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ORIGINAL ARTICLE

Metabolic syndrome and related variables, insulin resistance, leptin levels, and *PPAR-γ2* and leptin gene polymorphisms in a pedigree of subjects with bipolar disorder

Trino Baptista,[1] Ignacio Sandia,[2] Erika Fernandez,[3] Ligia Balzán,[3] Lissette Connell,[3] Euderruh Uzcátegui,[2] Ana Serrano,[2] Albis Pabón,[2] Félix Angeles,[2] Yarira Araque,[2] Heidy Delgado,[2] Alexy González,[2] Yonathan Alviarez,[2] Jose Piñero,[2] Enma A. de Baptista[4]

[1]*Department of Physiology, Los Andes University Medical School, Mérida, Venezuela.* [2]*Department of Psychiatry, Los Andes University Medical School, Mérida, Venezuela.* [3]*Institute of Clinical Research Dr. Américo Negrette, Zulia University Medical School, Maracaibo, Venezuela.* [4]*Department of Microbiology, Los Andes University Pharmacy School, Mérida, Venezuela.*

Objective: Evidence points to a high prevalence of metabolic dysfunction in bipolar disorder (BD), but few studies have evaluated the relatives of subjects with BD. We conducted a cross-sectional study in an extended family of patients with BD type I.
Methods: The available relatives of the same family were interviewed (DSM-IV-R) and assessed in fasting conditions for body mass index, constituent variables of the metabolic syndrome (MS), leptin levels, insulin resistance index, and single nucleotide polymorphisms (SNPs) for the leptin receptor and promoter and *PPAR-γ2* genes. The frequency of MS was compared with that recorded in the local general population.
Results: Ninety-three relatives of three adults with BD were evaluated (30 aged < 18 years, 63 aged > 18 years). The frequency of MS was similar to that of the general population. Significantly higher frequencies of abnormal glucose, total and low density cholesterol (LDL-c) levels (all $p < 0.05$), waist circumference ($p = 0.057$), and leptin and insulin resistance values (in adults only) were observed in the family. Adults with the QQ genotype of the leptin receptor displayed higher LDL-c levels than carriers of the R allele.
Conclusions: The associations among BD consanguinity, familial hypercholesterolemia, and leptin receptor SNPs reported herein should be replicated and extended in other pedigrees.

Keywords: Bipolar disorder; familiar hypercholesterolemia; insulin sensitivity; leptin; metabolic syndrome

Introduction

Convergent evidence points to a high frequency of physical ailments in subjects with bipolar disorders (BD). These include metabolic syndrome (MS), migraine, type 2 diabetes mellitus, thyroid disorders, chronic kidney disease, cancer, chronic pain, and obstructive airway disease, among many others.[1-8] Particular emphasis has been placed on characterizing the elevated frequency of MS among patients with BD[4,9] and the postulated role of psychotropic medication in its development and maintenance.[6,7,10,11]

Even though a genetically-based predisposition to metabolic dysfunction in BD has been consistently suggested,[12] few studies have evaluated metabolic status in relatives of patients with BD. Such studies might help clarify the role of environmental and genetic factors in the development of MS and related disorders in subjects with BD. In fact, a large study of a multigenerational kindred (508 relatives) including members with BD or schizophrenia in Quebec, Canada, showed obesity to be 2.5 times more frequent in antipsychotic-treated patients (30%) than in untreated family members (12%).[13] This finding suggests that medication plays a role in the development of obesity. By contrast, in a large epidemiological study comprising data from the general population (GP), the authors reported that drug-free BD relatives showed the highest frequency of MS, obesity, and abnormal glucose and diastolic blood pressure (BP) levels among several comparison groups, including drug-treated patients and their relatives.[7] In another study comprising subjects with schizophrenia or schizoaffective disorders and their first-degree relatives, the authors reported that, among the drug-treated groups, those receiving olanzapine displayed the highest blood levels of

Correspondence: Trino Baptista, Department of Physiology, Los Andes University Medical School, Tulio Febres Cordero Ave., n° 4-27, 5101-A, Mérida, Venezuela.
E-mail: trinbap@yahoo.com
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plasminogen activator inhibitor (PAI-1) and leptin, but levels were similar to those observed in their relatives after controlling for body mass index (BMI).[14]

These results indicate that variables other than medication play a role in the development of metabolic dysfunction in patients with BD and their relatives, such as shared environmental and/or genetic factors.

In the present article, we report a higher frequency of metabolic abnormalities in a single pedigree with several BD members compared to the local GP. In the same family, we also assessed potential associations between metabolic profile and the following single nucleotide polymorphisms (SNPs) that are involved in metabolism regulation: LEP2548/GA for the leptin promoter, LEPR Q223R for the leptin receptor, and Pro12/Ala for the peroxisome proliferator-activated receptor gamma (*PPAR-γ2*).

Methods

Subjects

The study was conducted in an extended rural family in Mérida, Venezuela, three members of which are being treated for BD type I by the first author (TB). The roots of this family were traced to a single man who died 9 years ago at the age of 80. All available blood relatives who lived in a circumscribed area (Bailadores) were contacted, and all provided written informed consent for voluntary participation. In subjects aged $<$ 18 years, the consent form was signed by one of their parents. All the evaluations were performed in a single visit.

The degree of consanguinity of each subject with the BP patients was defined as follows: first-degree relatives (parents, offspring, siblings), second-degree relatives (grandparent, grandchild, uncle, aunt, nephew, niece, half-sibling), or third-degree relatives (first cousin).[15]

The comparison groups were independent randomized samples of adults and child/adolescent subjects drawn from the local GP of Mérida. These data are published elsewhere.[7,16]

Procedure

The family consisted of 63 adults (aged $\geqslant$ 18 years), not including the subjects with BD, and 30 children/adolescents (aged $<$ 18 years). The GP samples consisted of 270 adults and 370 children/adolescents. All subjects were evaluated in fasting conditions. From each subject, a blood sample was obtained from cubital veins and was immediately centrifuged and stored at -4°C until analysis.

Waist circumference (WC), body weight, and height were measured with subjects wearing light clothing. The BMI was calculated as follows: weight (kg)/height $(m)^2$. Obesity was defined as a BMI $\geqslant$ 30 kg/m^2 in adults and as values above the 97th percentile of the BMI distribution in the GP for children/adolescents.[16] BP was calculated as the average of three measures, obtained with the subject in standing, sitting, and supine positions.

MS was diagnosed in adults according to the criteria of the Latin American Diabetes Association,[17] as follows: a) abdominal obesity (waist circumference $\geqslant$ 88 cm in women or $\geqslant$ 94 cm in men), plus at least two of the following criteria: b) triglycerides: $>$ 150 mg/dL or under specific treatment; c) high density lipoprotein cholesterol (HDL-c): $<$ 50 mg/dL in women or $<$ 40 mg/dL in men or under specific treatment; d) elevated BP: systolic $\geqslant$ 130 mm/Hg, diastolic $\geqslant$ 85 mm/Hg, or under specific treatment; and e) glucose levels: $\geqslant$ 100 mg/dL or under specific treatment.

In children/adolescents, MS was diagnosed following a modified version of the Cook et al. criteria if three or more of the following were positive: WC and BP $>$ 90th percentile; glucose $>$ 100 mg/dL; triglycerides $>$ 110 mg/dL; and HDL-c $<$ 40 mg/dL.[16]

A detailed interview was conducted to obtain demographic data, mental and physical health history, and determine degree of consanguinity with the BD subjects. Psychiatric diagnosis was performed with the Structured Interview for the DSM-IV[18] in adults and with the Kiddie-SADS-PL in children/adolescents.[19]

Chemical analysis

Glucose and lipid levels were measured using an enzymatic method (Humans, Germany). Insulin (detection limit: 1.76 μIU/mL) and leptin (detection limit: 1.0 ng/mL) were assessed by ELISA with commercial kits (DRG, Germany). The insulin resistance index (HOMA-IR) was calculated as follows: glucose (mmol/L) $\times$ insulin (μIU/mL)/22.5.

Genotyping

Genomic DNA was isolated from peripheral leukocytes.[20,21] Genotyping was conducted by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR).[22] We used the 2548G/A and Q223R nomenclature for the leptin promoter and receptor genes, respectively. The GG and QQ are the "wild-type" homozygous genotypes, whereas the (GA/AA) and (QR/RR) variants are the heterozygous and homozygous genotypes. The nomenclature for the *PPAR-γ2* gene polymorphism is Pro12/Ala; PRO/PRO is the "wild type" homozygous genotype, and the (PRO/ALA-ALA/ALA) variants are the heterozygous and homozygous genotypes respectively.

Statistical analysis

SPSS version 17.0 was used for all analyses. Data normality was estimated with the one-sample Kolmogorov-Smirnov test and Levene's test was used to assess the equality of variances. Categorical (MS, abnormal values of its constituting variables, and association with specific genotypes) and continuous data were analyzed with binary logistic regression and covariate analysis respectively, with age and BMI as covariates. Results were considered significant when $p \leqslant 0.05$.

Results

Demographic and clinical features

The subjects with BD were three sisters aged 43, 46, and 63 years. Most family members were their second-degree relatives, and they had no first-degree relatives among the child/adolescent sample (Tables 1 and 2).

The adult family members were younger than those in the GP sample. Females were more numerous in both groups (Table 1). The child/adolescent family members were numerically older than those of the GP, but the gender distribution was similar (Table 2). There were more African American subjects in the adult GP (Table 1). Ethnicity was not reported in the study of GP children/adolescents (Table 2).

The axis I diagnosis distribution (DSM-IV) in the family was as follows for adults: no diagnosis, n=40 (63.5%); mood disorders (excluding BD), n=9 (14.3%); anxiety disorders, n=10 (15.9%); others, n=4 (6.3%). In children/adolescents, the distribution of diagnoses was: no axis I diagnosis, n=27 (90%); attention deficit disorder, n=2 (6.7%); and alcohol abuse, n=1 (3.3%).

Metabolic syndrome and its constituting variables

The frequency of MS and obesity recorded in the adult members of the tested family was similar to that observed in the GP, but a significantly higher frequency of abnormal blood glucose, total and low-density lipoprotein cholesterol (LDL-c) levels, and WC (marginally significant) was recorded in the family adults ($p < 0.05$). By contrast, a significantly lower frequency of abnormal triglycerides, HDL-c, and BP levels was observed in the family adults (Table 3).

The frequency of abnormal LDL-c levels recorded in the family's children/adolescents was significantly higher than that observed in their GP counterparts. By contrast, the frequency of abnormal triglyceride and HDL-c levels was significantly lower than that recorded in the GP (Table 3).

Table 1 Sociodemographic features of adult subjects

Variable	Family (n=63)	General population (n=274)
Gender*		
Female	49 (77.8)	175 (63.9)
Male	14 (22.2)	99 (36.1)
Ethnicity†		
Mixed	18 (28.6)	15 (5.5)
AA	0.0 (0.0)	67 (24.5)
White	45 (71.4)	134 (48.9)
Native	0.0 (0.0)	58 (21.2)
Age (years), mean ± SD‡		
Female	38.7±13.7	41.3±15.3
Male	31.1±16.0	44.0±15.3
Degree of consanguinity with the BD subjects		
First degree	13 (20.6)	-
Second degree	39 (61.9)	-
Third degree	11 (17.5)	-

Data expressed as n (%), unless otherwise specified.
AA = African American; BD = bipolar disorder; SD = standard deviation.
* $\chi^2_{(1)} = 4.4$, $p = 0.03$; † $\chi^2_{(3)} = 61.5$, $p = 0.000$; ‡ $F_{(3)} = 3.7$, $p = 0.01$.

Table 2 Sociodemographic features of child/adolescent subjects

Variable	Family (n=30)	General population (n=370)
Gender*		
Female	15 (50.0)	177 (47.8)
Male	15 (50.0)	193 (52.2)
Ethnicity		
Mixed	11 (36.7)	-
AA	-	-
White	19 (63.3)	-
Native	-	-
Age (years), mean ± SD		
Female family members	11.9±3.7	
Male family members	11.3±3.6	
General population (males and females)		7.82±0.62
Degree of consanguinity with the BD subjects		
First degree	-	-
Second degree	22 (73.3)	-
Third degree	8 (26.7)	-

Data expressed as n (%), unless otherwise specified.
AA = African American; BD = bipolar disorder; SD = standard deviation.
* $\chi^2_{(1)} = 0.001$, $p = 0.9$.

Insulin resistance and leptin levels

Adults

The HOMA-IR was calculated in all subjects in both groups. The family members displayed significantly higher values than the GP did (Table 4).

Leptin was measured in all family members, but in only 12 males and 32 females of the GP subjects, since the latter was a probabilistic sample. Leptin levels were significantly higher among females in the adult family group (Table 4).

Children/adolescents

There are no normative data for the HOMA-IR and leptin levels in the Venezuelan child/adolescent GP. Thus, family data are presented in a purely descriptive manner (Table 4).

Regarding HOMA-IR values, besides the mean ± SEM presented in Table 4, we compared each individual value with the average reported in a local GP study and discriminated by BMI.[23] By considering 1 SD above or below the mean as abnormal, five subjects (16.7%) in the young family group were above this cutoff point. Interestingly, three of these individuals had normal or low BMI (data not shown).

Genetic analysis

This analysis was conducted in 90 family members only. There were no adults with the RR genotype of the leptin

Table 3 Frequency (percentage) of metabolic syndrome and abnormal values of its constituting variables

Adults	Family (n=63)	General population (n=274)	p-value*
Metabolic syndrome	19.0 (9.3-28.7)	27.4 (24.7-30.1)	0.4
Obesity	27 (16-38)	24.2 (19.1-29.3)	0.4
Waist circumference	53.2 (40.9-65.5)	41.2 (38.2-44.2)	0.057
Abnormal blood glucose (mg/dL)	27 (16-38)	9.1 (7.4-10.8)	0.000
Triglycerides (mg/dL)	6.3 (0.3-12.3)	39.1 (33.5-44.9)	0.000
HDL-c (mg/dL)	41.3 (29.1-53.5)	84.7 (80.4-88.9)	0.000
Blood pressure (mmHg)	6.3 (0.3-12.3)	32.1 (26.6-37.6)	0.001
Total cholesterol (mg/dL)	54 (41.7-66.3)	9.1 (5.7-13.5)	0.000
LDL-c (mg/dL)	85.7 (77.1-94.3)	21.9 (17.0-26.8)	0.000
Children/adolescents	**Family (n=30)**	**General population (n=370)**	**p-value***
Metabolic syndrome	3.3 (-3.6-9.7)	4.6 (2.5-6.7)	0.7
Obesity	13.3 (1.2-25.4)	9.7 (6.7-12.7)	0.9
Waist circumference	13.3 (1.0-25.6)	10 (7.0-13.0)	0.7
Abnormal blood glucose (mg/dL)	13.3 (1.0-25.6)	6.5 (4.0-9.0)	0.3
Triglycerides (mg/dL)	-	12.7 (9.3-16.1)	0.04
HDL-c (mg/dL)	10 (-0.3-20.7)	41.4 (36.4-46.4)	0.001
Blood pressure (mmHg)	10 (-0.3-20.7)	7.8 (5.1-10.5)	0.9
Total cholesterol (mg/dL)	30 (13.6-46.4)	-	-
LDL-c (mg/dL)	40 (22.5-57.5)	10.8 (7.6-14.0)	0.0001

Data expressed as percentage (95% confidence interval).
HDL-c = high-density lipoprotein cholesterol. LDL-c = low-density lipoprotein cholesterol.
* Probability on binary regression analysis, with age and body mass index as covariates.

receptor and no children/adolescents with the ALA/ALA genotype of the *PPAR-γ2* gene. The genotype distribution for the whole sample was as follows. For the *PPAR-γ2* gene: PRO/PRO = 58.9%; PRO/ALA = 38.9%; ALA/ALA = 2.2%; for the leptin receptor: QQ = 68.9%; QR = 28.9%; RR = 2.2%; for the leptin promoter: GG = 51.1%; GA = 43.3%; AA = 5.6%. None of these rates deviated from Hardy-Weinberg equilibrium ($p > 0.05$).

Adults

Subjects with the QR genotype of the leptin receptor gene displayed higher HOMA-IR values and a lower frequency of abnormal LDL-c than those carrying the QQ genotype ($p < 0.05$, Table 5).

Children/adolescents

Subjects with the wild-type genotype of the *PPAR-γ2* gene (PRO/PRO) displayed higher systolic and diastolic BP values than carriers of the PRO/ALA genotype ($p < 0.05$, Table 5). Similarly, those subjects with the wild-type genotype of the leptin receptor (QQ) showed a higher frequency of abnormal WC than those of the (QR/RR) group ($p < 0.05$, Table 5).

Influence of the degree of consanguinity

The relevance of this analysis is hindered by the small sample size, particularly when considering first-degree relatives. The second-degree relatives were significantly younger than the other groups (first-degree group, 52.2±13.5 years; second-degree group, 23.5±13.2 years; third-degree group, 30.0±16.9 years, $f_{(2)} = 22.1$, $p = 0.000$). Most differences among the three consanguinity groups were nonsignificant when age was entered as a covariate, either when considering absolute values or the proportion of abnormal levels.

The only significant findings were a lower proportion of abnormal WC and HDL-c values and lower absolute diastolic BP values in the second-degree group when compared with the other two groups ($p < 0.05$, data not shown).

Table 4 Insulin resistance index and leptin levels

	Family	General population	p-value
Adults*			
Insulin resistance index (HOMA-IR)	2.4±0.2	1.6±0.1	0.004
Leptin (females) (ng/mL)	22.1±1.4	17.9±1.7	0.036
Leptin (males) (ng/mL)	7.5±1.8	7.8±1.8	0.9
Children/adolescents†			
Insulin resistance index (HOMA-IR)	2.2±1.8	-	-
Leptin (females) (ng/mL)	13.1±12.7	-	-
Leptin (males) (ng/mL)	5.9±8.4	-	-

Data expressed as mean ± standard error.
HOMA-IR = insulin resistance index.
* p-value associated probability, general linear model with age and body mass index as covariates.
† HOMA-IR, n=29; leptin: females, n=15; males, n=13.

Table 5 Selective description of the association between SNPs and metabolic dysfunction

Polymorphism	Variable	Genotype		Statistics
Adults				
Leptin receptor Q223R	HOMA-IR	QQ 1.7 ± 0.5	QR 5.1 ± 1.0	$F_{(1)} = 8.3$, p = 0.006
Leptin receptor Q223R	LDL-c*	QQ 93.5 (87.4-99.6)	QR 71.4 (60.2-82.6)	$Wald_{(1)} = 4.2$, p = 0.03
Children/adolescents				
PPARγ2	Systolic BP	PRO/PRO 98.4 ± 2.6	PRO/ALA 89.1 ± 3.5	$F_{(1)} = 4.5$, p = 0.04
PPARγ2	Diastolic BP	PRO/PRO 64.9 ± 2.4	PRO/ALA 55.2 ± 3.1	$F_{(1)} = 5.9$, p = 0.02
Leptin receptor Q223R	WC*	QQ 70.1 ± 1.7	(QR + RR) 64.4 ± 1.8	$F_{(1)} = 5.4$, p = 0.02

Values expressed as mean ± SEM, unless otherwise specified. Statistics: univariate general linear model with age and body mass index as covariates.
BP = blood pressure; HOMA-IR = insulin resistance index; LDL-c = low density cholesterol; WC = waist circumference.
* Values expressed as percentage of subjects (95% confidence interval) with abnormal values. Statistics: binary logistic regression analysis with age and body mass index as covariates.

Discussion

In this study, we evaluated a single multigenerational pedigree with several members diagnosed with BD type I, instead of the most frequently used approach, which consists of assessing relatives in samples of unrelated BD patients. Since such studies can assess large numbers of genetically-related subjects, they may be particularly suitable for detection of putative markers of the disease.

A MEDLINE search conducted in May 2014 by the first author identified 297 family genetic studies of BD carried out since 1967; however, most were designed to explore the genetic basis of the psychiatric disorder itself and/or its related clinical features, but not its metabolic regulation. Hence, in this pioneering study, we expected to detect metabolic patterns and genotype distributions in relevant genes that could generate hypotheses to support further comparative studies.

Metabolic profile

This study showed that a significant proportion of relatives of subjects with BD displayed metabolic abnormalities. The frequency of abnormal blood glucose, total and LDL-c levels, WC (only marginally significant), insulin resistance index, and leptin levels (in women) were higher in the adult relatives of the subjects with BP than in the GP. These high leptin levels suggest an elevated rate of insulin resistance in the family.[24]

In the children/adolescents group, the frequency of abnormal LDL-c was also significantly higher than that recorded in the GP. Furthermore, 13% of the children/adolescent group exhibited abnormal fasting blood glucose levels (vs. 6.5% of the GP, Table 3), and 16.7% of them presented abnormal HOMA-IR. Conversely, the frequency of abnormal triglycerides, HDL-c, and BP (in adults only) was lower in the BD relatives than in the GP.

The lipid profile strongly suggests a mild familiar hypercholesterolemia (FH),[25] because 85.7% of adults and 40% of children/adolescents had abnormal LDL-c levels. This is an autosomal dominant disorder linked to abnormal LDL receptor functioning. More than 700 meaningful mutations of this receptor have been identified. This underlies the heterogeneity of the clinical picture, which is severe in homozygotes and mild in heterozygotes. The homozygote and heterozygote types have an estimated prevalence of 1 in 500 and 1 in 1 million respectively.[25] Elevated triglyceride and glucose levels and BP dysregulation – which are the other components of the MS – are not primarily linked to FH.[25]

Hence, it appears that this family carries simultaneously mild FH and some components of the MS, mainly carbohydrate dysregulation, expressed as abnormal fasting blood glucose levels and HOMA-IR indices. The MS is frequently observed in subjects with BD,[1-4] but the association between FH and BD has so far not been explored.

Numerous studies have analyzed the lipid profile of subjects with BD. A consistent finding is the high frequency of abnormal triglyceride and low HDL-c levels, as defined in the MS criteria.[1-4,7,8] As a matter of fact, this profile, along with abnormal WC and insulin levels, accounts for most of the metabolic differences observed between psychiatric and internal medicine patients.[26] Total and LDL-c levels are not formally included in the MS criteria and are often unreported, but they are among the strongest predictors of atherosclerosis and cardiovascular diseases.[27]

In a context different from BD, a negative correlation between blood cholesterol levels and impulsive, violent, and suicidal behaviors has been extensively explored, with conflicting results.[28,29] Some studies have also included low triglyceride levels in that pathogenic model.[30] Finally, low cholesterol levels have been reported during the manic state[31-33] and have been suggested as a trait rather than a state marker of the manic phase of BD.[33]

In short, in patients with BD, high cholesterol levels are linked to cardiovascular risk, whereas low levels are related to psychopathological traits. However, this is the

first report of FH in a BD pedigree. As stated above, this metabolic disorder differs from the MS because it does not primarily involve abnormal glucose, HDL-c, triglyceride, or BP values. The remaining discussion about our genetic findings will focus on their relationship with total and LDL-c levels.

Genetic analysis

Since our research group has studied the metabolic risk associated with SNPs in the leptin promoter/receptor and *PPAR-γ2* genes in Venezuelan GP and psychiatric patients before,[34-36] we explored this potential association in the family under study.

PPAR-γ2 gene

The ALA substitution of the *PPAR-γ2* gene (genotypes PRO/ALA and ALA/ALA) has been associated with a favorable metabolic profile in non-psychiatric populations, such as lower triglyceride levels and BP and higher HDL-c levels than the wild-type genotype (PRO/PRO).[34,37] There was only one significant result in our sample, and it is consistent with the above-mentioned findings: the child/adolescent population with the PRO/ALA genotype had lower BP values than those recorded in the PRO/PRO group.

No published studies have explored the association between *PPAR-γ2* polymorphisms and BP in children/adolescents. Jermendy et al.[38] reported lower post-challenge glucose and insulin values in obese children carrying the ALA allele, but no difference in BP was observed. Interestingly, when we discriminated between normal and abnormal BMI (according to the 97th percentile of the Venezuelan child/adolescent GP), a protective effect of the ALA allele was observed only in those subjects with normal BMI, for both systolic ($p = 0.01$) and diastolic ($p = 0.007$) BP. In any case, no particular *PPAR-γ2* SNP was associated with the high total and LDL-c levels reported in this pedigree.

Leptin promoter (2548G/A)

Early studies reported a positive association between excessive weight gain and SNPs for this gene,[39] but numerous contradictory reports that point to ethnicity and drug treatment as significant confounding variables were later published.[35] Similar conflicting results have been reported regarding the MS and its constituting variables.[40,41]

We did not find any significant association between the various 2548G/A SNPs and any of the metabolic variables under study.

Leptin receptor (Q223R)

Early reports showed that R allele carriers were less obese than non-carriers (reviewed elsewhere[35]). This association was confirmed here in children/adolescents when considering WC values.

The adult R allele carriers in the BD family displayed significantly lower LDL-c levels than the non-carriers. Hence, hypercholesterolemia in this pedigree appears to be associated with the wild-type genotype (QQ). This finding has not been previously reported.

In conclusion, the extended family of three subjects with BD exhibited higher total and LDL cholesterol levels, insulin resistance, leptin levels (in women only), abdominal obesity, and abnormal blood glucose levels than the GP. Most genetic-metabolic associations agreed with those described in drug-free subjects. These results confirm that relatives of patients with BD are also at high risk of metabolic dysfunction.

Also, we did not find any published study about the association between BD and familial hypercholesterolemia. This finding, along with its association with the QQ genotype of the leptin receptor, could thus be specific of this particular family. For this and other reasons, the present study should be replicated in different geographical locations and ethnic groups.

Finally, the small sample size made it difficult to analyze separate genotypes and the influence of the degree of consanguinity. Furthermore, approximately 25% of the extended family could not be contacted. Therefore, these results should be considered preliminary.

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Disclosure

The authors report no conflicts of interest.

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