle E de AM. Multivariate linkage analysis of blood pressure and body mass index. *Genet Epidemiol* 2004; **27**: 64–73.
37 Li W, Dong C, Li D, Zhao H, Price R. An obesity-related locus in chromosome region 12q23–24. *Diabetes* 2004; **53**: 812–820.
38 Saar K, Geller F, Ruschendorf F, Reis A, Friedel S, Schauble N *et al*. Genome scan for childhood and adolescent obesity in German families. *Pediatrics* 2003; **111**: 321–327.
39 Stone S, Abkevich V, Hunt SC, Gutin A, Russell DL, Neff CD *et al*. A major predisposition to locus for severe obesity, at 4p15–p14. *Am J Hum Genet* 2002; **70**: 1459–1468.
40 Comuzzie AG, Hixson JE, Almasy L, Mitchell BD, Mahaney MC, Dyer TD *et al*. A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. *Nat Genet* 1997; **15**: 273–276.
41 Frayling TM, Lindgren CM, Chevre JC, Menzel S, Wishart M, Benmezroua Y *et al*. Young-onset type 2 diabetes families are the major contributors to genetic loci in the diabetes UK Warren 2 genome scan and identify putative novel loci on chromosomes 8q21, 21q22 and 22q11. *Diabetes* 2003; **52**: 1857–1863.
42 Ohman M, Oksanen L, Kaprio J, Koskenvuo M, Mustajoki P, Rissanen A *et al*. Genome-wide scan of obesity Finnish sibpairs reveals linkage to chromosome Xq24. *J Clin Endocrinol Metab* 2000; **85**: 3183–3190.
43 Chagnon YC, Chen WJ, Perusse L, Chagnon M, Nadeau A, Wilkison WO *et al*. Linkage and association studies between the melanocortin receptors 4 and 5 genes and obesity-related phenotypes in the Quebec family study. *Mol Med* 1997; **3**: 663–673.
44 Li WD, Dong C, Li D, Garrigan C, Price RA. A quantitative trait locus influencing fasting plasma glucose in chromosome region 18q22–23. *Diabetes* 2004; **53**: 2487–2491.
45 Norman RA, Thompson DB, Foroud T, Garvey WT, Bennett PH, Bogardus C *et al*. Genome wide search for genes influencing percent body fat in Pima Indians: suggestive linkage at chromosome 11q21–q22. Pima diabetes gene group. *Am J Hum Genet* 1997; **60**: 166–173.
46 Adeyemo A, Luke A, Cooper R, Wu X, Tayo B, Zhu X *et al*. A genome-wide scan for body mass index among Nigerian families. *Obes Res* 2003; **11**: 266–273.
47 Platte P, Papanicolaou GJ, Johnston J, Klein CM, Doheny KF, Pugh EW *et al*. A study of linkage and association of body mass index in the Old Order Amish. *Am J Med Genet* 2003; **121C**: 71–80.
48 Norman RA, Tataranni PA, Pratley R, Thompson DB, Hanson RL, Prochazka M *et al*. Autosomal genomic scan for loci linked to obesity and energy metabolism in Pima Indians. *Am J Hum Genet* 1998; **62**: 659–668.
49 Hsueh WC, Mitchel B, Schneider J, St Jean P, Pollin TI, Ehm MG *et al*. Genome-wide scan of obesity in the Old order. *Amish J Clin Endocrinol Metab* 2001; **86**: 1199–1205.
50 Lindsay RS, Kobes S, Knowler WC, Bennett PH, Hanson RL. Genome-wide linkage analysis assessing parent-of-origin effects in the inheritance of type 2 diabetes and BMI in Pima Indians. *Diabetes* 2001; **50**: 2850–2857.
51 Arya R, Duggirala R, Jenkinson CP, Almasy L, Blangero J, O'Connell P *et al*. Evidence of a novel quantitative-trait locus for obesity on chromosome 4p in Mexican–Americans. *Am J Hum Genet* 2004; **74**: 272–282.
52 Dong C, Wang S, Li W, Li D, Zhao H, Price R. Interacting genetic loci on chromosomes 20 and 10 influence extreme obesity in humans. *Am J Hum Genet* 2003; **72**: 115–124.
53 Price RA, Li WD, Bernstein A, Crystal A, Golding EM, Weisberg SJ *et al*. A locus affecting obesity in human chromosome region 10p12. *Diabetologia* 2001; **44**: 363–366.
54 Hager J, Dina C, Francke S, Dubois S, Houari M, Vatin V *et al*. A genome-wide scan for human obesity genes reveals a major susceptibility locus on chromosome 10. *Nat Genet* 1998; **20**: 304–308.
55 Hinney A, Ziegler A, Oeffner F, Wedewardt C, Vogel M, Wulftange H *et al*. Independent confirmation of a major locus for obesity on chromosome 10. *J Clin Endocrinol Metab* 2000; **85**: 2962–2965.
56 Palmer LJ, Buxbaum SG, Larkin EK, Patel SR, Elston RC, Tishler PV *et al*. Whole genome scan for obstructive sleep apnea and obesity in African–American families. *Am J Respir Crit Care Med* 2004; **169**: 1314–1321.
57 Mitchell B, Cole SA, Comuzzie AG, Almasy L, Blangero J, MacCluer JW *et al*. A quantitative trait locus influencing BMI maps to the region of the beta-3 adrenergic receptor. *Diabetes* 1999; **48**: 1863–1867.
58 Gorlova O, Amos C, Wang N, Shete S, Turner S, Boerwinkle E. Genetic linkage and imprinting effects on body mass index in children and young adults. *Eur J Hum Genet* 2003; **11**: 425–432.
59 Chagnon YC, Rice T, Perusse L, Borecki IB, Ho-Kim MA, Lacaille M *et al*. HERITAGE family study. Genomic scan for genes affecting body composition before and after training in Caucasians from HERITAGE. *J Appl Physiol* 2001; **90**: 1777–1787.
60 Li W, Dong C, Li D, Zhao H, Price R. An obesity-related locus on chromosome region 12q23–24. *Diabetes* 2004; **53**: 812–820.
61 North KE, Rose KM, Borecki IB, Oberman A, Hunt SC, Miller MB *et al*. Evidence for a gene on chromosome 13 influencing postural systolic blood pressure change and body mass index. *Hypertension* 2004; **43**: 780–784.