VNN1 Gene Expression Levels and the G-137T Polymorphism Are Associated with HDL-C Levels in Mexican Prepubertal Children

Leonor Jacobo-Albavera[1,2], Pablo I. Aguayo-de la Rosa[1,2], Teresa Villarreal-Molina[3], Hugo Villamil-Ramírez[1,2], Paola León-Mimila[1,2], Sandra Romero-Hidalgo[3], Blanca E. López-Contreras[3], Fausto Sánchez-Muñoz[4], Rafael Bojalil[4,5], Juan Antonio González-Barrios[6], Carlos A. Aguilar-Salinas[7], Samuel Canizales-Quinteros[1,2]*

1 Unidad de Biología Molecular y Medicina Genómica, Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán" (INCMNSZ), Mexico City, Mexico, 2 Departamento de Biología, Facultad de Química, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico, 3 Instituto Nacional de Medicina Genómica (INMEGEN), Mexico City, Mexico, 4 Departamento de Inmunología, Instituto Nacional de Cardiología "Ignacio Chávez", Mexico City, Mexico, 5 Departamento de Cuidado de la Salud, Universidad Autónoma Metropolitana (UAM) Xochimilco, Mexico City, Mexico, 6 Laboratorio de Medicina Genómica, Hospital Regional "Primero de Octubre", Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado (ISSSTE), Mexico City, Mexico, 7 Departamento de Endocrinología y Metabolismo, INCMNSZ, Mexico City, Mexico

Abstract

Background: *VNN1* gene expression levels and the G-137T polymorphism have been associated with high density lipoprotein cholesterol (HDL-C) levels in Mexican American adults. We aim to evaluate the contribution of *VNN1* gene expression and the G-137T variant to HDL-C levels and other metabolic traits in Mexican prepubertal children.

Methodology/Principal Findings: *VNN1* mRNA expression levels were quantified in peripheral blood leukocytes from 224 unrelated Mexican-Mestizo children aged 6–8 years (107 boys and 117 girls) and were genotyped for the G-137T variant (rs4897612). To account for population stratification, a panel of 10 ancestry informative markers was analyzed. After adjustment for admixture, the TT genotype was significantly associated with lower *VNN1* mRNA expression levels ($P = 2.9 \times 10^{-5}$), decreased HDL-C levels ($\beta = -6.19$, $P = 0.028$) and with higher body mass index (BMI) z-score ($\beta = 0.48$, $P = 0.024$) in the total sample. In addition, *VNN1* expression showed a positive correlation with HDL-C levels ($r = 0.220$; $P = 0.017$) and a negative correlation with BMI z-score ($r = -0.225$; $P = 0.015$) only in girls.

Conclusion/Significance: Our data suggest that *VNN1* gene expression and the G-137T variant are associated with HDL-C levels in Mexican children, particularly in prepubertal girls.

Introduction

Low plasma levels of high-density lipoprotein cholesterol (HDL-C) is the most common dyslipidemia in Mexican children and adults [1–3], which is modulated by both genetic and lifestyle factors [4,5]. Several genetic variants affecting HDL-C levels have been identified through candidate gene/genome-wide association studies (GWAS) and replicated in different populations [6–8]. However, relatively few genome-wide quantitative gene expression studies identifying expression quantitative trait loci (eQTLs) for HDL-C levels have been reported [9–12]. Interestingly, these studies have identified some genes associated with HDL-C levels not previously associated with this trait by GWAS. In this regard, Göring et al. [9] reported that Vanin 1 (*VNN1*) gene expression

levels showed the strongest correlation with HDL-C concentrations in lymphocytes of Mexican-American adults. Moreover, the authors also observed that the functional G-137T polymorphism (rs4897612) was associated with both *VNN1* expression levels and HDL-C concentrations in this population, even though the polymorphism had not been previously associated with HDL-C levels in GWAS performed mainly in Caucasians [6–8]. Although there is no direct evidence of the role of the *VNN1* protein in HDL-C levels regulation, it is known that *VNN1* codes for pantetheinase and produces cysteamine, a potent antioxidant that prevents lipid peroxidation [13,14] and has been associated with other metabolic traits such as obesity in animal models [15]. *VNN1* thus can be considered as a reasonable candidate gene to modulate HDL-C levels and perhaps other metabolic traits.

In addition to genetic variation, it is known that environmental factors may modify gene expression [16,17]. Because environmental exposure is likely to be not as relevant in children than in adults [18,19], we sought to replicate the *VNN1* expression association with the G-137T variant and HDL-C levels in a population sample of Mexican prepubertal children, and to assess whether *VNN1* expression is also associated with other metabolic traits.

Materials and Methods

Subjects

We analyzed 224 healthy unrelated school-aged Mexican-Mestizo children (107 boys and 117 girls) aged 6 to 8 years, recruited from a summer camp for children of employees of the Mexican Health Ministry (Convivencia Infantil 2008 and 2009, Secretaría de Salud). Weight, height and waist circumference were measured in all participants. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. BMI z-scores were calculated using age and sex specific BMI reference data, as recommended by the Centers for Disease Control and Prevention [20]. Fat mass percentage was measured using a bioelectric impedance method (Quantum × impedance analyzer, RJL Systems, Detroit, MI). None of the participants had evidence of diabetes, thyroid, renal or liver disease. A parent of each child signed the consent form for participation. The project was approved by the Institutional Committee of Biomedical Research in Humans of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ).

Biochemical Parameters

Blood samples were drawn from all participants after a 12-hour fast. Total cholesterol (TC), triglyceride (TG), HDL-C and ApoA1 plasma levels were measured at the INCMNSZ with commercially available standardized methods as described by Villarreal-Molina et al. [21]. Hypoalphalipoproteinemia (HA) was defined as HDL-C levels<40 mg/dL [22].

VNN1 mRNA Expression Analysis

Total RNA was extracted from leukocytes using TRIzol (Invitrogen, Life Technologies, Carlsbad, CA, USA). To avoid DNA carryover, DNAse treatment was performed with DNAse I recombinant (Roche, Rotkreuz, Switzerland). To perform the expression analysis, 1000 ng of total RNA was reverse transcribed with TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA) using random hexamers, according to the protocol recommended by the manufacturer. Real-time PCR was performed in a LightCycler 2.0 (Roche, Rotkreuz, Switzerland), using LNA TaqMan probes from the Universal Probe Library (Roche, Rotkreuz, Switzerland), in combination with intron-spanning specific primers as described previously [23]. The following primers and probes were used to assess *VNN1* gene expression in human leucocytes: tcctgaggtgttgctgagtg (forward), agcgtccgtcagttgacac (reverse), and probe #80 (cat. no. 04689038001). Hipoxanthine phosphoribosyl transferase (*HPRT*) expression was measured as reference using *HPRT* primers tgatagatccattcctatgactgtaga (forward), caagacattctttccagt-taaagttg (reverse), and probe #22 (cat. no. 04688961001); *β-actin* (*ACTB*) was measured as reference using Universal ProbeLibrary Human *ACTB* Gene Assay (cat. no. 05046165001). All assays showed linearity and a coefficient of variation<10%. Relative quantification of gene expression was calculated with the Light-Cycler Software 4.0.

Single Nucleotide Polymorphisms Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp DNA Blood Kit (Qiagen, Valencia, California). The *VNN1* G-137T variant was genotyped using the TaqMan assay C_29857881_10 (ABI Prism 7900HT Sequence Detection System; Applied Biosystems, Foster City, CA). No discordant genotypes were observed in 30 duplicate samples analyzed by direct sequencing. *VNN1* G-137T genotype frequencies were tested for Hardy-Weinberg equilibrium, deviation was not observed in any group.

Because the Mexican population resulted from the admixture of mainly European (Spaniard) and Native American populations, it was necessary to assess whether any association could be confounded by population stratification. A panel of 10 ancestry informative markers (AIMs) distinguishing mainly Native American and European ancestry (δ for the minor allele>0.29), was screened in all participants (rs3340, rs1881826, rs2341823, rs4130405, rs1980888, rs1487214, rs726391, rs724729, rs292932 and rs1877751) [24,25]. Genotyping was performed using TaqMan assays (ABI Prism 7900HT Sequence Detection System; Applied Biosystems, Foster City, CA). Genotyping call rates of each ancestry informative marker exceeded 95%, and no discordant genotypes were observed in 48 duplicate samples. No ancestry informative marker showed significant departure from Hardy-Weinberg equilibrium.

Statistical Analysis

Because gene expression, HDL-C levels and the effect of some polymorphisms on metabolic traits are known to have gender differences [26–29], all analyses were performed on the entire sample and stratified by gender. Differences in anthropometric and biochemical parameters were analyzed using Student's t-test. Because *VNN1* gene expression and triglyceride levels were not normally distributed, they were log transformed for analysis. Correlations between *VNN1* gene expression and metabolic parameters were analyzed using Pearson's correlation and partial correlation was used to examine the correlations between gene expression and metabolic phenotypes adjusting for admixture. Differences in anthropometric and biochemical parameters according to G-137T genotype were tested by linear regression analysis. Associations were analyzed using additive (Table S1),

Table 1. Anthropometric and biochemical parameters according to gender.

Characteristic	Total subjects (224)	Boys (107)	Girls (117)	P^a
Age, years	7.73±0.97	7.84±0.97	7.63±0.96	0.194
BMI z-score	0.99±0.98	1.0±1.0	0.98±0.97	0.846
FM, %	30.03±10.52	28.37±11.60	31.52±9.25	0.041
TG, mg/dL	98.03±59.09	90.95±50.38	104.50±65.62	0.087
TC, mg/dL	175.83±31.39	173.36±29.90	178.09±32.66	0.261
HDL-C, mg/dL	48.58±10.56	49.15±10.36	48.06±10.75	0.442
ApoA1, mg/dL	168.52±21.74	171.08±20.57	166.40±22.53	0.120
HA (%)	19.6	16.8	22.2	0.310^b

Data are means ± s.d. or n (%). BMI, body mass index; FM, percent fat mass; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; HA, hypoalphalipoproteinemia.
[a]P-values were calculated by t-test;
[b]X² test.
doi:10.1371/journal.pone.0049818.t001

Table 2. Correlation of VNN1 mRNA levels in leukocytes with metabolic phenotypes.

Metabolic phenotypes	Whole Population (n = 224)			Boys (n = 107)			Girls (n = 117)		
	r	P	P^a	r	P	P^a	r	P	P^a
BMI z-score	−0.144	0.032	0.034	−0.062	0.528	0.518	−0.225	0.015	0.015*
FM, %	−0.118	0.080	0.085	−0.068	0.490	0.525	−0.145	0.118	0.119
TG, mg/dL	−0.149	0.026	0.028	−0.062	0.525	0.576	−0.208	0.025	0.023
TC, mg/dL	−0.056	0.402	0.396	−0.047	0.631	0.643	−0.051	0.586	0.586
HDL-C, mg/dL	0.086	0.198	0.211	−0.071	0.465	0.448	0.220	0.017	0.017
ApoA1, mg/dL	0.042	0.546	0.566	−0.106	0.308	0.308	0.129	0.170	0.175

BMI, body mass index; FM, percent fat mass; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol.
[a]P-values adjusted for admixture.
*Significant after Bonferroni correction.
doi:10.1371/journal.pone.0049818.t002

dominant (Table S2) and recessive models for the T allele, being the recessive model the most significant. Comparison of VNN1 expression levels between G-137T genotypes was performed using Mann-Whitney U test. All statistical analyses were performed using SPSS version 15.0 (Chicago, IL) and a P-value of less than 0.05 was considered statistically significant. Genetic ancestry estimates were computed from genotypes for 10 AIMs using the program ADMIXMAP [30]. Because the Mexican-Mestizo population derived mainly from Native American and European populations, the model used included two primary parental populations. All analyses were adjusted for admixture and for BMI-z score when appropriate. To address multiple testing, we first determined the average pairwise correlation for all measurements (6 measurements, correlation = 0.38); with this correlation we calculated Bonferroni's correction using the freely available Simple Interactive Statistical Analyses Software (http://www.quantitativeskills.com/sisa/) and a P-value below 0.017 was

considered significant. The study power to detect association of G-137T with HDL-C levels was estimated using QUANTO software (v1.2.4, http://hydra.usc.edu/GxE/) and reached 82.8%, assuming a recessive model with a minor allele (T) frequency of 0.35, beta for HDL-C levels of −6.19 (SE = 2.20) and sample size of 224 individuals. Statistical power stratified by gender was 53.5% for boys and 54.1% for girls.

Results

Anthropometric and Biochemical Characteristics

Table 1 describes the anthropometric characteristics and lipid profile of the children stratified by gender. Fat mass percentage was significantly higher in girls ($P = 0.041$), but no significant gender differences in BMI-z score or lipid parameters were observed.

VNN1 Expression Analysis

VNN1 expression levels were not significantly correlated with HDL-C levels in the entire study population, but showed a significant negative correlation with BMI z-score and TG levels ($P = 0.032$ and 0.026, respectively) (Table 2). On stratification according to gender, a significant positive correlation of VNN1 expression levels with HDL-C ($P = 0.017$), and significant negative correlations with BMI z-score and TG levels were observed exclusively in girls ($P = 0.015$ and $P = 0.025$). All associations remained significant by further adjustment for admixture, but only the association with BMI-z score was significant after correction for multiple testing.

G137T Variant, VNN1 Expression Levels and Metabolic Parameters

The overall frequency of the T-137 allele was 35%. The TT genotype was significantly associated with lower VNN1 expression levels ($P = 2.9 \times 10^{-5}$ after adjusting for admixture) (Figure 1). Multiple linear regression analyses to predict VNN1 expression levels revealed that only TT genotype contributed independently to explain 4.8% of the variance at VNN1 gene expression ($P = 0.004$). In addition, the TT genotype was associated with lower HDL-C and ApoA1 levels ($P = 0.005$ and 0.044, respectively), and with higher BMI z-score, percent fat mass, TG and TC levels in the whole sample ($P < 0.05$, Table 3). After adjusting for BMI z-score and admixture, only associations with TC and HDL-C remained significant in the whole sample ($P = 0.034$ and 0.028, respectively). On stratification according to gender, a significant
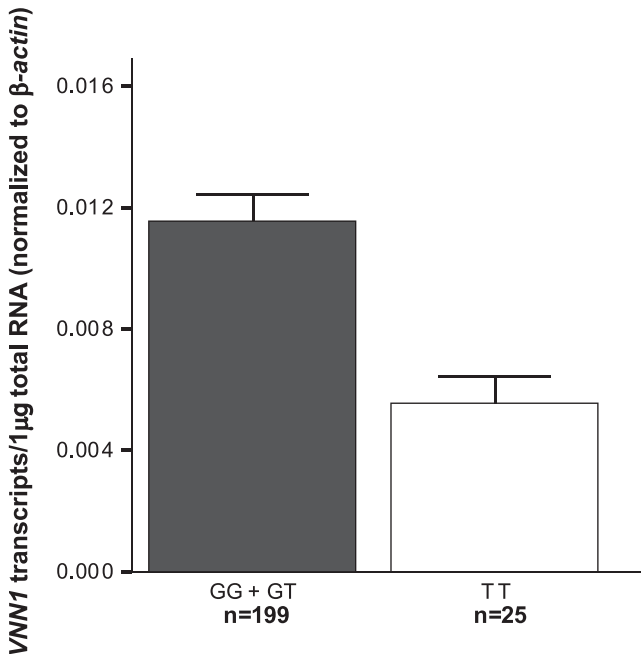

Figure 1. Effect of G-137T variant on VNN1 expression levels in Mexican prepubertal children. The results are presented as the global mean ± the standard error.
doi:10.1371/journal.pone.0049818.g001

Table 3. Association of G-137T variant with metabolic parameters stratified by gender.

Parameters	All children (n = 224)			Boys (n = 107)			Girls (n = 117)		
	Effect (SE)	P	Pª	Effect (SE)	P	Pª	Effect (SE)	P	Pª
BMI z-score	0.48 (0.21)	0.024	0.024	0.69 (0.36)	0.061	0.063	0.38 (0.26)	0.146	0.156
FM, %	5.66 (2.25)	0.012	0.012*	6.45 (4.24)	0.131	0.123	4.56 (2.46)	0.067	0.072
TG, mg/dL	25.76 (12.45)	0.040	0.194	−1.65 (17.63)	0.926	0.371	39.53 (17.34)	0.024	0.079
TC, mg/dL	16.35 (6.58)	0.014	0.034	10.64 (10.41)	0.309	0.423	18.93 (8.64)	0.031	0.057
HDL-C, mg/dL	−6.19 (2.20)	0.005	0.028	−2.10 (3.62)	0.562	0.994	−8.47 (2.80)	0.003	0.010*
ApoA1 mg/dL	−9.66 (4.77)	0.044	0.093	−5.95 (8.72)	0.497	0.969	−12.44 (5.98)	0.040	0.093

Effect values are presented as effect for two T copies (recessive model), standard error (SE). Genotype frequencies: all children (GG, 41.1%; GT, 47.7%; TT, 11.2%); boys (GG, 42.1%; GT, 49.5%; TT, 8.4%); girls (GG, 40.2%; GT, 46.1%; TT, 13.7%). BMI, body mass index; FM, percent fat mass; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol.
ªP-values adjusted for admixture in all tests and for BMI z-score when appropriate.
*Significant after Bonferroni correction.
doi:10.1371/journal.pone.0049818.t003

association of TT genotype with lower HDL-C levels was observed exclusively in girls ($P = 0.010$).

Discussion

Several epidemiological studies report that the onset of metabolic disease is occurring at increasingly earlier ages [31,32]. In the Mexican population, low HDL-C levels is the most common dyslipidemia in both adults and adolescents [1–3], and was also found to be common in prepubertal children aged 6–8 years (20%), showing that low HDL-C levels are present since childhood in this population.

Several studies have shown that variation in gene expression is probably an important mechanism influencing HDL-C levels [9–11,33]. Göring et al. observed that VNN1 expression levels showed the strongest correlation with HDL-C concentrations in lymphocytes of Mexican-American adults, ($r = 0.28$, $P = 4 \times 10^{-9}$). Similarly, we observed a positive correlation of VNN1 expression levels in leukocytes with HDL-C levels in Mexican prepubertal children, which was significant only in girls ($r = 0.22$, $P = 0.017$). We also observed a negative correlation of VNN1 expression levels with triglyceride levels and BMI z-score in the whole sample, again with gender-specific differences. Although the mechanism by which VNN1 function may affect these metabolic traits is largely unknown, there is experimental evidence that Vanin 1 is a central regulator of lipid biosynthesis by controlling the flux through the fatty acid and/or cholesterol biosynthetic pathways [34]. Because to our knowledge this is the only study analyzing the effects of VNN1 expression levels on metabolic traits in children, further studies are required to confirm this finding and to further characterize the gender differences observed.

The VNN1 T-137 allele frequency in the Mexican children population (35%) was similar to the 33% frequency reported in Mexican-American population and lower than in European populations (67%) [35]. Several studies have reported an effect of genetic ancestry on both global gene expression and associations of gene variants with disease [36,37]. Because the Mexican population is the result of admixture, mainly of Spanish and Native American populations [25,38], we included ancestry as a confounding factor to test for associations. Although the panel of AIMs analyzed is small (10 AIMs), the mean admixture proportions of the population were estimated as 65% Native American and 35% European, in agreement with previous studies in the population of Mexico City using 69 AIMs [25]. All

associations reported in the present study remained significant after adjusting for admixture, suggesting that population stratification was not an important confounding factor in our analysis.

The TT genotype was significantly associated with lower VNN1 expression and HDL-C levels, in accordance with the findings of Göring et al. in Mexican-American adults. Although experimental evidence strongly supports that this promoter polymorphism has functional consequences with respect to binding transcription factors [9], this genotype contributed independently to explain only 4.8% of the variance at VNN1 gene expression. While this is consistent with the previously reported range of the effect of SNPs on gene expression variability [11], the genetic and environmental factors explaining most of VNN1 gene expression variability remain to be determined.

Interestingly, associations of the TT genotype with higher TG and TC levels were observed in girls. However, these associations lost significance after adjusting for BMI z-score, suggesting they may be mediated by an effect on body fat. To our knowledge, there is no previous experimental evidence of the role of VNN1 in human adipocyte function, however it was identified as an obesity-related gene in mice [15] and is known to induce lipolysis in rat adipose tissue [39]. Because VNN1 gene variants have not been previously found to be associated with obesity in GWAS [40,41] and because the sample number analyzed here is reduced, these associations should be interpreted with caution. Additional studies are required to confirm and further understand the role of VNN1 in human obesity.

The gender differences observed are noteworthy, particularly because it involved prepubertal children. Mexican prepubertal girls showed significantly higher fat mass percentage than boys, which could explain at least part of the gender differences observed for genetic associations and correlations. Gender differences in the effect of polymorphisms on lipid traits have been previously observed in prepubertal children [28], apparently explained by dehydroepiandrostenone-sulfate (DHEA-S) level variation [42]. Because we did not measure DHEA-S levels, we cannot rule out whether this factor modulates the effect of VNN1 gene expression levels and the G-137T variant on metabolic traits in this study.

In conclusion, our data suggest that VNN1 gene expression levels and the G-137T variant are associated with lipid traits (particularly HDL-C levels) in Mexican prepubertal girls. However, these results should be interpreted with caution and further studies with larger samples sizes are required to confirm these findings.

Supporting Information

Table S1   Association of G-137T variant with metabolic parameters stratified by gender (additive model).
(DOC)

Table S2   Association of G-137T variant with metabolic parameters stratified by gender (dominant model).
(DOC)

Author Contributions

Conceived and designed the experiments: LJA SCQ. Performed the experiments: LJA PIAR HVR PLM. Analyzed the data: LJA PIAR SRH SCQ. Contributed reagents/materials/analysis tools: BELC FSM RB JAGB CAAS. Wrote the paper: LJA PIAR TVM SCQ.

References

1. Aguilar-Salinas CA, Olaiz G, Valles V, Torres JM, Gómez Pérez FJ, et al. (2001) High prevalence of low HDL cholesterol concentrations and mixed hyperlipidemia in a Mexican nationwide survey. J Lipid Res 42: 1298–307.
2. Posadas-Sánchez R, Posadas-Romero C, Zamora-González J, Mendoza-Pérez E, Cardoso-Saldaña G, et al. (2007) Lipid and lipoprotein profiles and prevalence of dyslipidemia in Mexican adolescents. Metabolism 56: 1666–72.
3. Flores-Dorantes T, Arellano-Campos O, Posadas-Sánchez R, Villarreal-Molina T, Medina-Urrutia A, et al. (2010) Association of R230C ABCA1 gene variant with low HDL-C levels and abnormal HDL subclass distribution in Mexican school-aged children. Clin Chim Acta 411: 1214–7.
4. Qasim A, Rader DJ (2006) Human genetics of variation in high-density lipoprotein cholesterol. Curr Atheroscler Rep 8: 198–205.
5. Lusis AJ, Fogelman AM, Fonarow GC (2004) Genetic basis of atherosclerosis: part II: clinical implications. Circulation 110: 2066–71.
6. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, et al. (2008) Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet 40: 161–69.
7. Kooner JS, Chambers JC, Aguilar-Salinas CA, Hinds DA, Hyde CL, et al. (2008) Genome-wide scan identifies variation in MLXIPL associated with plasma triglycerides. Nat Genet 40: 149–51.
8. Kathiresan S, Melander O, Guiducci C, Surti A, Burtt NP, et al. (2008) Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet 40: 189–97.
9. Göring HH, Curran JE, Johnson MP, Dyer TD, Charlesworth J, et al. (2007) Discovery of expression QTLs using large-scale transcriptional profiling in human lymphocytes. Nat Genet 39: 1208–16.
10. Ma J, Dempsey AA, Stamatiou D, Marshall KW, Liew CC (2007) Identifying leukocyte gene expression patterns associated with plasma lipid levels in human subjects. Atherosclerosis 191: 63–72.
11. Zeller T, Wild P, Szymczak S, Rotival M, Schillert A, et al. (2010) Genetics and beyond–the transcriptome of human monocytes and disease susceptibility. PLoS One 5: e10693.
12. Inouye M, Silander K, Hamalainen E, Salomaa V, Harald K, et al. (2010) An immune response network associated with blood lipid levels. PLoS Genet 6: e1001113.
13. Yamazaki K, Kuromitsu J, Tanaka I (2002) Microarray analysis of gene expression changes in mouse liver induced by peroxisome proliferator- activated receptor alpha agonists. Biochem Biophys Res Commun 290: 1114–22.
14. Di Leandro L, Maras B, Schininà ME, Dupré S, Koutris I, et al. (2008) Cystamine restores GSTA3 levels in vanin-1 null mice. Free Radic Biol Med 44: 1088–96.
15. Schadt EE, Lamb J, Yang X, Zhu J, Edwards S, et al. (2005) An integrative genomics approach to infer causal associations between gene expression and disease. Nature Genet 37: 710–7.
16. Crujeiras AB, Parra D, Milagro FI, Goyenechea E, Larrarte E, et al. (2008) Differential expression of oxidative stress and inflammation related genes in peripheral blood mononuclear cells in response to a low-calorie diet: a nutrigenomics study. OMICS 12: 251–61.
17. Idaghdour Y, Storey JD, Jadallah SJ, Gibson G (2008) A genome-wide gene expression signature of environmental geography in leukocytes of Moroccan Amazighs. PLoS Genet 4: e1000052.
18. Brown WM, Beck SR, Lange EM, Davis CC, Kay CM, et al. (2003) Framingham Heart Study.Age-stratified heritability estimation in the Framingham Heart Study families. BMC Genet 4 Suppl 1: S32.
19. Dumitrescu L, Brown-Gentry K, Goodloe R, Glenn K, Yang W, et al. (2011) Evidence for age as a modifier of genetic associations for lipid levels. Ann Hum Genet 75: 589–97.
20. Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, et al. (2002) 2000 CDC growth charts for the United States: methods and development. Vital Health Stat 246: 1–190.
21. Villarreal-Molina MT, Aguilar-Salinas CA, Rodríguez-Cruz M, Riaño D, Villalobos-Comparan M, et al. (2007) The ATP-binding cassette transporter A1

R230C variant affects HDL cholesterol levels and BMI in the Mexican population: association with obesity and obesity-related comorbidities. Diabetes 56: 1881–7.
22. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (2011) Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. Pediatrics 128 Suppl 5: S213–56.
23. Mouritzen P, Noerholm M, Nielsen PS, Jacobsen N, Lomholt C, et al. (2005) ProbeLibrary: a new method for faster design and execution of quantitative real-time PCR. Nat Methods 2: 313–316.
24. Bonilla C, Parra EJ, Pfaff CL, Dios S, Marshall JA, et al. (2004) Admixture in the Hispanics of the San Luis Valley, Colorado, and its implications for complex trait gene mapping. Ann Hum Genet 68: 139–53.
25. Martinez-Marignac VL, Valladares A, Cameron E, Chan A, Perera A, et al. (2007) Admixture in Mexico City: implications for admixture mapping of type 2 diabetes genetic risk factors. Hum Genet 120: 807–19.
26. Ellegren H, Parsch J (2007) The evolution of sex-biased genes and sex-biased gene expression. Nat Rev Genet 8: 689–98.
27. Feitosa MF, Myers RH, Pankow JS, Province MA, Borecki IB (2009) LIPC variants in the promoter and intron 1 modify HDL-C levels in a sex-specific fashion. Atherosclerosis 204: 171–7.
28. López-Simón L, de Oya M, Lasunción MA, Riestra P, Benavente M, et al. (2009) Genetic determinants of plasma HDL-cholesterol levels in prepubertal children. Clin Chim Acta 403: 203–6.
29. Weiss LA, Pan L, Abney M, Ober C (2006) The sex-specific genetic architecture of quantitative traits in humans. Nat Genet 38: 218–22.
30. Hoggart CJ, Parra EJ, Shriver MD, Kittles RA, Clayton DG, et al. (2003) Control of confounding of genetic associations in stratified populations. Am J Hum Genet 72: 1492–504.
31. Saland JM (2007) Update on the metabolic syndrome in children. Curr Opin Pediatr 19: 183–91.
32. Nathan BM, Moran A (2008) Metabolic complications of obesity in childhood and adolescence: more than just diabetes. Curr Opin Endocrinol Diabetes Obes 15: 21–9.
33. Cookson W, Liang L, Abecasis G, Moffatt M, Lathrop M (2009) Mapping complex disease traits with global gene expression. Nat Rev Genet 10: 184–94.
34. Kaskow BJ, Proffitt JM, Blangero J, Moses EK, Abraham LJ (2012) Diverse biological activities of the vascular non-inflammatory molecules - the Vanin pantetheinases. Biochem Biophys Res Commun 417: 653–8.
35. International HapMap Consortium.The International HapMap Project (2003) Nature 426: 789–96.
36. Storey JD, Madeoy J, Strout JL, Wurfel M, Ronald J, et al. (2007) Gene-expression variation within and among human populations. Am J Hum Genet 80: 502–9.
37. Yang HC, Wang PL, Lin CW, Chen CH, Chen CH (2012) Integrative analysis of single nucleotide polymorphisms and gene expression efficiently distinguishes samples from closely related ethnic populations. BMC Genomics 13: 346.
38. Lisker R, Ramirez E, Briceno RP, Granados J, Babinsky V (1990) Gene frequencies and admixture estimates in four Mexican urban centers. Hum Biol 62: 791–801.
39. Bocos C, Herrera E (1998) Pantethine stimulates lipolysis in adipose tissue and inhibits cholesterol and fatty acid synthesis in liver and intestinal mucosa in the normolipidemic rat. Environ Toxicol Pharmacol 6: 59–66.
40. Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, et al. (2009) Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet 41: 18–24.
41. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, et al. (2010) Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet 42: 937–48.
42. Garcés C, Benavente M, Cano B, Viturro E, Ortega H, et al. (2003) Effects of dehydroepiandrosterone-sulfate on the ApoE genotype influence on plasma lipid levels in prepubertal children. J Clin Endocrinol Metab 88: 3997–4000.