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Original Article

Association of RBP4 genetic variants with childhood obesity and cardiovascular risk factors

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Background: Recent data suggest that retinol-binding protein 4 (*RBP4*) gene variants could be associated with a risk of obesity and its co-morbidities, such as metabolic syndrome, which increases the risk of developing type 2 diabetes mellitus and cardiovascular disease.
Objectives: The present study examined the potential association of *RBP4* single nucleotide polymorphisms (SNPs) with childhood obesity and its metabolic complications.
Methods: Four *RBP4* SNPs, rs3758538 (3944A>C), rs3758539 (4406G>A), rs12265684 (12177G>C) and rs34571439 (14684T>G), were genotyped in a population of 180 Spanish Caucasian children (97 obese and 83 normal-weight children). Association of *RBP4* SNPs with obesity, metabolic risk factors (blood pressure, triglycerides, high-density lipoprotein cholesterol, insulin resistance) and markers of vascular inflammation, such as high-sensitive C-reactive protein (hs-CRP), was tested.
Results: We found SNP rs3758538 to be associated with obesity ($p = 0.007$). Specifically, each copy of the minor allele C was associated with an increased risk of obesity, by more than twofold, in respect of being homozygous for the major allele A (odds ratio = 2.4; 95% confidence interval = 1.2–4.8). The rs3758538 and rs34571439 *RBP4* SNPs correlated with plasma RBP4 levels. The SNPs rs12265684 and rs34571439 correlated with plasma triglyceride levels. The rs34571439 was also associated to hs-CRP levels. Marginal association of *RBP4* SNPs with plasma high-density lipoprotein levels (rs34571439), blood pressure (rs12265684) and insulin resistance (rs3758539) was also observed.
Conclusions: These findings suggest that childhood obesity may be associated with variations in *RBP4* gene. The presence of selective SNPs in the *RBP4* gene may account for metabolic complications.

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Obesity-associated diseases, formerly considered to be exclusively an 'adult' condition, are now growing in children and can lead to a shortened lifespan. Although the escalating rate of obesity is likely to be a result of changes in human behavior and lifestyle, the importance of genetic factors in the development of co-morbidities, such as type 2 diabetes mellitus, metabolic syndrome or fatty liver disease, among others, is noteworthy (1).

It is well-established that adipokines, biologically active factors secreted by adipose tissue, play an important role in the development of obesity-related complications not only in adults but also in children and adolescents (2). One such adipokine is the retinol-binding protein 4 (RBP4), a member of the lipocalin family of proteins that transport vitamin A from the liver to target tissues. RBP4 is synthesized primarily in the liver and, to a lesser extent, in adipose tissue

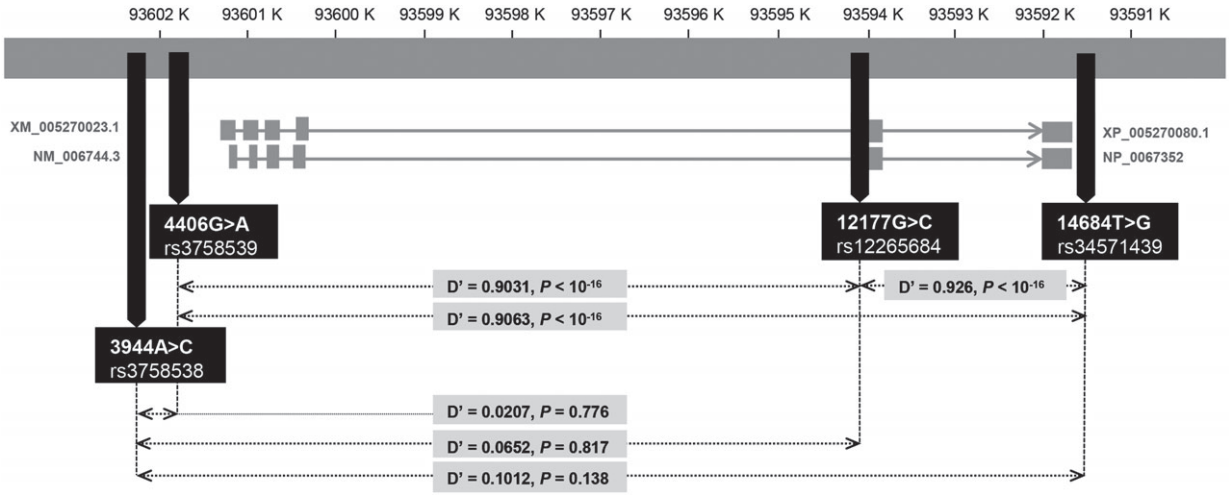

Fig. 1. Linkage disequilibrium between retinol-binding protein 4 single nucleotide polymorphism (*RBP4* SNP) pairs, evaluated via the Lewontin's standardized coefficient D′. Values of D′ ranged between 0 and 1. A value of 0 for D′ indicates that the examined SNPs are independent of each other, whereas a value of 1 demonstrates complete dependency.

and other organs such as kidney and ovaries (3). RBP4 has been correlated with several conditions related to obesity, such as type 2 diabetes mellitus and metabolic syndrome (4, 5). Recently, our group showed that increased plasma RBP4 levels might potentially identify obese children at an increased risk for developing co-morbidities, mainly because of its association with markers of oxidative stress (6).

Given these facts, the *RBP4* gene may represent a susceptibility gene to obesity-related sequelae. In this sense, some studies have reported that common variations in the *RBP4* gene increase the risk of metabolic disease in human. Specifically, rs3758538 (3944A>C) and rs3758539 (4406G>A), both in the promoter 5′ flanking region; the intronic rs12265684 (12177G>C) between Exons 4 and 5; and rs34571439 (14684T>G) located in the 3′ untranslated region of the gene (Fig. 1) have been shown to be linked to insulin resistance (7), type 2 diabetes (8, 9) and several parameters of lipid metabolism (10, 11) in adult populations. However, limited and contradictory data exist regarding the influence of *RBP4* single nucleotide polymorphisms (SNPs) in children and adolescents (12). At this regard, the present study was conducted in children and aimed to determine the association of the four variants previously described of the *RBP4* gene [SNPs rs3758538, rs3758539, rs12265684 and rs34571439 (Table S1, Supporting Information)] with obesity and development of related metabolic complications.

Patients and methods

Study subjects

The study population included 180 Caucasian children (96 females) between 5 and 17 years of age who were consecutively recruited from the outpatient Gastroenterology and Nutrition Clinic of the Department of Pediatrics, Dr. Peset University Hospital. The local Research Ethics Committee approved the study, and informed consent was obtained from all parents and subjects. The body mass index (BMI) z-score, based on age and sex, was calculated according to the WHO Child Growth Standards (13). A total of 97 children presented with obesity (defined as a BMI z-score ≥ 2), and 83 children presented with normal weight. Metabolic risk factors were evaluated. Blood pressure (BP) was measured at the time of consultation in the dominant arm, and the mean arterial pressure {[(2 × diastolic BP) + systolic BP]/3} was considered for analysis. Biochemical metabolic syndrome components (lipid profile, glucose, insulin) were determined by automated methods (Architect C 16000; Abbott Diagnostics, Abbott Park, IL, USA). The Homeostasis model assessment for insulin resistance (HOMA-IR) index was calculated as fasting insulin levels (IU/l) x fasting glucose (mmol/L)/22.5. Plasma RBP4 and high-sensitive C-reactive protein (hs-CRP) levels were measured by immunonephelometry in a Beckman Image 800 analyzer (Beckman Coulter Canada Inc, Mississauga, Canada). Exclusion criteria for the study were the following: non-Caucasian ethnicity, genetic or endocrine disorders and medication use. Subjects were also excluded if there were symptoms or signs of infectious or inflammatory illness, which was confirmed by clinical history, physical examination and laboratory tests. Clinical, anthropometric and analytical data of the included children are shown in Table S2.

Genotyping

The four SNPs of the *RBP4* gene were genotyped in the genomic DNA of all children. Genomic DNA was

extracted from peripheral blood using the QIAamp DNA Blood Mini Kit from Qiagen, (Izasa, Madrid, Spain) according to the manufacturer's protocol. Genotyping of the *RBP4* SNPs was performed by real time PCR, using TaqMan® SNP Genotyping on demand assays, which are commercially supplied by Applied Biosystems (Life Technologies, Carlsbad, CA, USA). The assays were performed according to the manufacturer's instructions. Briefly, each sample reaction was composed of 2.5 μL of TaqMan® Genotyping Master Mix from Applied Biosystems (Life Technologies), 0.12 μL of TaqMan® probe assay, and 2.5 μL of DNA sample at 5 μg/mL. The thermal cycler conditions were as follows: an initial stage of 50°C for 2 min, a second stage of 95°C for 10 min and a third stage consisting of 45 cycles of 95°C for 15 sec and 60°C for 1 min. Thermal cycling and detection were performed in a Fast-Real time PCR system 7900HT from Applied Biosystems (Life Technologies).

Statistical analysis

Statistical tools for genotype and haplotype analysis of SNPs (Hardy–Weinberg equilibrium, linkage disequilibrium, genotype/haplotype distributions and association tests) were provided by SNPStats software, available in http://bioinfo.iconcologia.net/SNPstats/. The Kolmogorov–Smirnov test was used for testing normality, and skewed variables (such as hs-CRP) were log-transformed prior to using parametric tests. All tests were adjusted by age and gender. For association with obesity, an unmatched case (obese)-control (normal weight) design is assumed. The logistic regression analysis is summarized by genotype frequencies, proportions, odds ratio (OR) and 95% confidence interval (CI). For the association tests of quantitative variables, a unique population was assumed and linear regression models were used to assess the proportion of variation in the response explained by the SNPs. Data are presented as the means, standard error of the mean, mean differences with respect to a reference category (the most frequent genotype or haplotype), and 95% CI of the differences. Five genetic models were considered (codominant, dominant, recessive, additive and overdominant). The Akaike information criterion (AIC) and Bayesian Information Criterion (BIC) were used to choose the genetic model that best fit the data. The preferred model is that with the lowest AIC and BIC values. Following Bonferroni correction, p-values of ≤ 0.0125 were considered statistically significant, and those between 0.05 and 0.0125 were regarded as marginally significant. Haplotype association test was performed in cases in which the association was statistically significant for more than one SNP.

Linkage disequilibrium between *RBP4* SNP pairs was evaluated by means of Lewontin's standardized coefficient D′. Values of D′ ranged between 0 and 1. A value of 0 for D′ indicates that the examined SNPs are independent of each other, whereas a value of 1 indicates that they are in complete linkage disequilibrium.

Results

Genotyping

Successful genotyping of the four *RBP4* SNPs was achieved in 100% of the samples. All genotype frequencies were consistent with those expected from Hardy–Weinberg equilibrium. We calculated the linkage disequilibrium for each pair of SNPs. We found that three of four SNPs (rs3758539, rs12265684 and rs34571439) were in strong linkage disequilibrium (D′ statistic values approximately 0.90, $p < 10^{-16}$) and poorly related to the other SNP [the 5′-near gene rs3758538 (Fig. 1)].

Association between RBP4 SNPs with obesity and other metabolic risk factors

We analyzed the genotype effect of the four *RBP4* SNPs on obesity (Table 1) and other variables related to metabolic syndrome (carbohydrate metabolism, lipid profile, BP) (Table 2). We found that SNP rs3758538 was associated with obesity and BMI z-score in an additive model. Specifically, each copy of the C allele was associated with an increased risk of obesity, by more than twofold ($p = 0.007$) and with an increase of 0.81 points in the BMI z-score ($p = 0.022$) in respect of being homozygous for the major allele A. For the other SNPs, although we observed a higher BMI z-score and an increase in the OR in homozygous minor allele carriers with respect to the major allele carriers, such association did not reach statistical significance.

Regarding lipid metabolism, we found three of four SNPs (SNPs rs3758539, rs12265684 and rs34571439) to be associated with triglyceride levels in the dominant model. Individuals carrying the minor allele (homozygous or heterozygous) had increased plasma triglyceride levels with respect to those children who were homozygous for the major allele. The significance was mainly achieved for the intronic SNP rs12265684. Children carrying the minor allele C (GC and CC genotypes) had an increase of 18.5 mg/dL in triglyceride values compared with those children who were homozygous for the major allele G ($p = 0.002$). Significant results were also obtained for SNP rs34571439 ($p = 0.007$), whereas SNP rs3758539 was marginally associated ($p = 0.017$). The non-coding SNP rs34571439 was the only SNP to be marginally associated with plasma high-density lipoprotein cholesterol (HDL-C) levels, under an additive model. We found that each copy of the

Table 1. Association of *RBP4* SNPs with childhood obesity

SNP ID	Genotype	Normal weight n (%)	Obese n (%)	OR (95% CI)	p-value*	Best model†
rs3758538	A/A	70 (84.3)	69 (71.1)	2.4 (1.2–4.8)	**0.007**	Additive
	A/C	13 (15.7)	23 (23.7)			
	C/C	0 (0)	5 (5.2)			
rs3758539	G/G	52 (62.6)	57 (58.8)	2.5 (0.5–13.0)	0.24	Recessive
	G/A	29 (34.9)	34 (35.0)			
	A/A	2 (2.4)	6 (6.2)			
rs12265684	G/G	57 (68.7)	60 (61.9)	4.3 (0.5–37.5)	0.14	Recessive
	G/C	25 (30.1)	32 (33.0)			
	C/C	1 (1.2)	5 (5.2)			
rs34571439	T/T	56 (67.5)	60 (61.9)	5.3 (0.6–45.0)	0.07	Recessive
	T/G	26 (31.3)	31 (32.0)			
	G/G	1 (1.2)	6 (6.2)			

CI, confidence interval; OR, odds ratio; RBP4, retinol-binding protein 4; SNP, single nucleotide polymorphism.
*Following Bonferroni correction, p-values ≤ 0.0125 were considered statistically significant (in bold in the Table), and those between 0.05 and 0.0125 were regarded as marginally significant.
†The Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were calculated to select the best inheritance model for each specific polymorphism. The preferred model is that with the lowest AIC and BIC value. p-values ≤ 0.0125 were considered statistically significant (in bold in the Table).

G minor allele was associated with a decrease of 2.7 mg/dL in HDL-C plasma values ($p = 0.045$) compared with homozygous genotype for the major allele T.

We found a significant (although marginally) association between the SNP rs3758539 and insulin and HOMA-IR values under a recessive model ($p = 0.040$). Children who were homozygous AA had higher insulin levels and HOMA-IR values than carriers of the major allele G (GG and GA genotypes).

With respect to BP, we found that the SNPs rs3758538 and rs12265684 were marginally associated ($p = 0.043$ and $p = 0.038$, respectively) with mean arterial pressure, minor allele carriers having higher values of mean arterial pressure than major allele carriers.

Association between *RBP4* SNPs and plasma RBP4 and hs-CRP protein levels

The four SNPs studied showed association with plasma RBP4 levels (Table 3), but only the SNPs rs3758538 and rs34571439 result in a significant contribution in an additive model to higher RBP4 plasma levels. Specifically, each copy of the minor allele C of SNP rs3758538 produces an increase of 0.5 mg/dL in RBP4 values compared with the levels of carriers of homozygous major allele A. Similarly, in the case of rs34571439 SNP, carriers of the minor allele G (T/G, G/G) had an increase of 0.5 mg/dL of RBP4 plasma values compared with those of the T/T wild-type homozygous subjects. We additionally performed a haplotype association analysis considering these two SNPs (Table S3). The haplotype with the minor allele in the two SNPs (3944C, 14684G), which had a frequency of 3%, is associated with an increase of 1.4 mg/dL in serum RBP4 values compared with the most common haplotype (3944A, 14684T) (95% CI = 0.41–2.36 mg/dL, $p = 0.006$).

We also performed an association analysis between *RBP4* SNPs and the marker of inflammation hs-CRP (Table 3). Linear regression analysis showed the four SNPs to be associated with hs-CRP under a recessive model. Individuals who were homozygous for the minor allele had significantly higher hs-CRP levels (increase of 0.40–0.50 mg/dL) compared with carriers of major allele. However, significant association was only achieved for rs34571439 SNP ($p = 0.011$).

Discussion

There is evidence of association of elevated plasma RBP4 levels with obesity and insulin resistance (14, 15) although there are also other studies that have been unable to establish these associations (16). Recent research supports a relevant role of the *RBP4* gene in childhood and adult obesity (17, 18). However, controversy remains regarding the role of *RBP4* SNPs underlying the risk for obesity and associated co-morbidities (12, 16). In the current study, we sought to investigate the relationship between variation in the *RBP4* gene and obesity and metabolic traits in children. We found the SNP rs3758538 to be correlated with obesity and higher BMI z-score. Carriers of the minor allele C were associated with higher BMI z-scores and a higher risk of obesity, according to an additive model. To our knowledge, this is the first report of an association of this SNP with childhood obesity. The influence of the rs3758538 polymorphism on obesity risk could be ascribed to its influence on modulating *RBP4* expression, with minor allele C associated with a higher *RBP4* expression and higher risk of obesity. Enhanced *RBP4* gene expression may be

Table 2. Association of *RBP4* SNPs with parameters of metabolic risk*

	SNP ID	Genotype	n	Mean (SEM)	Difference (95% CI)†	p-value‡	Best model
BMI z-score	rs3758538	A/A	139	2.01 (0.20)	0.81 (0.12–1.50)	0.022	Additive
		A/C	36	2.69 (0.41)			
		C/C	5	3.59 (0.53)			
	rs3758539	G/G	109	2.04 (0.21)	0.41 (−0.29–1.12)	0.14	Additive
		G/A	63	2.29 (0.31)			
		A/A	8	3.48 (1.16)			
	rs12265684	G/G	117	2.06 (0.21)	0.48 (−0.15–1.10)	0.14	Additive
		G/C	57	2.31 (0.31)			
		C/C	6	3.57 (1.53)			
	rs34571439	T/T	116	2.05 (0.20)	0.45 (−0.16–1.06)	0.14	Additive
		T/G	57	2.31 (0.33)			
		G/G	7	3.52 (1.29)			
Triglycerides (mg/dL)	rs3758538	A/A	139	79.6 (3.5)	−7.3 (−20.7–6.1)	0.29	Dominant
		A/C–C/C	41	72.6 (4.4)			
	rs3758539	G/G	109	72.5 (3.0)	14.1 (2.5–25.6)	0.017	Dominant
		G/A–A/A	71	86.6 (5.6)			
	rs12265684	G/G	117	71.8 (3.0)	18.5 (6.8–30.2)	**0.002**	Dominant
		G/C–C/C	63	89.9 (5.8)			
	rs34571439	T/T	116	72.3 (3.0)	16.2 (4.6–27.9)	**0.007**	Dominant
		T/G–G/G	64	88.6 (5.8)			
HDL-C (mg/dL)	rs3758538	A/A	139	49.7 (0.9)	−2.5 (−6.0–1.1)	0.18	Dominant
		A/C	36	46.9 (1.4)			
		C/C	5	49.8 (5.15)			
	rs3758539	G/G	109	49.8 (0.99)	−1.6 (−4.2–0.9)	0.21	Additive
		G/A	63	48.5 (1.26)			
		A/A	8	45.5 (4.06)			
	rs12265684	G/G	117	50.0 (1.0)	−2.3 (−5–0.5)	0.10	Additive
		G/C	57	48.0 (1.26)			
		C/C	6	44.3 (5.16)			
	rs34571439	T/T	116	50.2 (0.97)	−2.7 (−5.3–−0.1)	0.045	Additive
		T/G	57	47.9 (1.27)			
		G/G	7	43.4 (4.45)			
HOMA-IR	rs3758538	A/A	175	2.8 (0.1)	0.5 (−1.2–2.4)	0.54	Recessive
		A/C–C/C	5	3.3 (0.5)			
	rs3758539	G/G	172	2.7 (0.1)	1.5 (0.1–3.0)	0.040	Recessive
		G/A–A/A	8	4.2 (1.8)			
	rs12265684	G/G	174	2.8 (0.2)	−0.5 (−2.2–1.1)	0.51	Recessive
		G/C–C/C	6	2.2 (0.6)			
	rs34571439	T/T	173	2.8 (0.2)	−0.3 (−1.8–1.2)	0.71	Recessive
		T/G–G/G	7	2.5 (0.5)			
Mean arterial pressure (mm Hg)	rs3758538	A/A	175	82.6 (0.72)	8.8 (0.4–17.3)	0.043	Recessive
		A/C–C/C	5	91.4 (3.1)			
	rs3758539	G/G	109	81.8 (0.90)	2.7 (−0.2–5.5)	0.07	Dominant
		G/A–A/A	71	84.4 (1.2)			
	rs12265684	G/G	117	81.8 (0.8)	2.7 (0.2–5.2)	0.038	Additive
		G/C	57	84.7 (1.4)			
		C/C	6	86.3 (1.9)			
	rs34571439	T/T	173	82.6 (0.7)	4.9 (−2.3–12.2)	0.18	Recessive
		T/G–G/G	7	87.6 (2.0)			

BMI, body mass index; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; RBP4, retinol-binding protein 4; SEM, standard error of the mean; SNP, single nucleotide polymorphism.

*To convert triglycerides to mmol/L multiply by 0.0113; HDL-C by 0.0259.

†Maximum significative difference between means.

‡Following Bonferroni correction, p-values ≤ 0.0125 were considered statistically significant (in bold in the Table), and those between 0.05 and 0.0125 were regarded as marginally significant.

Table 3. Association of *RBP4* SNPs with RBP4 and hs-CRP plasma levels

	SNP ID	Genotype	n	Mean (SEM)	Difference (95% CI)*	p-value†	Best model
RBP4 (mg/dL)	rs3758538	A/A	139	4.25 (0.09)	0.51 (0.17–0.84)	**0.004**	Additive
		A/C	36	4.77 (0.24)			
		C/C	5	5.22 (0.73)			
	rs3758539	G/G	109	4.23 (0.09)	0.38 (0.04–0.73)	0.031	Dominant
		G/A–A/A	71	4.61 (0.17)			
	rs12265684	G/G–C/C	123	4.24 (0.10)	0.44 (0.08–0.80)	0.018	Overdominant
		G/C	57	4.68 (0.17)			
	rs34571439	T/T	116	4.21 (0.10)	0.50 (0.15–0.85)	**0.006**	Dominant
		T/G–G/G	64	4.70 (0.17)			
hs-CRP (mg/L)	rs3758538	A/A–A/C	175	1.84 (0.26)	0.49 (0.04–0.94)	0.033	Recessive
		C/C	5	3.46 (1.18)			
	rs3758539	G/G–G/A	172	1.85 (0.27)	0.42 (0.06–0.77)	0.024	Recessive
		A/A	8	2.56 (0.54)			
	rs12265684	G/G–G/C	174	1.84 (0.27)	0.50 (0.09–0.91)	0.019	Recessive
		C/C	6	3.17 (0.72)			
	rs34571439	T/T–T/G G/G	173	1.83 (0.27)	0.50 (0.12–0.88)	**0.011**	Recessive
			7	3.07 (0.62)			

CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; RBP4, retinol-binding protein 4; SNP, single nucleotide polymorphism.
*Maximum significative difference between means.
†Following Bonferroni correction, p-values ≤ 0.0125 were considered statistically significant (in bold in the Table), and those between 0.05 and 0.0125 were regarded as marginally significant.

linked to adipogenic transcription factors that facilitate the transport and storage of fatty acids (19) affecting the size and recruitment of adipocytes.

However, the mechanism by which RBP4 influences the occurrence of obesity and its co-morbidities remains poorly understood. Previous reports suggested that RBP4 affects glucose metabolism through conferring insulin resistance (20). Subsequent studies have demonstrated a correlation between high plasma RBP4 levels and type 2 diabetes mellitus (21, 22). However, other authors have failed to confirm this finding, and some have even shown an inverse correlation (23). In our work, we found only SNP rs3758539 to be marginally associated with insulin resistance under a recessive model, where individuals carrying two copies of the minor allele A have higher values for HOMA-IR than major allele carriers. Our results agree with those of others who have reported that homozygous carriers of the A allele of this SNP are associated with an increased risk of type 2 diabetes (7, 8, 24), whereas a study conducted in children (12) has failed to find any association with carbohydrate metabolism. This discrepancy may be due to higher HOMA-IR values in children of our study. Therefore, these individuals would be more likely to develop type 2 diabetes later in life.

Regarding lipid metabolism, we found association between three SNPs (rs3758539, rs12265684 and rs34571439) and triglyceride levels. Carriers of one or two copies of the minor allele have higher triglyceride values. There is growing evidence that RBP4 may play a role in the pathophysiology of lipid homeostasis through either hepatic production or catabolism of very low-density lipoproteins (25). Thus, in turn, alleles that increase *RBP4* expression, such as the minor allele G in rs34571439 could increase the susceptibility to dyslipidemia. We have not found association of triglyceride levels with rs3758538 contrary from a previous study in Chinese population (10). In this study, the variant rs3758538 exhibited association with reduced plasma triglyceride levels. The same authors reported a marginal association of C allele of rs3758538 with lower plasma RBP4 values. The reason for the discrepancies with our results is unclear but ethnic differences may be in cause. In addition, we also found SNP rs34571439 to be marginally associated with HDL-C levels, with minor allele G associated to lower levels of HDL-C. Thus, the SNP rs34571439 could provide valuable information about vascular risk associated with atherogenic dyslipidemia, since the minor allele G confers an increased risk of having higher levels of triglycerides and lower levels of HDL-C. It is noteworthy that RBP4 has been recently identified as a HDL-C associated protein (26). It may influence lipid metabolism and contributes to the formation of small HDL-C that are related to cardiovascular risk (27).

An important novel aspect of our study was the finding that homozygous carriers of the minor allele of the rs34571439 variant, that are associated with higher RBP4 plasma levels, is also correlated with higher levels of plasma C-reactive protein values. To our knowledge, this is the first report of an association between genetic variants of the *RBP4* gene and hs-CRP

plasma levels. Several works have found a correlation between baseline elevations of hs-CRP levels and future risk of coronary events (28). Furthermore, there is growing evidence that RBP4 is involved in vascular inflammation and risk of cardiovascular disease (29). Recently, serum levels of RBP4 have also been associated with the presence and severity of coronary artery disease (30). It seems that increased RBP4 expression might directly contribute to the progression of endothelial/vascular inflammation via activation of NADPH oxidase and NF-κB pathways (31).

Taken together, these initial findings support a role for *RBP4* genomic variance to the risk of childhood obesity and its metabolic complications. This influence appears to be based on the effects of the polymorphisms on plasma RBP4 levels. However, we are aware of several limitations of our study. It should be noted that the small sample size of our series does not allow to draw firm conclusions. Furthermore, some associations observed in the present study are marginal and require further validation in a larger series. By contrast, we have used the Bonferroni correction that is highly restrictive, and the study in pediatric patients, in the early stages of metabolic syndrome, offers the possibility to assess potential associations without confounders habitually present in adults. Genetic studies in a larger and independent pediatric cohort are needed. Thus, the relationship between obesity, adipose *RBP4* expression, circulating levels of RBP4, dislipidemia and insulin resistance needs to be evaluated in future studies.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of the genotyped retinol-binding protein 4 single nucleotide polymorphisms (*RBP4* SNPs)

Table S2. Clinical and biochemical parameters of children included in the study

Table S3. Haplotype association (rs3758538 and rs34571439) with retinol-binding protein 4 (RBP4) levels

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