José Suazo, María Isabel Hodgson, Ana María Obregón, Macarena Valladares, Gerardo Weisstaub, Paola Amador and José Luis Santos*

Prevalence of metabolic syndrome in obese Chilean children and association with gene variants of the leptin-melanocortin system

Abstract: Metabolic syndrome (MS) related to adult type 2 diabetes mellitus and cardiovascular disease is prevalent among obese children/adolescents. Genetic variants of the leptin-melanocortin system have been associated with components of MS. The aim of our study is to estimate the prevalence of MS (according to Cook's criteria) in a Chilean cross-sectional sample of 259 obese children (47.1% girls, aged 6–12 years), and to assess the association between common genetic variants of leptin-melanocortin pathway genes (*LEP*, *LEPR*, *POMC*, *MC3R* and *MC4R*) with components of the MS using logistic regression. We observed an overall MS prevalence of 26.3% (32.2% in girls and 21.1% in boys) in obese Chilean children. No associations were detected between genetic variants of leptin-melanocortin genes and MS components. MS prevalence among our obese children sample is similar to those previously described in Chile, demonstrating the increased risk of diseases in adulthood that obese children carry.

Keywords: association; childhood obesity; leptin-melanocortin genes; metabolic syndrome.

***Corresponding author: José Luis Santos,** Alameda 340, Gastroenterology Building, 4th floor, Santiago, Chile, Phone: +56-2-3543862, Fax: +56-2-6338298, E-mail: jsantos@med.puc.cl

José Suazo, María Isabel Hodgson, Ana María Obregón, Macarena Valladares and José Luis Santos: Department of Nutrition, Diabetes and Metabolism, School of Medicine, Pontifical Catholic University of Chile, Santiago, Chile

Gerardo Weisstaub and Paola Amador: Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile

Introduction

Metabolic syndrome (MS) is defined as a combination of central obesity, elevated blood pressure, dyslipidemia, and increased glycemia. Although the true biological significance of this phenotype cluster is controversial (1), MS is considered as a risk factor for developing cardiovascular disease and type 2 diabetes, with both being conditions related to insulin resistance (IR) (2, 3).

Prevalence for MS varies according to age, sex, and ethnic origin in different populations, with rates ranging from 23% to 39% in adults (4). Some authors have estimated an overall prevalence of MS in children and adolescents of 4.2% and 8.6%, respectively (5, 6). These rates increase considerably when only obese individuals are considered: 19.6%, 23.3%, and 28.7%, usually showing higher prevalence in boys than in girls (5–7).

The prevalence of adult obesity has increased in developing and developed countries (from 30% in 2000 to 35% in 2010). In children and adolescents this prevalence increased from ~14% to ~17% in the same period (8). In Chile, due to epidemiological and nutrition transition, the prevalence of childhood obesity has tripled over the last two decades, becoming a major nutritional disease (9, 10). As a result of the reported relation between overweight/obesity and IR, research has been focused in MS as a predictor of adulthood diseases (11, 12).

Ethnic differences have been reported in MS prevalence in childhood and adolescence. Thus, populations with a higher degree of Amerindian admixture have shown the highest rates, whereas African-American individuals have shown the lowest (5, 6). These findings allow for proposing an involvement of genetic factors in this syndrome. In this context, Park et al. (13) and Tang et al. (14) have described a familial aggregation for MS, reporting genetic correlations for its individual components.

Genetic influences have been detected for each of the MS components in genome-wide association studies (GWAS) (15). As a result of the complexity of MS, many metabolic or regulatory pathways are necessarily involved in this multifactorial phenotype. In this context Sookoian and Pirola identified several molecular pathways related to MS using bioinformatic tools (16). One of these networks is the leptin-melanocortin system.

The leptin-melanocortin system is a crucial regulator of the complex equilibrium between food intake

and energy expenditure. Leptin, an adipocyte-derived hormone, stimulates the expression of pro-opiomelanorcotin (POMC) in first order neurons at the hypothalamic arcuate nucleus. One of the post-traslational products of POMC, the α-melanocyte-stimulating hormone (α-MSH), binds to the melanocortin -4 receptor (MCR4) in second order neurons generating an anorexigenic stimulus. However, absence of leptin is associated with higher expression of neuropeptide Y (NPY) and the MC4R ligand agouti-related protein (AGRP). These molecules act by producing an orexigenic response in leptin-deficiency states (17, 18). In this context, mice deficient for *Pomc* and *Mc4r* genes also exhibit several characteristics related to MS, with the exception of arterial hypertension (19–21).

Mutations in genes coding for leptin, leptin receptor, and other proteins and receptors related to leptin-melanocortin, such as *LEP*, *LEPR*, *POMC*, *MC3R* and *MC4R*, have been shown to be involved in early-onset monogenic forms of obesity (18). In Chilean children, an association between obesity, eating behavior and common polymorphisms of *MC3R* and *MC4R* have been described (22, 23). Common polymorphisms –2548G>A in the 5′ flanking region of *LEP*, Gln223Arg within *LEPR* and Val-103Ile within *MC4R* have previously shown significant association with MS (24–27).

The purpose of the present study is to estimate the prevalence of MS in a sample of Chilean obese children and also to evaluate the association between common variants of the leptin-melanocortin pathway genes with MS and its individual components.

Materials and methods

Subjects

In a cross-sectional study, we recruited 259 Chilean children (122 girls and 137 boys, aged 6–12 years old) with a body mass index (BMI) ≥95th percentile according to National Center for Health Statistics/Centers for Disease Control and Prevention (NCHS/CDC) (28). In this sample, the gender-specific maturational stage distribution was: Tanner 1, 59.1% and 64.2%; Tanner 2, 17.4% and 27.6%, Tanner 3, 18.3% and 6%, Tanner 4, 3.5% and 2.2%; and Tanner 5, 1.7% and 0%, in girls and boys, respectively. These children were recruited from the outpatient unit of the Pediatrics Department at the Institute of Nutrition and Food Technology (INTA), University of Chile, from the outpatient unit of the School of Medicine of the Pontificia Universidad Católica de Chile, and through an open invitation made in schools from our public education system in the metropolitan area of Santiago. All the parents signed an inform consent document approved by the Institutional Review Boards.

Anthropometric and metabolic measurements

Anthropometric and blood pressure measurements were carried out by trained personnel using standardized techniques (29). Height and weight were measured in light clothing, and BMI was calculated (kg/m^2). Waist circumference was measured using a non-elastic tape just above the uppermost lateral border of iliac crest, at the end of a normal expiration (30). The criterion for obesity was defined as BMI above the 95th percentile according to the CDC/NCHS 2000 reference curve (CDC, 2000). For this purpose, BMI z-score was calculated for all children (31). Blood samples were drawn to measure serum concentration of fasting glucose (enzymatic colorimetric test; Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany), fasting insulin (radioinmmunoassay; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA), leptin and leptin soluble receptor (ELISA; R&D Systems, Minneapolis, MN, USA) triglycerides (TG), total cholesterol, high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol (all of them by enzymatic-colorimetric assays; Roche Diagnostics GmbH, Mannheim, Germany) (Table 1).

Metabolic syndrome diagnosis

Diagnosis of MS was defined by the presence of at least three out of five components of the Cook's phenotype: waist circumference (WC) above sex-gender and age 90th percentile, serum TG concentration over 110 mg/dL, serum HDL-cholesterol lower than 40 mg/dL, blood fasting glucose (FG) over 100 mg/dL, and systolic or diastolic blood pressure (BP) over the 90th percentile for gender, age, and height (5, 29).

Genetic analysis of leptin-melanocortin gene variants

Genomic DNA extraction was carried out using a commercial kit (QIAamp DNA Blood Mini Kit; Qiagen Sciences Inc, Germantown, MD, USA). We determined genotypes of the single nucleotide polymorphisms (SNPs) Lys109Arg (rs1137100), Gln223Arg (rs1137101), Lys656Asn (rs8179183), and InsCTTTA (rs75054066) for the *LEPR* gene; InsGCCGCTGCT (rs10654394) for the *POMC* gene; rs17782313 for the *MC4R* gene, and –239A>G (rs11575886), Thr6Lys (rs3746619), Val81Ile (rs3827103), InsCAGACC (rs74181042) for the *MC3R* gene. We also tested the tetranucleotide repeat (TTTC)n for the *LEP* gene. Genotypes for the selected SNPs were determined by polymerase chain reaction followed by restriction fragment length polymorphism technique (PCR-RFLP) as previously described (22, 23, 32, 33), with the exception of *MC4R* rs17782313 that was genotyped by Taqman allele discrimination assay (Applied Biosystems ID c_32667060). Genotypes for *LEP* (TTTC)n were determined by PCR and capillary electrophoresis analysis according to Snoussi et al. (34).

Statistical analysis

Arithmetic means for all of the quantitative variables were compared between study groups by Student's t-test. According to Cook et al. (5),

proportions for MS and MS components were also compared between girls and boys by means of the χ^2-test. Genotype and allele frequencies were estimated by simple proportions. Hardy-Weinberg equilibrium was evaluated based on a goodness-of-fit χ^2-test. Association between MS and its components and SNPs genotypes was evaluated using logistic regression, estimating odds ratio (OR) with 95% confidence interval (95% CI) for each genotype. Statistical analyses were carried out with the program STATA 12.0 (STATA Corp., Station College, TX, USA).

Results

Table 1 shows the means (±standard deviations) for several anthropometric and metabolic characteristics in the obese Chilean children stratified by gender. Boys presented many measurements with means statistically higher than the ones registered among girls: weight (p=0.006), height (p=0.009), BMI (p=0.032), BMI z-score (p=0.005), waist circumference (p=0.007), blood fasting glucose (p=0.001), serum HDL cholesterol (p<0.001), and systolic blood pressure (p=0.012).

Table 2 shows the proportion of individuals with diagnosis of MS and each of its components according to Cook's phenotype. Elevated levels of fasting glucose where significantly more frequent in boys than in girls (p=0.037), while the opposite figure was observed for the prevalence of low HDL cholesterol (p=0.0001). No significant differences were observed between girls and boys for the other MS components. The overall MS prevalence in this cohort was estimated at 26.3% (95% CI: 20.8%–31.7%). When the MS prevalence was estimated by gender, girls showed a greater prevalence than boys: 32.2% and 21.1%, respectively (p=0.04) (Table 2).

Allele and genotype frequencies for all of the polymorphisms included in this study are listed in Table 3. All SNPs showed genotype frequencies that were concordant with Hardy-Weinberg expectations (data not shown). Association analysis was performed between genotypes for all of the studied markers and MS and its components. In this context, Table 4 only shows the significant results for these analyses adjusted by age and stratified by gender. Carriers of *MC3R* genotypes 6Thr/6Lys (OR 3.46, 95% CI: 1.41–8.47, p=0.006) and 81Val/81Ile (OR 3.63, 95% CI: 1.46–8.99, p=0.005) present an increasing risk for MS for all children (Table 4). In addition, these genotypes are associated with MS for girls (OR 4.33, 95% CI: 1.24–15.1, p=0.022 and OR 5.13, 95% CI: 1.38–19.1, p=0.015, respectively) but not for boys. As a result of the strong linkage disequilibrium found between these two *MC3R* variants (35), they essentially provided the same information, and consequently, only the results for Thr6Lys will be presented from now on.

The association analysis of each MS component and the included markers present significant results for some of them and are listed in Table 4. Among these results, we can highlight that, in addition to MS, *MC3R* 6Thr/6Lys genotype seems to be a risk factor for serum TG >110 mg/dL (OR 2.91, 95% CI: 1.18–7.19, p=0.020), which was not significant when the analysis was performed by each gender.

Table 1 Anthropometric and metabolic characteristics of obese Chilean children.

	All children (n=259)	Girls (n=122)	Boys (n=137)	p-Value[a]
Age, years	9.57±1.77	9.38±1.77	9.73±1.77	N.S.
Weight, kg	51.9±13.9	49.4±13.0	54.1±14.4	0.006
Height, cm	139.9±11.3	138.0±11.5	141.7±10.9	0.009
BMI, kg/m^2	26.1±4.1	25.5±3.5	26.5±4.5	0.032
BMI z-score	2.12±0.33	2.06±0.32	2.18±0.33	0.005
Waist circumference, cm	85.9±10.6	84.1±10.3	87.7±10.7	0.007
Fasting insulin, μU/mL	9.75±9.77	9.82±7.48	9.70±11.37	N.S.
Fasting glucose, mg/dL	88.9±10.9	86.6±8.6	90.9±12.3	0.001
HOMA-IR	2.16±2.18	2.12±1.68	2.19±2.53	N.S.
Leptin, ng/mL	32.7±20.2	32.8±19.3	32.7±20.9	N.S.
Soluble leptin receptor, ng/mL	31.6±6.8	31.1±7.1	31.2±6.7	N.S.
Total cholesterol, mg/dL	163.7±30.3	161.8±31.2	165.4±29.6	N.S.
LDL cholesterol, mg/dL	95.4±24.4	93.7±24.4	96.7±24.4	N.S.
HDL cholesterol, mg/dL	45.9±9.9	42.7±8.8	48.8±9.9	<0.001
Triglycerides, mg/dL	104.9±58.1	110.7±61.9	99.8±54.1	N.S.
Diastolic blood pressure, mm Hg	67.5±10.9	66.9±12.1	68.1±9.7	N.S.
Systolic blood pressure, mm Hg	104.8±12.5	102.7±12.7	106.7±11.9	0.012

[a]Uncorrected p-value in the comparison of mean scores of girls and boys using Student's t-test. Values are shown as mean±standard deviation. HOMA-IR, homeostasis model assessment-insulin resistance; LDL, low density lipoprotein; HDL, high density lipoprotein.

Table 2 Prevalence of children with components of the metabolic syndrome[a] (95% CI).

	All children	Girls	Boys
Waist circumference >90th percentile	97.2 (95.2–99.2)	97.4 (94.6–100.3)	97.0 (94.1–99.8)
Diastolic blood pressure >90th percentile	16.8 (12.1–21.4)	15.0 (8.5–21.6)	18.3 (11.6–24.9)
Systolic blood pressure >90th percentile	19.3 (14.3–24.2)	20.3 (12.9–27.8)	18.3 (11.6–24.9)
Blood hypertension[b]	25.8 (20.3–31.3)	26.5 (18.4–34.7)	25.2 (17.7–32.6)
Fasting glucose >100 mg/dL	11.7 (7.7–15.7)	7.8 (2.9–12.7)	15.1 (9.0–2.1)[c]
HDL cholesterol <40 mg/dL	30.2 (24.4–35.9)	42.6 (33.6–51.6)	19.2 (12.4–26.0)[c]
Triglycerides >110 mg/dL	31.8 (26.0–37.6)	34.7 (26.1–43.5)	29.2 (21.4–37.0)
%MS prevalence (95% CI)	26.3 (20.8–31.7)	32.2 (23.7–40.6)	21.1 (10.1–27.9)[c]

[a]According to the criteria described by Cook et al. (5). [b]Diastolic blood pressure >90th percentile or Systolic blood pressure >90th percentile or both. [c]p-value <0.05 for girls proportions versus boys proportions using *z*-test. HDL, high density lipoprotein.

In addition, two *LEPR* markers are associated to triglycerides levels. Thus, 223Gln/223Arg seems to be a protective factor just for boys (OR 0.36, 95% CI: 0.14–0.95, p=0.040), while 656Lys/656Asn could be considered as a risk factor for the whole sample (OR 2.21, 95% CI: 1.12–4.35, p=0.021). With respect to HDL levels, *LEPR* 223Gln/223Arg genotype seems to have a protective role for levels <40 mg/dL in the total sample (OR 0.31, 95% CI: 0.10–0.92, p=0.036).

Table 3 Allele and genotype frequencies for *LEP, LEPR, POMC, MC3R* and *MC4R* polymorphisms in Chilean obese children.

Gene	SNP	Genotype	All Children	Girls	Boys
LEP	(TTTC)n	ClassI/ClassI	0.246	0.227	0.262
		ClassI/ClassII	0.497	0.545	0.456
		ClassII/ClassII	0.257	0.227	0.282
LEPR	Lys109Arg	109Lys/109Lys	0.583	0.522	0.633
		109Lys/109Arg	0.377	0.422	0.339
		109Arg/109Arg	0.040	0.056	0.028
	Gln223Arg	223Gln/223Gln	0.291	0.256	0.321
		223Gln/223Arg	0.528	0.555	0.505
		223Arg/223Arg	0.181	0.188	0.174
	Lys656Asn	656Lys/656Lys	0.704	0.678	0.725
		656Lys/656Asn	0.276	0.311	0.248
		656Asn/656Asn	0.020	0.011	0.027
	InsCTTTA	Del/Del	0.678	0.700	0.661
		Del/Ins	0.292	0.289	0.293
		Ins/Ins	0.030	0.011	0.046
POMC	InsGCCGCTGCT	Del/Del	0.952	0.963	0.943
		Del/Ins	0.048	0.037	0.057
MC3R	−239A>G	AA	0.910	0.878	0.936
		AG	0.085	0.122	0.055
		GG	0.005	0.000	0.009
	Thr6Lys	6Thr/6Thr	0.874	0.845	0.899
		6Thr/6lys	0.121	0.144	0.101
		6Lys/6lys	0.005	0.011	0.000
	Val81Ile	81Val/81Val	0.879	0.856	0.899
		81Val/81Ile	0.116	0.133	0.101
		81Ile/81Ile	0.005	0.011	0.000
	InsCAGACC	Del/Del	0.678	0.567	0.771
		Del/Ins	0.297	0.389	0.220
		Ins/Ins	0.025	0.044	0.009
MC4R	rs17782313	TT	0.728	0.678	0.772
		TC	0.248	0.288	0.213
		CC	0.024	0.034	0.015

SNP, single nucleotide polymorphisms.

Table 4 Significant results for the association between metabolic syndrome and its components, and *LEP*, *LEPR*, *POMC*, *MC3R* and *MC4R* polymorphisms.

Gene	Genotype	All Children[a]			Girls[a]			Boys[a]		
		OR	(95% CI)	p-Value[b]	OR	(95% CI)	p-Value[b]	OR	(95% CI)	p-Value[b]
Metabolic syndrome										
MC3R	6Thr/6Lys	3.46	(1.41–8.47)	0.006	4.33	(1.24–15.1)	0.022	2.38	(0.61–9.39)	0.213
	81Val/81Ile	3.63	(1.46–8.99)	0.005	5.13	(1.38–19.1)	0.015	2.38	(0.61–9.39)	0.213
Serum TG >110 mg/dL										
LEPR	223Gln/223Arg	0.94	(0.47–1.87)	0.868	2.71	(0.91–8.14)	0.074	0.36	(0.14–0.95)	0.040
	656Lys/656Asn	2.21	(1.12–4.35)	0.021	2.02	(0.79–5.19)	0.143	2.59	(0.95–7.02)	0.060
MC3R	6Thr/6Lys	2.91	(1.18–7.19)	0.020	2.94	(0.84–10.2)	0.090	2.59	(0.71–9.62)	0.153
	81Val/81Ile	2.48	(1.01–6.15)	0.049	2.26	(0.64–7.96)	0.204	2.60	(0.71–9.62)	0.153
Serum HDL <40 mg/dL										
LEPR	223Gln/223Arg	0.31	(0.10–0.92)	0.036	0.37	(0.09–1.50)	0.163	0.14	(0.02–1.31)	0.086
MC3R	6Thr/6Lys	2.91	(1.21–7.02)	0.017	3.13	(0.89–10.9)	0.073	2.56	(0.67–9.80)	0.170
	81Val/81Ile	3.11	(1.27–7.61)	0.013	3.68	(0.99–13.7)	0.052	2.56	(0.67–9.80)	0.170
Blood pressure >90th percentile										
LEPR	656Lys/656Asn	0.41	(0.18–0.97)	0.043	0.21	(0.57–0.82)	0.025	0.78	(0.25–2.41)	0.668

[a]Adjusted by age. [b]Uncorrected p-value. OR, odds ratio.

However, *MC3R* 6Thr/6Lys genotype is associated to HDL <40 mg/dL only for all children (OR 2.91, 95% CI: 1.21–7.02, p=0.017). Finally, *LEPR* 656Lys/656Asn could have a protective role for BP for all children (OR 0.41, 95% CI: 0.18–0.97, p=0.043) and for girls (OR 0.21, 95% CI: 0.57–0.82, p=0.025). Considering the number of markers analyzed (11 SNPs) plus the association test performed for each one (for all children, for girls, and for boys), none of the results mentioned will remain significant after the application of any method for multiple comparisons.

Discussion

The increased prevalence of overweight/obesity among children and adolescents has become a major health burden for Western countries. This problem is not only related to direct consequences for health systems but it is also associated with an increase in the risk of developing adulthood pathologies, such as type 2 diabetes mellitus and cardiovascular disease. These diseases are strongly linked to IR, which can also explain the features of MS (11, 12). Consequently, the prevalence of MS is higher in obese children/adolescents than the overall population in this age range, both in developed and developing countries. Thus, among normal children/adolescent, MS prevalence is reported between 0.2% and 14.1% while among obese individuals, the prevalence range is between 21% and 43% (36–41). These different proportions could be explained by factors such as different definitions of MS, age range, ethnic origin and, in the case of obese populations, the severity of this condition (36–38, 42, 43). This several-fold increase in the frequency of MS among obese children and adolescents makes it necessary to focus our preventive efforts in this population, who have an increased risk of developing metabolic and cardiovascular diseases.

Our results show that in a population of children (6–12 age old), the prevalence of MS (Cook's criteria) was estimated at 26.3% with a significant difference among genders (32.2% for girls and 21.1% boys). This difference could be explained by the fact that the girls showed a higher frequency of pubertal development than the boys. The increase in the IR associated with age and pubertal development has been well documented (44), this could influence the MS prevalence reported herein. Consequently, in our sample, pre-pubertal children (Tanner 1) showed lower mean values of fasting insulin and homeostasis model assessment-insulin resistance (HOMA-IR) than pubertal ones (Tanner >1) (Student's t-test, p=0.014 and 0.016, respectively). In addition, when these variables were compared by gender, the differences are more evident when pre-pubertal girls are compared to pubertal girls (Student's t-test, p=0.0002 and 0.0002 for fasting insulin and HOMA-IR, respectively).

As we previously mentioned, there is heterogeneity of the samples analyzed, and the estimation of MS prevalence for obese children/adolescent makes it difficult to make comparisons between other reports and our results. To our knowledge, based on Cook's criteria, there are three reports on MS prevalence in Chilean children/adolescents:

two of them from the city of Santiago (our capital city) by Burrows et al. (45) and by Eyzaquirre et al. (46), and the third from the cities of Conception and Coronel (located south of Santiago) by Bustos et al. (47). In comparison with the present study, Burrows et al. describe a similar prevalence to ours, with 29.8% of obese children having MS in a sample of obese children and adolescents with a wider age range (6–16 years old) (45). Similar to our study, the most prevalent MS component in Burrows' sample was central obesity, followed by hypertriglyceridemia, while the least frequent was hyperglycemia. Although there were no statistical significant differences by gender, a higher prevalence for girls vs. boys was also detected by the study of Burrows et al. (28.9% vs. 24.1%, respectively) (45). This tendency, like our report, could be explained by the higher proportion of girls presenting greater pubertal development than boys.

In a Chilean sample of obese children and adolescent (5–18 years old), a report described a SM prevalence of 22.7% (46), a figure that is lower than the results from our report. Another difference with our study is the higher prevalence of MS found in boys compared to girls (37.6% and 18.4%, respectively). These authors do not comment about the Tanner's stages of their sample but they state that obesity in boys was more severe than girls, which may account for the gender differences in the sample. This study shows once again that the most prevalent MS component was abdominal obesity, followed by hypertriglyceridemia, and the less prevalent was hyperglycemia. Finally, a third study by Bustos et al. detected a MS prevalence of 37.5% in obese adolescents ranged 10–18 years old (43.7% among boys and 33% among girls) (47). Similar to our report, the most common MS component was abdominal obesity while the least frequent was elevated fasting glycemia. The age range of this sample may be related to the higher MS prevalence detected when it is compared with the prevalence detected by us. It is worth noting that the three previous Chilean studies detailed herein and ours show an important proportion of hypertensive obese children/adolescents (around 25%). This can be linked with the risk in developing adult cardiovascular diseases mentioned above.

Another issue that could influence the prevalence of MS mentioned above is the severity of obesity (considering the BMI z-score as its estimator) in the Chilean children/adolescent samples. However, this relation is unclear because, for example, the report by Bustos et al. presents the greater MS prevalence but does not show the greater BMI z-score (47). However, the lower prevalence reported by Eyzaguirre et al. (46) exhibits a BMI z-score higher than Bustos et al. (47) and ours. A description of the ethnic origin of our sample was not considered, nor did the previous reports mention this. Our sample, Burrows' sample and Bustos' sample all have individuals that were recruited from the public education and health systems. On the contrary, the sample analyzed by Eyzaguirre et al. was recruited in a private clinic from Santiago, Chile (46). Based on genetic markers such as blood groups, different ethnic backgrounds (Amerindian admixture index) have been postulated for populations attending the public and private Chilean health systems (48, 49). There has been published a tendency for a higher prevalence among Hispanic (Amerindian-Caucasian admixed) populations compared to African and Caucasian ones (5, 42, 43) that may influence the different MS prevalence rates detected in the Chilean samples.

Previous evidence showed that about half of the variance for IR is as a result of genetic factors, which is concordant with the observed familial aggregation and genetic correlations reported for MS (13, 14). Based on GWAS and reported monogenic cases of obesity, the leptin-melanocortin pathway has been postulated as a candidate for MS features. As previously mentioned, the central leptin-melonocortin system is involved in energy homeostasis, fat mass deposit and metabolism, and blood pressure modulation (50–54). Consequently, the possible involvement of genes in this system in MS expression can be suggested. The second aim of the present work was to perform the association analyses between variants for genes involved in this pathway (*LEP*, *LEPR*, *POMC*, *MC3R* and *MC4R*) and MS and their components.

There are some examples of previous studies showing a relation between some of MS components and variants within the genes analyzed herein. The microsatellite marker D20s32, close to *MC3R*, was associated with IR in Maori families affected with early onset type 2 diabetes and obesity. This association was independent of age (55). In the USA, children and adolescent double homozygous for *MC3R* Lys6 and Ile81 showed higher waist circumference measurements in comparison to double wild-type and double heterozygous individuals (56). Carriers of Lys6-Val81 haplotype show a decrease in diastolic and systolic blood pressure in a population of adolescents from South Africa (57). Although there is no evidence of direct association between *LEPR* 223Arg/223Arg genotype and dyslipidemia, this genotype shows a direct effect over BMI in obese patients (58). Similar to Gln223Arg, there is a lack of consensus about the role of *LEPR* Lys656Asn in obesity and its related phenotypes (59). In a sample of adolescents, Lys656Asn could have an effect over the total content of adiposity in the later life of these individuals (60).

Our results show that certain genotypes are significantly associated with MS and some of its components.

However, all of their p-values are insufficient to remain significant after the application of any methods of multiple comparison corrections. Thus, despite the association of evidence mentioned above in other populations, we did not have evidence of the involvement of genes related to the leptin-melanocortin system in MS features. This could be a reflection of genetic heterogeneity, a common characteristic for complex traits closely related to the ethnic origin of the populations compared, and/or the relation between population size and the minor allele frequency for many of the variants analyzed here.

In summary, our estimation of MS prevalence among obese children/adolescents are similar to those previously described by Burrows et al. in Chile (45), and support the risk of metabolic diseases in adulthood that obese children and adolescents may develop. In addition, there was no evidence of an association between variants at genes related to the leptin-melanocortin system in the present report.

Acknowledgments: The authors wish to thanks Dr. Raquel Burrows and Dr. Cecilia Albala, without their collaboration this study would not have been possible. This study was supported by the National Fund for Science and Technology Development of Chile (FONDECYT) grant 1061096.

Received March 6, 2013; accepted May 14, 2013; previously published online July 2, 2013

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