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A genetic contribution to circulating cytokines and obesity in children

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Abstract

Cytokines are considered to be involved in obesity-related metabolic diseases. Study objectives are to determine the heritability of circulating cytokine levels, to investigate pleiotropy between cytokines and obesity traits, and to present genome scan results for cytokines in 1030 Hispanic children enrolled in VIVA LA FAMILIA Study. Cytokine phenotypes included monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF- α), leptin, adiponectin, soluble intercellular adhesion molecule-1 (sICAM-1), transforming growth factor beta 1 (TGF- β 1), C-reactive protein (CRP), Regulated upon Activation, Normal T-cell Expressed and Secreted (RANTES) and eotaxin. Obesity-related phenotypes included body mass index (BMI), fat mass (FM), truncal FM and fasting serum insulin. Heritabilities ranged from 0.33 to 0.97. Pleiotropy was observed between cytokines and obesity traits. Positive genetic correlations were seen between CRP, leptin, MCP-1 and obesity traits, and negative genetic correlations with adiponectin, ICAM-1 and TGF- β 1. Genome-wide scan of sICAM-1 mapped to chromosome 3 (LOD=3.74) between markers *D3S1580* and *D3S1601*, which flanks the adiponectin gene (*ADIPOQ*). Suggestive linkage signals were found in other chromosomal regions for other cytokines. In summary, significant heritabilities for circulating cytokines, pleiotropy between cytokines and obesity traits, and linkage for sICAM-1 on chromosome 3q substantiate a genetic contribution to circulating cytokine levels in Hispanic children.

Keywords

cytokines; childhood obesity; genome-wide scan; inflammation

1. Introduction

Obesity is now recognized as a low-grade inflammatory state resulting from secretion of cytokines, chemokines and hormone-like factors such as leptin, adiponectin and resistin from excess adipose tissue [1]. Elevated levels of several proinflammatory cytokines including leptin

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[2], interleukin-6 (IL-6) [3], tumor necrosis factor-alpha (TNF- α) [3], and C-reactive protein (CRP) [4] have been reported not only in obese adults but also children.

CRP is a major acute phase protein secreted by the liver in response to a variety of inflammatory cytokines. In NHANES III, overweight children had higher CRP and white blood cell counts, suggesting a state of low-grade systemic inflammation [5]. CRP was significantly associated with waist-to-hip ratio, body mass index (BMI), and high density lipoprotein cholesterol (HDL) in children [6].

Intercellular Adhesion Molecule-1 (ICAM-1) is a member of the immunoglobulin superfamily that mediates lymphocyte adhesion. It is expressed in many cell lineages including vascular endothelial cells, epithelial cells, fibroblasts, tissue macrophages, and lymphocytes, and it is upregulated in adipose tissue and serum in an animal model of dietary obesity [7]. ICAM-1 is inducible by TNF- α . Soluble intracellular adhesion molecule-1 (sICAM-1) is found in serum. Its concentration fell after weight loss in children [8]. Transforming growth factor beta 1 (TGF- β 1) is a polypeptide member of the transforming growth factor beta superfamily of cytokines. It is a secreted protein that performs many cellular functions, including the control of cell growth, cell proliferation, cell differentiation and apoptosis. Most immune cells (or leukocytes) secrete TGF- β 1 and higher levels are seen in obesity. Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES) is chemotactic for T cells, eosinophils and basophils and plays an active role in recruiting leukocytes into inflammatory sites and increases in obese individuals [9,10]. Eotaxin, another chemokine, was increased in both obese animals and humans [11].

Inflammatory cytokines and adipokines are proposed to link obesity to insulin resistance and the development of metabolic diseases [12]. Power et al. [13] and Nemet et al. [14] reported that TNF- α is overexpressed in both obesity and diabetes. There is considerable evidence linking leptin and cytokine production [15,16]. Adiponectin, an adipokine with anti-inflammatory and insulin-sensitizing properties, has independent negative associations with obesity and hyperinsulinemia/insulin resistance in adults and children [17,18]. Adiponectin counteracts the actions and circulating levels of TNF- α . Part of the mechanism by which TNF- α induces insulin resistance could involve a reduction in adiponectin. Monocyte chemoattractant protein 1 (MCP-1) is a chemokine involved in the activation of monocytes and subsequent atherosclerotic development; elevated serum MCP-1 values seen in obesity may contribute to cardiovascular diseases and type 2 diabetes [19].

The genetic influence on cytokines and childhood obesity has not been studied before. In obese diabetic adults, the -238G>A genotype of *TNF- α* affects postprandial free fatty acids levels [20]. Long et al. [21] found that *TGF- β 1* variants were related to obesity traits. To the best of our knowledge, no report has been published on the genetic relationships between inflammatory factors and obesity-related phenotypes or on the genetic locations regulating cytokine levels in a pediatric population.

The objectives of this report are to determine the heritability of circulating cytokine levels, to investigate pleiotropy between cytokines and obesity traits, and to present genome scan results for cytokines in a large cohort of Hispanic children enrolled in the VIVA LA FAMILIA Study.

2. Methods

2.1. Subjects and study design

The Viva La Familia Study was designed to investigate the genetic and environmental factors affecting obesity and its comorbidities in 1030 Hispanic children from 319 families [22]. Subjects were ascertained through an overweight proband between the ages 4-19 y, whose BMI

$\geq 95^{\text{th}}$ percentile [23] and FM $\geq 85^{\text{th}}$ percentile [24,25]. All enrolled children and parents gave written informed consent or assent. The protocol was approved by the Institutional Review Boards for Human Subject Research for Baylor College of Medicine and Affiliated Hospitals and for Southwest Foundation for Biomedical Research.

Phenotypic assessments were conducted at the Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX. Body composition was measured and a 12-h fasting blood sample was obtained from the children for DNA extraction and biochemistries.

2.2. Anthropometry and body composition

A digital balance was used to measure body weight to the nearest 0.1 kg and a stadiometer to measure height to the nearest 1 mm. Body composition was estimated by dual-energy x-ray absorptiometry (DXA) using a Hologic Delphi-A whole-body scanner (DXA, Delphi-A, Hologic, Inc., Waltham, MA). Fat mass (FM) and truncal FM were determined using the manufacturer's software.

2.3. Fasting blood biochemistries

A fasting blood sample was drawn for biochemical determinations. Fasting serum concentrations of CRP was measured by enzyme-linked immunosorbent assay (ELISA) (interassay CV 6%) (Alpco Diagnostic, Windham, NH); sICAM-1 was measured using a duoset kit (CV 6.1%) (R & D Systems, Minneapolis, MN); TGF- β 1 (CV 12.8%), MCP-1 (CV 5%), eotaxin (CV 11.5%), IL-6 (CV 6.4%) and RANTES (CV 6.7%) were measured by ELISA (R & D Systems, Minneapolis, MN). TNF- α was measured by ELISA (CV 8.2%) (Biosource, Camarillo, CA). Latent TGF- β 1 was converted to immunoreactive TGF- β 1 by adding 1:1 2.5 N acetic acid/10 M urea and then neutralizing the acidified sample with 2.7 N NaOH/1 M HEPES. The neutralized sample was measured by ELISA (R & D Systems, Minneapolis, MN) (CV 12.8%). Leptin (CV 8.3%), adiponectin (CV 3.6%) and insulin (CV 10%) were measured using radioimmunoassay (RIA) kit from Linco Research (division of Millipore, St. Charles, MO).

2.4. Genotyping

DNA was extracted and prepared using the Genomic DNA Purification Kit (Promega, Madison, WI). Fluorescently labeled polymerase chain reaction (PCR) primer pairs amplified autosomal microsatellites from the ABI PRISM Linkage Mapping Set-MD10 Version 2.5 (Applied Biosystems, Foster City, CA). Each marker was amplified in a separate PCR reaction and later the PCR products were pooled onto an ABI PRISM 3100 Genetic Analyzer for laser-based automated genotyping. Genotypes were scored utilizing the Genotyper software package (Applied Biosystems). The chromosomal maps in this project were based upon marker locations specified by the Marshfield Center for Medical Genetics and DeCode.

2.5. Statistical analyses

The variance components decomposition method was applied for quantitative genetic analysis and genome wide scans in this study [26,27]. The total phenotypic variance (σ_p^2) of the traits is partitioned into additive genetic (σ_G^2) and environmental (σ_E^2) components:

$$\sigma_p^2 = \sigma_G^2 + \sigma_E^2$$

The environmental component includes environmental factors, the non-additive genetic component, and measurement errors. The portion of the total phenotypic variance contributed by additive genetic factors is denoted by heritability (h^2):

$$h^2 = \frac{\sigma_G^2}{\sigma_P^2}$$

Bivariate analysis is an extension of the univariate analysis, which decomposes the phenotypic relationships (ρ_P) between two traits into genetic (ρ_G) and environmental correlations (ρ_E):

$$\rho_P = \rho_G \sqrt{h_1^2} \sqrt{h_2^2} + \rho_E \sqrt{(1 - h_1^2)} \sqrt{(1 - h_2^2)}$$

Here, h_1^2 and h_2^2 represent the heritabilities of the two traits. The bivariate phenotype is a linear function of the individual's phenotypic values, the population means, the additive genetic and environmental effects [28]. A null model where the genetic correlation is constrained to zero is compared with an alternative model where all parameters are estimated using maximum likelihood technique. Twice the difference of logarithm likelihood of the two models asymptotically distributes as a χ^2 with one degree of freedom. A significant genetic correlation indicates that a common set of genes influence more than one trait.

Multipoint linkage analysis decomposes the phenotypic covariance into additive genetic effects specific to a quantitative trait locus (QTL), residual additive genetic effects, and random environmental effects. To determine the statistical significance, logarithm of the odds (LOD) was calculated by the difference between log10 of the likelihood ratio statistic. Sex, age, age-squared, and their interactions were incorporated into analysis as covariates. Tanner age was not incorporated into the analyses as a covariate because its effect was captured by the above covariates. These genetic analyses have been implemented in the SOLAR 2.0 software (Southwest Foundation for Biomedical Research, San Antonio, TX). If the data were not normally distributed (kurtosis >1.9), a log transformation was performed before further analyses.

3. Results

All the measured cytokines were significantly heritable with heritabilities ranging from 0.33 to 0.97 (Table 1). As shown previously [22], the obesity traits (BMI, FM, truncal FM and insulin) were highly heritable in this cohort. The genetic correlations between cytokines and obesity-related traits were estimated and those with p-values $p < 0.1$ are presented in Table 2. CRP and leptin were positively correlated with BMI, FM, truncal FM and insulin. The genetic correlation ($\sigma_G^2 = 0.24$) between MCP-1 and BMI was borderline ($p = 0.08$). Negative genetic correlations were seen between adiponectin, sICAM-1 and TGFB1 and obesity traits (BMI, FM, truncal FM).

The genetic relationships among cytokines were investigated and those with p-values < 0.1 are presented in Table 3. Moderate positive genetic correlations were found for CRP and leptin, leptin and MCP-1, sICAM-1 and TNF- α , TGF- β 1 and eotaxin, TGF- β 1 and RANTES, eotaxin and MCP-1, eotaxin and RANTES, and MCP-1 and RANTES. A negative genetic correlation was seen between eotaxin and TNF- α .

The univariate genome wide scans are summarized in Table 4. A QTL for sICAM-1 levels was identified on chromosome 3 between markers *D3S1580-D3S1601* with a significant LOD score of 3.74. The string plot and linkage plot for chromosome 3 are presented in Figures 1 and 2, respectively. Suggestive evidence for linkage (LOD > 2) was found on chromosomes 4, 7, and 19 for TGF- β 1, eotaxin, and TNF- α , respectively. IL-6 and RANTES levels showed suggestive evidence for linkage to two different locations on chromosome 9. Adiponectin and MCP-1 levels were linked to two distinct regions on chromosome 11. For any significant genetic correlation observed in this study, a bivariate genome-wide scan was performed, but none was found to improve the evidence for linkage relative to the univariate scans.

4. Discussion

Circulating levels of cytokines were highly heritable in this cohort of non-overweight and overweight Hispanic children. Positive genetic correlations were seen between CRP, leptin, and MCP-1 and obesity traits, while negative genetic correlations were detected between adiponectin, sICAM-1, and TGF- β 1 and obesity traits. This evidence of pleiotropy indicates that a common set of genes contribute to the regulation of cytokines and the expression of obesity phenotypes in Hispanic children. The positive correlations indicate that genetic factors associated with obesity also result in increasing levels of CRP, leptin, and MCP-1, while the negative correlations implied that genetic factors associated with obesity also result in decreasing levels of adiponectin, sICAM-1, and TGF- β 1.

The genome-wide scans of cytokines identified a QTL for sICAM-1 (LOD=3.74) levels on chromosome 3 between markers *D3S1580* and *D3S1601*, which flanks the adiponectin gene (*ADIPOQ*). This gene region was previously reported to be associated with circulating levels of adiponectin in the Amish Family Diabetes Study [29]. The *ADIPOQ* insertion-deletion mutation in the 3' UTR and two other SNPs were found to be responsible for the association [29]. SNPs in the proximal promoter region of the *ADIPOQ* gene were associated with type 2 diabetes in Swedish Caucasians [30]. In addition to the promoter region, SNPs in exon 3 were associated with adiponectin levels and type 2 diabetes in French adults [31].

Another strong positional candidate gene for the sICAM-1 linkage signal is a protein known as the interleukin 1 receptor associated protein (IL1RAP). IL1RAP resides between *D3S1580* and *D3S1601*. The cellular response to the pro-inflammatory cytokine interleukin 1 is mediated through the interleukin 1 receptor (ILR1) and IL1RAP. Interleukin-1 signaling is known to upregulate ICAM-1 gene expression and the release of sICAM-1 from vascular endothelial cells [32]. It is possible that variation in the availability or structure of IL1RAP could impact the production of sICAM-1 in endothelial cells exposed to IL-1. However, in the study by Dupuis et al. [33], sICAM-1 levels were linked to chromosome 1 at 229 cM (LOD=2.09), less than 5 cM from the interleukin-10 (*IL10*) gene. Therefore, it is likely that alleles at multiple loci may contribute to the regulation of sICAM-1 levels.

In our study, CRP circulating levels were linked to chromosome 5 between markers *D5S2115* and *D5S436* (LOD = 1.7). We did not replicate the linkage signal found in the HERITAGE Family Study, where chromosome 20q13 was found to be linked to plasma CRP levels in healthy white adults [34]. Also, in our study, *D11S1314* on chromosome 11 showed suggestive linkage with MCP-1 levels in contrast to the study by Dupuis et al. [33], in which significant linkage to MCP-1 was found on chromosome 1 (LOD = 4.27).

To the best of our knowledge, no genome-wide scan results of IL-6, TNF- α , RANTES, eotaxin, and TGF- β 1 have been reported previously. Although our linkage signals for these phenotypes did not reach the significant LOD score of 3, they do suggest the involvement of their respective chromosomal regions in the expression of these traits. Cytokines levels display wide ranges of

intra- and inter-individual variations, unquestionably, due to environmental as well as genetic factors. Substantial variability in circulating levels offsets efforts to identify the chromosomal locations influencing cytokine levels.

In summary, significant heritabilities for circulating cytokines, pleiotropy between cytokines and obesity traits, and linkage for sICAM-1 on chromosome 3q substantiate a genetic contribution to circulating cytokine levels in Hispanic children.

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References

1. Calabro P, Yeh ET. Obesity, inflammation, and vascular disease: the role of the adipose tissue as an endocrine organ. *Subcell Biochem* 2007;42:63–91. [PubMed: 17612046]
2. Dubey S, Kabra M, Bajpai A, Pandey RM, Hasan M, Gautam RK. Serum leptin levels in obese Indian children relation to clinical and biochemical parameters. *Indian Pediatr* 2007;44:257–62. [PubMed: 17468520]
3. Halle M, Korsten-Reck U, Wolfarth B, Berg A. Low-grade systematic inflammation in overweight children: impact of physical fitness. *Exerc Immunol Rev* 2004;10:66–74. [PubMed: 15633587]
4. Giannini C, de Giorgis T, Scarinci A, Ciampani M, Marcovecchio ML, Chiarelli F. Obese related effects of inflammatory markers and insulin resistance on increased carotid intima media thickness in pre-pubertal children. *Atherosclerosis*. 2007 Aug 4;Epub ahead of print
5. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Low-grade systemic inflammation in overweight children. *Pediatrics* 2001;107:e13. [PubMed: 11134477]
6. Gillum RF. Association of serum C-reactive protein and indices of body fat distribution and overweight in Mexican American children. *J Natl Med Assoc* 2003;95:545–52. [PubMed: 12911252]
7. Brake DK, Smith EO, Mersmann H, Smith SW, Robker R. ICAM-1 expression in adipose tissue: Effects of diet-induced obesity in mice. *Am J Physiol Cell Physiol* 2006;291:C1232–C1239. [PubMed: 16807303]
8. Clifton PM, Keogh JB, Foster PR, Noakes M. Effects of weight loss on inflammatory and endothelial markers and FMD using two low-fat diets. *Int J Obes (Lond)* 2005;29:1445–51. [PubMed: 16172619]
9. Maury E, Ehala-Aleksejev K, Guiot Y, Detry R, Vandenhoof A, Brichard SM. Adipokines oversecreted by omental adipose tissue in human obesity. *Am J Physiol Endocrinol Metab*. 2007 Jun 19;Epub ahead of print
10. Wu H, Ghosh S, Perrard XD, Thakker G, Sweeney JF, Chan LCB, et al. T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. *Circulation* 2007;115:1029–38. [PubMed: 17296858]
11. Vasudevan AR, Wu H, Xydakis AM, Jones PH, Smith EO, Sweeney JF. Eotaxin and obesity. *J Clin Endocrinol Metab* 2006;91:256–61. [PubMed: 16263831]
12. Dedoussis GV, Kaliora AC, Panagiotakos DB. Genes, diet and type 2 diabetes mellitus: a review. *Rev Diabet Stud* 2007;4:13–24. [PubMed: 17565412]
13. Power C, Miller SK, Alpert PT. Promising new casual explanations for obesity and obesity-related diseases. *Biol Res Nurs* 2007;8:223–33. [PubMed: 17172321]
14. Nemet D, Wang P, Funahashi T, Matsuzawa Y, Tanaka S, Engelman L, et al. Adipocytokines, body composition, and fitness in children. *Pediatr Res* 2003;53:148–52. [PubMed: 12508095]

15. Juge-Aubry CE, Meier CA. Immunomodulatory actions of leptin. *Mol Cell Endocrinol* 2002;194:1–7. [PubMed: 12242022]
16. Meier CA, Bobbioni E, Gabay C, Assimacopoulos-Jeannet F, Golay A, Dayer JM. IL-1 receptor antagonist serum levels are increased in human obesity: a possible link to the resistance to leptin? *J Clin Endocrinol Metab* 2002;87:1184–8. [PubMed: 11889184]
17. Stefan N, Bunt JC, Salbe AD, Funahashi T, Matsuzawa Y, Tataranni PA. Plasma adiponectin concentrations in children: relationships with obesity and insulinemia. *J Clin Endocrinol Metab* 2002;87:4652–6. [PubMed: 12364452]
18. Stefan N, Stumvoll M. Adiponectin—its role in metabolism and beyond. *Horm Metab Res* 2002;34:469–74. [PubMed: 12384822]
19. Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. *Proc Natl Acad Sci* 2003;100:7265–70. [PubMed: 12756299]
20. Fontaine-Bisson B, Wolever TM, Chiasson JL, Rabasa-Lhoret R, Maheux P, Josse RG. Tumor necrosis factor alpha -238G>A genotype alters postprandial plasma levels of free fatty acids in obese individuals with type 2 diabetes mellitus. *Metabolism* 2007;56:649–55. [PubMed: 17445540]
21. Long J-R, Liu P-Y, Lu Y, Xiong D-H, Elze L, Recker RR, et al. APOE and TGF-beta1 genes are associated with obesity phenotypes. *J Med Genet* 2003;40:918–24. [PubMed: 14684691]
22. Weigensberg MJ, Ball GDC, Shaibi GQ, Cruz ML, Goran MI. Decreased β -cell function in overweight Latino children with impaired fasting glucose. *Diabetes Care* 2005;28:2519–24. [PubMed: 16186290]
23. Must A, Dallal GE, Dietz WH. Reference data for obesity: 85th and 95th percentiles of body mass index (wt/ht²) and triceps skinfold thickness. *Am J Clin Nutr* 1991;53:839–46. [PubMed: 2008861]
24. Ellis KJ. Body composition of a young, multiethnic, male population. *Am J Clin Nutr* 1997;66:1323–31. [PubMed: 9394682]
25. Ellis KJ, Abrams SA, Wong WW. Body composition of a young, multiethnic female population. *Am J Clin Nutr* 1997;65:724–31. [PubMed: 9062521]
26. Amos CI. Robust variance-components approach for assessing genetic linkage in pedigrees. *Am J Hum Genet* 1994;54:535–43. [PubMed: 8116623]
27. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 1998;62:1198–211. [PubMed: 9545414]
28. Lange K, Boehnke M. Extensions to pedigree analysis. IV. Covariance components models for multivariate traits. *Am J Med Genet* 1983;14:513–24. [PubMed: 6859102]
29. Pollin TI, Tanner K, O'Connell JR, Ott SH, Damcott CM, Shuldiner AR. Linkage of plasma adiponectin levels to 3q27 explained by association with variation in the APM1 gene. *Diabetes* 2005;54:268–74. [PubMed: 15616038]
30. Gu HF, Abulaiti A, Ostenson CG, Humphreys K, Wahlestedt C, Brookes AJ. Single nucleotide polymorphisms in the proximal promoter region of the adiponectin (APM1) gene are associated with type 2 diabetes in Swedish Caucasians. *Diabetes* 2004;53:S31–S35. [PubMed: 14749263]
31. Vasseur F, Helbecque N, Dina C, Lobbens S, Delannoy V, Gaget S, et al. Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. *Hum Mol Genet* 2002;11:2607–14. [PubMed: 12354786]
32. Pober J. Cytokine-mediated activation of vascular endothelium. *Am J Pathol* 1988;133:416–422.
33. Dupuis J, Larson MG, Vasan RS, Massaro JM, Wilson PW, Lipinska I. Genome scan of systematic biomarkers of vascular inflammation in the Framingham Heart Study: evidence for susceptibility loci on 1q. *Atherosclerosis* 2005;182:307–14. [PubMed: 16159603]
34. Lakka TA, Rankinen T, Rice T, Leon AS, Rao DC, Skinner JS. Quantitative trait locus on chromosome 20q13 for plasma levels of C-reactive protein in healthy whites: the HERITAGE Family Study. *Physiol Genomics* 2006;27:103–7. [PubMed: 16822830]

Figure 1.
Genome-wide scan results of sICAM-1, indicating the chromosomal location on the x-axis and the LOD score on the y-axis.

Figure 2.
The multipoint linkage analyses of sICAM-1 on chromosome 3.

Table 1

Descriptive analyses of cytokines and obesity traits in Hispanic children

Trait	Boys	Girls	Heritability
CRP (ng/ml)	1215 ± 1412 (491) ^a	1176 ± 1404 (493)	0.33(0.08) ^b
IL-6 (pg/ml)	2.00 ± 2.14 (493)	2.20 ± 2.10 (494)	0.41(0.08)
TNF-α (pg/ml)	8.50 ± 2.48 (491)	8.29 ± 2.48 (494)	0.94(0.02)
Leptin (ng/ml)	15.3 ± 13.5 (493)	20.5 ± 15.8 (495)	0.36(0.08)
Adiponectin (ng/ml)	12727 ± 5916 (493)	14010 ± 6469 (495)	0.97(0.06)
RANTES (pg/ml)	53772 ± 30451 (481)	50568 ± 29920 (488)	0.61(0.07)
MCP-1 (pg/ml)	317 ± 117 (493)	309 ± 117 (494)	0.62(0.08)
Eotaxin (pg/ml)	48.7 ± 15.0 (491)	45.8 ± 15.0 (492)	0.68(0.07)
sICAM-1 (pg/ml)	284.7 ± 120.9 (494)	273.4 ± 103.1 (493)	0.67(0.07)
TGF-β1 (pg/ml)	8558 ± 5320 (492)	8731 ± 4994 (491)	0.84(0.05)
BMI (kg/m ²)	25.9 ± 7.9 (509)	24.3 ± 7.2 (516)	0.39(0.08)
Fat mass (kg)	19.0 ± 12.4 (497)	18.8 ± 12.0 (506)	0.33(0.07)
Truncal fat mass (kg)	8.1 ± 6.1 (477)	8.5 ± 6.2 (480)	0.31(0.08)
Insulin ((μU/mL)	22.0 ± 18.4 (493)	22.9 ± 18.9 (495)	0.40(0.08)

Abbreviations: CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; RANTES, Regulated upon Activation, Normal T-cell Expressed, and Secreted; MCP-1, monocyte chemoattractant protein-1; sICAM-1, soluble intercellular adhesion molecule-1; TGF-β1, transforming growth factor beta 1; BMI, body mass index.

^a Mean ± SD (N);

^b All heritabilities p<0.01, with covariates age, age², sex, age*sex, age²*sex

Table 2
The significant genetic correlations between cytokines and obesity traits^a

Trait 1	Trait 2	rhoE	sE	P	rhoG	sG	P
CRP	Fat mass	0.57	0.06	<0.01	0.43	0.14	0.02
CRP	Truncal fat mass	0.55	0.06	<0.01	0.43	0.14	0.02
CRP	Insulin	0.49	0.07	<0.01	0.43	0.14	0.01
CRP	BMI	0.59	0.06	<0.01	0.48	0.13	0.01
Leptin	Fat mass	0.75	0.03	<0.01	0.88	0.05	<0.01
Leptin	Truncal fat mass	0.71	0.04	<0.01	0.88	0.06	<0.01
Leptin	Insulin	0.75	0.04	<0.01	0.59	0.10	<0.01
Leptin	BMI	0.81	0.03	<0.01	0.78	0.06	<0.01
Adiponectin	Truncal fat mass	-0.93	0.07	<0.01	-0.22	0.12	0.07
Adiponectin	BMI	-0.97	0.03	<0.01	-0.20	0.10	0.07
sICAM-1	Fat mass	0.60	0.10	<0.01	-0.33	0.13	0.01
sICAM-1	Truncal fat mass	0.61	0.10	<0.01	-0.34	0.13	0.01
sICAM-1	BMI	0.60	0.11	<0.01	-0.28	0.13	0.02
TGF-β1	Fat mass	0.36	0.14	0.01	-0.22	0.12	0.06
TGF-β1	Truncal fat Mass	0.38	0.14	0.008	-0.24	0.13	0.06
MCP-1	BMI	-0.03	0.11	0.9	0.24	0.13	0.08

Abbreviations: CRP, C-reactive protein; sICAM-1, soluble intercellular adhesion molecule-1; TGF-β1, transforming growth factor beta 1; MCP-1, Monocyte chemoattractant protein-1; BMI, body mass index; rhoE, environmental correlation; sE, standard error of rhoE; rhoG, genetic correlation; sG, standard error of rhoG.

^a Genetic correlations between cytokines and obesity traits with p<0.10. Negative genetic correlations are shown in bold.

Table 3
The significant genetic correlations between cytokines^a

Trait1	Trait2	rhoE	sE	p	rhoG	sG	p
CRP	Leptin	0.68	0.05	<0.01	0.42	0.13	0.022
Leptin	Adiponectin	-0.95	0.05	<0.01	-0.24	0.10	0.034
Leptin	sICAM-1	0.49	0.10	<0.01	-0.22	0.13	0.080
Leptin	MCP-1	0.00	0.11	0.97	0.30	0.13	0.026
Adiponectin	TGF-β1	0.39	0.31	0.26	-0.09	0.06	0.073
Adiponectin	RANTES	0.36	0.21	0.11	-0.22	0.09	0.025
Adiponectin	TNF-α	0.45	0.24	0.17	-0.15	0.06	0.021
sICAM-1	TNF-α	-0.39	0.23	0.12	0.19	0.06	0.004
TGF-β1	Eotaxin	0.09	0.18	0.62	0.19	0.08	0.022
TGF-β1	RANTES	-0.13	0.17	0.45	0.18	0.09	0.039
Eotaxin	MCP-1	-0.27	0.14	0.05	0.28	0.10	0.005
Eotaxin	RANTES	-0.35	0.11	0.00	0.16	0.10	0.097
Eotaxin	TNF-α	0.26	0.18	0.21	-0.22	0.07	0.003
MCP-1	RANTES	0.36	0.11	0.01	0.45	0.09	<0.01

Abbreviations: CRP, C-reactive protein; sICAM-1, soluble intercellular adhesion molecule-1; TGF-β1, transforming growth factor beta 1; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor-α; RANTES, Regulated upon Activation, Normal T-cell Expressed, and Secreted; BMI, body mass index; rhoE, environmental correlation; sE, standard error of rhoE; rhoG, genetic correlation; sG, standard error of rhoG.

^a Genetic correlations between cytokines with p<0.10. Negative genetic correlations are shown in bold.

Table 4

The univariate genome-wide scans of cytokines in Hispanic children

Trait	Chromosome	Location cM	Markers	LOD
CRP	5	141	D5S2115 -D5S436	1.7
IL-6	9	9	D9S288	2.18
TNF- α	19	47	D19S226-D19S414	2.6
Leptin	12	114	D12S346-D12S78	1.96
Adiponectin	11	134	D11S4151	2.41
RANTES	9	125	D9S1776	2.07
MCP-1	11	80	D11S1314	2.17
Eotaxin	7	127	D7S486-D7S530	2.35
sICAM-1	3	209	D3S1580-D3S1601	3.74
TGF- β 1	4	21	D4S2935-D4S403	2.32

Abbreviations: CRP, c-reactive protein; IL-6, interleukin-6; TNF- α , tumor necrosis factor-alpha; RANTES, Regulated upon Activation, Normal T-cell Expressed, and Secreted; MCP-1, monocyte chemoattractant protein-1; sICAM-1, soluble intercellular adhesion molecule-1; TGF- β 1, transforming growth factor beta 1. Covariates included in the analysis were age, age², age*sex, age²*sex.