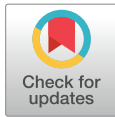




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ORIGINAL ARTICLE

## Association Between the Brain-derived Neurotrophic Factor Val66Met Polymorphism and Overweight/Obesity in Pediatric Population

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**Background.** The brain-derived neurotrophic factor (BDNF) rs6265 (G196A; Val66Met) single nucleotide polymorphism has been associated with BMI and obesity in distinct populations, both adult and pediatric, with contradictory results involving either Val or Met as the risk variant.

**Aim of the Study.** To determine the association between the BDNF Val66Met polymorphism and BMI in Mexican children and adolescents.

**Methods.** BDNF Val66Met genotyping by restriction fragment length polymorphism and nutritional status characterized by their BMI-for-age z-scores (BAZ) from pediatric volunteers ( $n = 498$ ) were analyzed by Fisher's exact test association analysis. Standardized residuals ( $R$ ) were used to determine which genotype/allele had the major influence on the significant Fisher's exact test statistic. Odds ratios were analyzed to measure the association between genotype and normal weight ( $\geq -2$  SD  $< +1$  SD) and overweight ( $\geq +1$  SD, including obesity, Ow + Ob) status with 95% confidence intervals to estimate the precision of the effect as well as 95% credible intervals to obtain the most probable estimate.

**Results.** Comparisons between GG (Val/Val), GA (Val/Met) and AA (Met/Met) genotypes or Met homozygotes vs. Val carriers (combination of GG and GA genotypes) showed significant differences ( $p = 0.034$  and  $p = 0.037$ , respectively) between normal weight and the combined overweight and obese pediatric subjects. Our data showed that children/adolescents homozygous for the A allele have increased risk of overweight compared to the Val carriers (Bayes OR = 4.2, 95% CI\*\*[1.09–33.1]).

**Conclusion.** This is the first study showing the significant association between the BDNF rs6265 AA (Met/Met) genotype and overweight/obesity in Mexican pediatric population. © 2018 IMSS. Published by Elsevier Inc.

**Key Words:** BDNF, BMI z-score, Body weight, Overweight, Obesity.

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## Introduction

### *Obesity Epidemic*

Obesity is a global increasing epidemic for both children (1) and adults (2) that compromises human well being. The most critical comorbidities related to adipose tissue excess range from rheumatological conditions to type 2 diabetes mellitus, cardiovascular disease, and increased risk of cancer (3). According to the World Health Organization (WHO) (4), overweight and obesity currently affects 1.9 billion adults and 41 million children under the age of five all around the world, accounting as the fifth leading risk for global deaths with at least 2.8 million adults dying each year as a result of these conditions. Up to the past decade, developing countries such as Mexico, China and Thailand have had the most dramatic increase in obesity (5). Recently, it was published that Mexico has the second prevalence of obesity in the adult population with 22 million obese (30%) in addition to the 26 million adults with overweight, while ranking fourth in children (6). The most recent results from the Mexican health and nutritional survey (ENSANUT, 2012) indicate an overall overweight (Ow) or obesity (Ob) prevalence of 28.8% in children <19 years of age (7,8). In the last 24 years, the highest prevalence was observed among children and adolescents living in urban areas and those from the highest socioeconomic level, while the rate of increase was higher in the lowest socioeconomic status (8).

In general, it is assumed that obesity results from a combination of genetic susceptibility, increased availability and consumption of high-energy foods as well as a decreased requirement and performance of physical activity as a consequence of modern life styles (9). Obesity is a complex condition determined by an intricate interplay of genetic and environmental factors (10). Genetic variants are estimated to account for a range between 40–70% of the heritability of BMI (11,12), including single mutations as well as single nucleotide polymorphisms (SNPs) causing from severe impairment in appetite regulation and early-onset overweight to slightly increased BMI or early-onset obesity (11).

### *BDNF and Obesity*

As for genetic susceptibility, known single-gene mutations (13,14) or syndromes (15) may explain only a small fraction (~5%) of childhood-onset obesity. However, as mentioned previously, obesity can mainly be the result of the imbalance between caloric intake and energy expenditure, so by studying the genes involved in appetite regulation we will be able to unravel the essential molecular network involved in obesity.

One such molecule that has been associated with body weight regulation is the brain-derived neurotrophic factor (BDNF). BDNF is a member of the neurotrophin family of small secreted proteins with major roles in central nervous system (CNS) development. Current data from

Ensembl shows that BDNF is located at locus 11p14.1, extends over approximately 67 kb, contains 12 exons with 9 functional promoters for tissue and brain-region specificity and originates 19 transcripts by alternative splicing (16). Information about the pro-BDNF proteolytic processing, mature BDNF and its receptors p75<sup>NTR</sup> and Trkb, respectively, has been thoroughly reviewed elsewhere (17).

Although it is widely expressed among several tissues (18), BDNF is abundant in the CNS (19,20), predominantly in the hippocampus, amygdala, cerebral cortex, and hypothalamus (21–23). BDNF plays a critical role in nervous system development and function (24,25), and particularly, exerts an anorexigenic function in the brain (26). BDNF molecular alterations have been implicated in conditions affecting body weight such as eating disorders (27,28). One of these variations affecting BDNF is the Val66Met single nucleotide polymorphism (G196A; SNP rs6265). In particular, the 66Met (A variant) allele is biologically relevant as it alters the intracellular processing, trafficking and activity-dependent secretion of BDNF (29,30), and has been associated with several clinical traits such as early seizures, bipolar affective disorders, obsessive-compulsive disorders, eating disorders, BMI, and obesity (17).

As with adults (31), studies involving children and adolescents attempting to examine the association between the BDNF rs6265 polymorphism and age-and-sex specific nutritional status characterized by their BMI-for-age z-scores (BAZ) have shown contradictory results. Some of them have found association between this SNP and childhood BAZ at the upper tail of the BMI distribution in children with European ancestry (32), as well as for BMI and obesity in Chinese (33–35), European American (36), and Croatian (37) children; while others reported no association with BMI in Spanish (38), with BAZ in Mexican children (39), and with extreme obesity in German children and adolescents (40).

The BDNF 66Met (A allele) presents greater plausibility of being associated with BMI increase and overweight/obesity, as it is a functional variant that generates subcellular translocation and activity-dependent secretion deficiencies of BDNF which could resemble the BDNF deficiencies associated with obesity (41,42). However, several articles have pointed to the Val66 allele (G variant) as the risk allele associated with BMI or obesity risk (33–35,43), while others point to the Met66 allele (A variant) (37,44,45), and even to the heterozygous genotype AG (37,46). In example, it has been observed in German children that 66Met carriers, although associated with lower BMI, had an increased calorie intake and reported higher carbohydrates and proteins consumption (47), while in Chinese children carriers of the A allele are at increased risk of obesity when moderate to low physical activity levels are reported (45).

At present, association studies involving BDNF rs6265 and BMI in children and adolescents are still scarce and conflicting. Therefore, the aim of this study was to analyze the relationship and determine the association between the

BDNF Val66Met polymorphism and nutritional status characterized by their BMI-for-age z-scores (BAZ) in Mexican pediatric subjects.

## Material and Methods

### Subject Recruitment and Sample Collection

Samples were obtained from Mexican pediatric volunteers ( $n = 498$ ), 282 girls (56.6%) and 216 boys (43.4%) between 5–17 years old (312 overweight children and 186 normal weight controls) without any metabolic condition reported. This study was performed at Unidad de Investigación Médica en Genética Humana (UIMGH), Hospital de Pediatría, at Centro Médico Nacional Siglo XXI from Instituto Mexicano del Seguro Social (CMN Siglo XXI, IMSS). Our protocol was reviewed and approved by the Ethical Committee of IMSS and assigned with the registry number R-2009-3603-9; both children and parents provided written informed consent for participation in the study before any study-related procedures were performed.

### Biological Parameters

Nutritional status categories for children and teenagers were diagnosed by calculating both their individual BMI as  $\text{weight}(\text{kg})/\text{height}^2(\text{m}^2)$  as well as their BMI-for-age z-scores for either girls or boys (BAZ), following WHO's growth reference data for 5–19 years (48) and employing the WHO AnthroPlus software (WHO 2007 R macro package) (49). Participants were then classified in two main BAZ categories: normal weight ( $> -2 \text{ SD} < + 1 \text{ SD}$ ;  $n = 186$ ) and overweight group, including overweight and obesity ( $\geq + 1 \text{ SD}$ ;  $n = 312$ ). Only BAZ values between  $-3$  and  $+ 5$  z-scores were considered valid and included in this analysis (7).

### Genotyping

Five milliliters of blood from each fasting participant were collected by a standard method in an EDTA tube. For the DNA preparation a commercial kit (Illustra blood genomic-Prep Mini Spin Kit, GE Healthcare) was used.

The BDNF Val66Met SNP rs6265 genotype (G196A) was obtained, as previously described (50), using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method with forward (5'-ACTCTGGA-GAGCGTGAAT-3') and reverse (5'-ATACTGTACACAC ACGCTC-3') primers, and further digestion of the PCR product with NlaIII enzyme (Cat. No. R0125S, New England Biolabs). From the five possible restriction fragments for this Val66Met amplicon, the genotype was identified by the size and distribution of three bands: 243 bp for the G variant (Val), 168 bp and 75 bp bands for the A variant (Met), and these three bands for GA heterozygotes (Val/Met), on 2.5% (w/v) agarose gel electrophoresis. A random selection

of 15 samples was performed for validation with genomic DNA sequencing.

### Statistical Analysis

The data analysis was carried out with free and commercial software, R and SPSS, respectively as well as online tools for statistical computation and visualization (51,52). Baseline characteristics for quantitative variables are presented as arithmetic mean and standard deviation ( $\text{mean} \pm \text{SD}$ ), and evaluated using one-way analysis of variance (ANOVA), while simple frequencies ( $n$ ) and percentages (%) were used for qualitative variables.

Allele and genotypic frequencies were analyzed for compliance with  $\chi^2$  Hardy-Weinberg equilibrium (HWE) with an online calculator for biallelic markers that includes an analysis for ascertainment bias for dominant/recessive models due to biological or technical causes (52) for both the whole pediatric sample as well as for each of the two BAZ categories.

To evaluate the possible association between the BDNF rs6265 polymorphism and BAZ nutritional status, genotype frequencies of the pediatric population were compared against the two main nutritional categories: normal weight and overweight, including obesity. To determine if there were significant differences in the frequency of occurrence for each genotype: GG (Val/Val), GA (Val/Met), and AA (Met/Met) or allele (Val or Met) in a particular nutritional group, we performed  $\chi^2$  statistics and Fisher's exact test. Standardized residuals ( $R$ ) were obtained as a measure of the strength of the difference between observed and expected values to determine how significant the frequencies are to the  $\chi^2$  value and which frequencies had the major influence on the significant  $\chi^2$  test statistic; positive or negative standardized residuals indicate that there are more or fewer genotype counts (or frequencies) than expected, respectively. Alternatively, the AA (Met/Met) and GA (Val/Met) groups were combined into Met carriers and compared against the homozygous GG group (Val homozygotes). Both the HWE and the frequency of each genotype according to their classification into a BAZ group were plotted in *de Finetti diagrams* to visualize the proportions and possible significant deviations from HWE of the biallelic marker as implemented in the program DeFinetti (53) as well as to explore the clustering trends between genotypes and BAZ categories (54).

To establish the magnitude of the association between Val66Met genotypes and nutritional groups, we performed logistic regression analysis considering genotypes or alleles as independent variables and BAZ categories as dependent variables comparing Normal weight vs. Overweight and Obesity combined (Ow + Ob). Odds ratios (ORs) with 95% confidence intervals (CI 95%) were obtained for precision. All results were considered statistically significant when two-tailed Fisher's exact test  $p$ -value was  $< 0.05$ .

A Bayesian analysis was performed with JASP using default priors (55) to determine the relative plausibility of the data under the null hypothesis versus the alternative through the Bayes factor (BF), when comparing the null hypothesis (H0) of no association between Val66Met genotypes and BAZ, as well as the alternative hypothesis (H1) as the association between them. In addition, the Bayesian OR with a 95% credible interval (CI\*\*) was obtained.

## Results

We recruited 498 Mexican children and adolescents attending the Unidad de Investigación Médica en Nutrición from the Hospital de Pediatría at CMN Siglo XXI, IMSS (Mexico City). The phenotypic characteristics and BDNF Val66Met genotypes of the pediatric participants, 282 girls (56.6%) and 216 boys (43.4%), ranging between 5 and 17 years (mean age:  $12.2 \pm 2.02$  years) are shown in Table 1. According to the BAZ categories, 37.3% of the participants had normal weight and 62.6% were overweight (corresponding to 26.1% overweight and 36.5% obesity). The BDNF Val66Met allele frequencies were 0.85 and 0.15 for G (Val) and A (Met), respectively, while the genotype frequencies were 71.7% for GG (Val/Val,  $n = 357$ ), 25.9% for GA (Val/Met,  $n = 129$ ), and 2.4% for AA (Met/Met,  $n = 12$ ). Hardy-Weinberg equilibrium (HWE) criteria under a model of ascertainment was met ( $\chi^2 = 0.007$ ;  $df = 1$ ;  $p = 0.93$ ), indicating no deviation from the Hardy-Weinberg equilibrium in this study, and that our data had no gain/losses bias in the genotype counts. There were no differences in genotype distribution between female and male subjects (Table 1). Our results are similar to those reported previously for other pediatric populations (56–58), and as expected under HWE.

**Table 1.** Baseline phenotypes and BDNF Val66Met (G196A) genotypes of Mexican children and adolescents ( $n = 498$ ) ranging between 5–17 years

Phenotypes	Participants	Frequency $n$ (%)	
Age (mean $\pm$ SD years)	$12.2 \pm 2.02$	498 (100)	
Sex	Female (girls)	282 (56.6)	
	Male (boys)	216 (43.4)	
Nutritional Status (BAZ)	Normal weight	186 (37.3)	
	Overweight	130 (26.1)	
	Obesity	182 (36.5)	
		(%) within gender <sup>b</sup>	
	Genotype	Population <sup>a</sup>	
BDNF Val66Met	GG (Val/Val)	357 (71.7)	
	GA (Val/Met)	129 (25.9)	
	AA (Met/Met)	12 (2.4)	
		Female	Male
	GG (Val/Val)	209 (74.1)	148 (68.5)
	GA (Val/Met)	67 (23.8)	62 (28.7)
	AA (Met/Met)	6 (2.1)	6 (2.8)

BAZ, BMI-for-age z-scores.

<sup>a</sup>HWE:  $\chi^2 = 0.007$ ;  $df = 1$ ;  $p = 0.93$ .

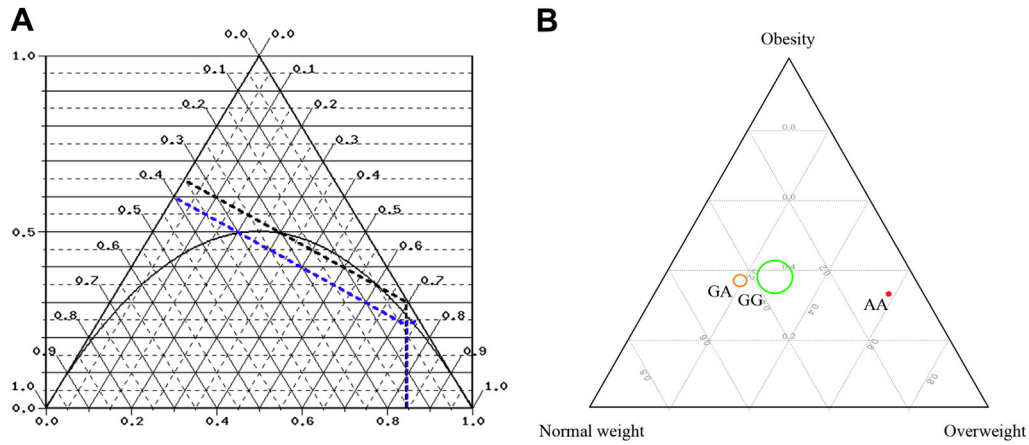
<sup>b</sup> $\chi^2 = 1.9$ ;  $df = 2$ ;  $p = 0.38$ .

Figure 1 illustrates the *de Finetti* distributions (53,54) of the BDNF rs6265 polymorphism according to (A) the test for deviation from HWE of both the normal weight and overweight group (HWE:  $p = 0.053$  and  $p = 0.124$  with Fisher's exact test, respectively), as well as (B) the frequency and location of the pediatric sample according to both their genotypes and BAZ classification. These visualizations showed that not only the whole sample but each of the two main BAZ groups, normal weight and overweight group, are in HWE, but also that there is a clear trend towards clustering closer to the overweight or obesity groups when bearing the AA genotype.

The aim of this study was to determine a possible association between BDNF rs6265 and nutritional status. We observed significant differences between the frequencies of the BDNF GG (Val/Val), GA (Val/Met) and AA (Met/Met) genotypes ( $p = 0.034$ ), as well as between the Met homozygotes in comparison to the combined Val/Val and Val/Met genotypes grouped into the Val carriers ( $p = 0.037$ ) depending on the normal weight or overweight status (Table 2). The frequency of the Val and Met alleles or the Met carriers (the combined Val/Met and Met/Met genotypes in comparison to the homozygous Val/Val genotype) did not show statistical differences. Although the absolute standardized residuals ( $R$ ) values were not  $\geq 1.96$  which would indicate that the frequency of that group is significantly contributing to the difference between proportions, both the lowest and highest  $R$  values were detected for the AA genotype ( $R = -1.6$  and  $+1.3$ ) in the normal weight and overweight group (Ow + Ob), respectively, when compared against GG and GA. Similar results were obtained for the lowest and highest  $R$  values from Met homozygotes ( $R = -1.4$  and  $1.1$ ) when compared against the Val carriers in the normal weight and Ow + Ob group, respectively. These results indicate that the significant difference between proportions is mainly the result of the lower and higher frequency of the AA (Met/Met) genotype among subjects in the normal weight (0.5%) and overweight group (3.5%), respectively (Table 2).

Given the contradictory results reported for the BDNF rs6265 SNP in pediatric as well as adult populations in which either variant has been associated with BMI or Ow + Ob-related conditions, we explored several genotype combinations considering either the A or G variant as the risk allele to obtain the directions of the effects. Our results showed that when considering the A variant as the risk allele, the AA (Met/Met) genotype increased more than six times the risk of being classified within the overweight group (Ow + Ob) when compared against each genotype: GG (6.22,  $p = 0.039$ ), AG (8.43,  $p = 0.015$ ), or both GA + GG (6.76,  $p = 0.028$ ). On the other hand, when considering the G variant as the risk allele, several combinations (GG vs AA, GA vs AA and GG + GA vs AA) showed that the common variant G had a significant





**Figure 1.** De Finetti ternary diagrams for Mexican pediatric population ( $n = 498$ ) of BDNF rs6265. These visualizations represent both the Hardy-Weinberg equilibrium of the bi-allelic marker in the pediatric population as well as their clustering into normal weight ( $> -2$  SD  $< +1$  SD) and overweight ( $\geq +1$  SD  $< +2$  SD) and obesity ( $\geq +2$  SD) phenotypes according to their BDNF rs6265 genotype. (A) Ternary diagram for the single nucleotide polymorphism with a Hardy-Weinberg parabola for normal weight (black dotted line,  $p = 0.053$ ) and overweight group ( $\geq +1$  SD; blue dotted line,  $p = 0.124$ ) Mexican children (<https://ihg.gsf.de/cgi-bin/hw/finetti2.pl>). (B) Ternary plot visualizing the location and frequency of genotypes in relation tonutritional status classified by WHO's BAZ cut-offs. The genotypes GG ( $n = 357$ ), GA ( $n = 129$ ) and AA ( $n = 12$ ) are shown with green and orange circles, and red dot, respectively, in proportion with the observed counts per BAZ category. (A color figure can be found in the online version of this article.)

protective effect against being classified in the overweight (Ow + Ob) group (Figure 2A).

Further, to estimate a more credible OR we performed a Bayesian analysis. Results of the Bayesian analysis are only shown for the association between AA vs GG + GA when compared against the BAZ groups (Figure 2B). First, to quantify the evidence provided by the observed data in favor of one hypothesis over the other, we calculated the Bayes factor (BF), where values  $> 1$  indicate evidence in

favor of the alternative hypothesis (H1: the probability of AA (Met/Met) genotype is higher in the overweight group). The Bayes factor for the alternative hypothesis ( $BF_{10}$ ) was 3.151 suggesting that these data are 3.151 more likely to be observed under the alternative hypothesis (H1). The Bayesian OR for this association was 4.22, indicating the most probable associated risk value for Ow + Ob with a 95% credible interval of 1.09–33.1.

**Table 2.** The BDNF genotype and allele count and frequencies (%) in Mexican children and adolescents ( $n = 498$ ) subdivided into two main BAZ groups, normal weight ( $\geq -2 < +1$  SD) and overweight group ( $\geq +1$  SD, including overweight and obesity)

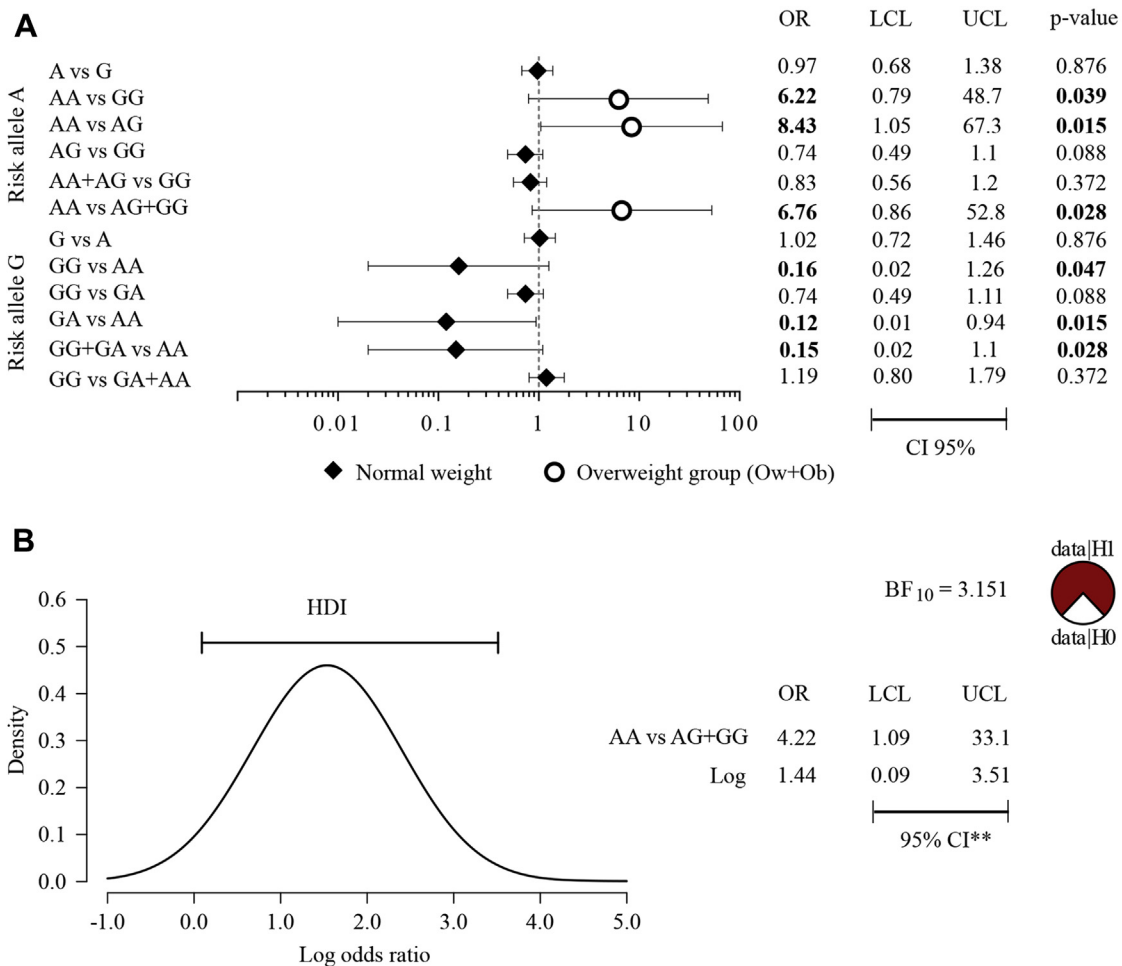
BDNF Val66Met genotype	Normal weight $n$ (%)	Overweight group <sup>a</sup> $n$ (%)
GG (Val/Val)	129 (69.4)	228 (73.1)
GA (Val/Met)	56 (30.1)	73 (23.4)
AA (Met/Met)	1 (0.5)	11 (3.5)
Fisher's exact test $p = 0.034$		
$R = -1.6$ and $+1.3$ for AA genotype in the normal and overweight groups, respectively		
Val allele	314 (84.4)	529 (84.8)
Met allele	58 (15.6)	95 (15.2)
Fisher's exact test $p = 0.93$		
Val carriers	185 (99.5)	301 (96.5)
Met homozygotes	1 (0.5)	11 (3.5)
Fisher's exact test $p = 0.037$		
$R = -1.4$ and $+1.1$ for Met homozygotes in the normal and overweight groups, respectively		
Met carriers	57 (30.6)	84 (26.9)
Val homozygotes	129 (69.4)	228 (73.1)
Fisher's exact test $p = 0.411$		

<sup>a</sup> $\geq +1$  SD, including overweight and obese children;  $R$  = absolute value of the standardized residual.

## Discussion

To our knowledge, this is the first study in Mexican pediatric population showing a significant association between the BDNF Val66Met (rs6265) Met homozygous and nutritional status characterized by their BMI-for-age z-scores (BAZ).

According to an update of our previous bibliometric analysis for BDNF Val66Met in three main databases: Web of Science, Pubmed and Scopus (59), a previous study in healthy Mexican school-aged children between 6–15 years of age recruited from a summer camp (60) did not find association between this SNP and BMI for-age z-scores (39); however, the authors analyzed this association as a linear function of BAZ, instead of considering nutritional status as well established overweight and obesity categories. In addition, they considered the G variant as the risk allele and only reported the risk allele frequency, but not the frequency of the AA (Met/Met) genotype, which could have allowed a comparison between our samples. At least across 58 global populations, the derived Met allele exhibits a wide allelic distribution variability ranging from 0–72% (61); in our sample, this frequency is 15.2%.



**Figure 2.** Risk estimation between BDNF rs6265 genotypes and BAZ categories in Mexican pediatric population. The image shows (A) a forest plot visualizing the magnitude and precision of the association of the effect of either allele A or G at rs6265 in BDNF on overweight and obesity combined, and (B) the 95% highest density interval for the association between AA vs GG + GA when compared against the BAZ groups, where every point inside the area under the curve between the limits has higher credibility (probability density) than any point outside the interval. The piechart visualizes the Bayes factor where the red predominance over white indicate evidence for the alternative hypothesis. ORs and *p*-values in bold indicate statistical significance by two-tailed Fisher's exact test. Both X-axis represents values at Log10 scale. BAZ, BMI for-age-and-sex z-scores; OR, odds ratio; CI 95%, 95% confidence interval; LCL, lower confidence (or credible) interval; UCL, upper confidence (or credible) interval; Ow, overweight; Ob, obesity; BF<sub>10</sub>, Bayes factor for the alternative hypothesis; H0, null hypothesis; H1, alternative hypothesis; HDI, highest density interval; 95% CI\*\*, 95% credible interval. (A color figure can be found in the online version of this article.)

Given the low frequency of the AA (Met/Met) genotype in our sample, employing BAZ categories resulted in the increase of statistical power, allowing us to detect its association with overweight and obesity. Indeed, WHO's standards were chosen since they depict normal (non-obese) childhood growth and can be used to assess children, regardless of ethnicity, socioeconomic status and type of feeding. In addition, it has been reported as a more sensitive criterion to identify overweight and obesity than CDC and IOTF recommendations since they derived from more recent data in which the BMI distribution of the reference populations has shifted towards the right due to an increase in BMI (62–64). Not only the frequency of the AA (Met/Met) genotype in our population is similar to that

observed in Croatian children (37), 2.4 and 3%, respectively, but also its association with overweight/obesity. Although difficult to explain, discrepancies observed in the literature about the risk allele associated with BMI, overweight, and obesity may be the result of differences in the clinical criteria for patients selection, allele distribution between populations, strategies for data processing and analysis (ie. BMI-for-age or nutritional status, national or international BAZ cut-offs), gene-environment factors, and the effect of other genes. For example, it has been shown that the effect of several SNPs associated with BMI, overweight and obesity in Europeans does not replicate completely in Mexican children, implying that distinct population genetic susceptibility variations may account for these outcomes

(65), in the context of particular gene-environment or gene-diet interactions which would require further exploration.

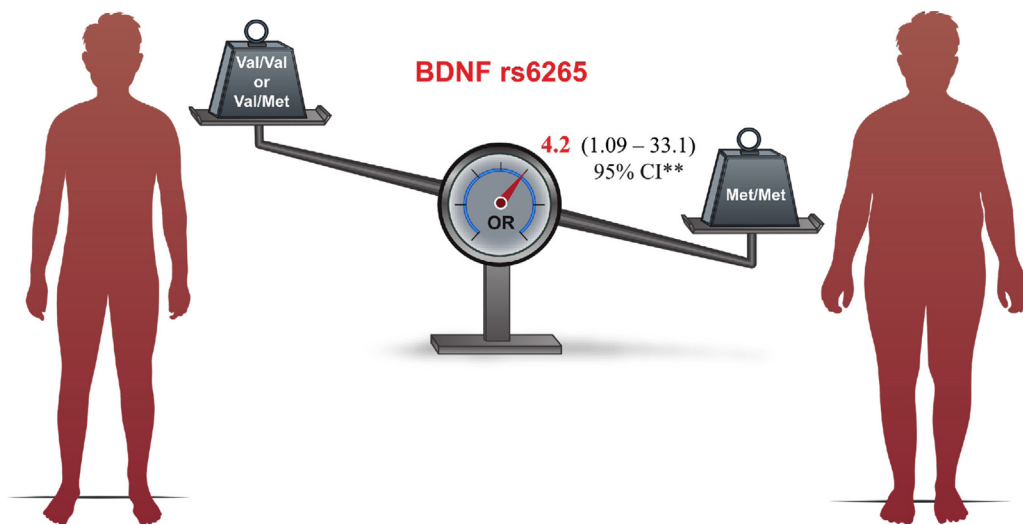
From the beginning, we hypothesized that the association between the AA genotype with the overweight BAZ category was a more plausible outcome given the clear functional effects of this SNP, in which in an homozygous state the molecular function would be completely altered, while compensated in an heterozygous state. As mentioned previously, the 66Met variant reduces the expression of BDNF, which inhibits excessive calorie uptake and promotes energy expenditure; then, the 66Met variant impairs the normal function of these systems with direct impact in either BMI or Ow + Ob-related outcomes (30). We tested this hypothesis by considering either the A or G allele as the risk variant. As biologically expected, we observed that the significant risk of being overweight (Ow + Ob) was observed only in participants bearing the AA (Met/Met) genotype (Figure 3). It is worth mention that the only children with the AA genotype classified in the normal weight group had a BMI-for age z-score = 0.96, which is almost in the cut-off point for the overweight group ( $\geq +1$  SD) classification. In contrast, either one or two copies of the Val allele, showed a significant protective effect against overweight (Figure 2A).

Our results are partially in line with recent studies that have shown a significant association between obesity (BMI percentile) for Caucasian children and adolescents of the same ethnic (Croatian) background and one or two Met alleles of the BDNF Val66Met polymorphism, each of them increasing their BMI, and with a significant risk for obesity in children bearing the Val/Met genotype (37). However, no significant differences in the distribution of the BDNF Met carriers compared to Val homozygotes were observed for adults from the same ethnic (Croatian)

background for normal weight, overweight and obese categories, neither gain or changes during a 35 years of follow-up with three time check-up periods ( $43.4 \pm 4.4$ ,  $53.4 \pm 4.5$ , and  $77.2 \pm 4.5$  years; mean age in years  $\pm$  SD for each period) (66). A recent systematic review and meta-analysis assessing the association of BDNF polymorphisms and BMI, as a representative index of overweight and obesity, has concluded that the rs6265 SNP can be considered as a genetic determinant of obesity (31). Nevertheless, a previous analysis from data of the Brain Resource International Database showed trends towards a lower BMI in adults from 18–82 years bearing the Met/Met genotype compared to the Val/Val and Val/Met genotypes as well as when comparing the Met homozygotes to the Val carriers (67).

According to these observations, it results intriguing the possibility that subjects with Met/Met genotype may shift their BMI according to their life span, in which young Met/Met subjects have an increased probability of showing a higher BAZ which will be attenuated in adulthood until shifting towards a lower BMI in old age.

Factors and mechanisms involved in this Met homozygous-BMI shift still remain to be elucidated. In fact, follow-up studies considering changing eating patterns, physical activity or sedentary behaviors, differential mechanisms and distinct combination of factors across lifespan regulating food intake and caloric expenditure between children, adults, and elderly subjects from distinct populations must be considered. Clearly, brain-regulating hormonal signals like leptin and insulin (68), with physiological effects particularly in the hippocampus, amygdala, cerebral cortex, and hypothalamus, brain regions with abundant BDNF expression (20–22), some of them involved in weight regulation and food intake via its actions



**Figure 3.** BDNF Val66Met is a genetic risk factor for overweight and obesity in Mexican children and adolescents. The AA (Met/Met) genotype relative to Val carriers (GG and GA genotypes) increased four times (Bayes OR = 4.2, 95% CI\*\*[1.09–33.1]) the risk of overweight, including obesity. OR, odds ratio; CI\*\*, credible interval. (A color figure can be found in the online version of this article.)

on specific hypothalamic nuclei (69), should be considered in further molecular combinatorial analysis, as the homeostatic imbalance of these nutritional signals has been associated to weight loss in lean older adults (70).

## Conclusion

Finally, we have confirmed BDNF Val66Met, particularly the AA genotype (Met variant), as a genetic risk factor for nutritional BAZ status in Mexican children and adolescents. However, studies on this polymorphism in Mexican pediatric population should be replicated in a larger sample and include the variables mentioned above that may be involved in the association discrepancies reported among populations. Further research should be encouraged towards BDNF and functional variants such as Val66Met associated with energy metabolism, food regulation and BMI, particularly in countries like Mexico widely affected by this health-threatening condition.

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## Conflicts of interest statement

The authors declare no conflict of interest.

## References

1. Wang Y, Lobstein T. Worldwide trends in childhood overweight and obesity. *Int J Pediatr Obes* 2006;1:11–25.
2. Caballero B. The global epidemic of obesity: an overview. *Epidemiol Rev* 2007;29:1–5.
3. Lawrence VJ, Kopelman PG. Medical consequences of obesity. *Clin Dermatol* 2004;22:296–302.
4. World Health Organization. Obesity and overweight - Fact sheet. World Health Organization; 2016. Available at: <http://www.who.int/mediacentre/factsheets/fs311/en>. Accessed September 29, 2017.
5. Popkin BM, Gordon-Larsen P. The nutrition transition: worldwide obesity dynamics and their determinants. *Int J Obes Relat Metab Disord* 2004;28(Suppl 3):S2–S9.
6. Davila-Torres J, Gonzalez-Izquierdo JJ, Barrera-Cruz A. [Obesity in Mexico]. *Rev Med Inst Mex Seguro Soc* 2015;53:240–249.
7. Gutiérrez JP, Rivera-Dommarco J, Shamah-Levy T, et al. Encuesta Nacional de Salud y Nutrición 2012. Resultados Nacionales. Instituto Nacional de Salud Pública; 2012. Available at: <http://ensanut.insp.mx/informes/ENSANUT2012ResultadosNacionales.pdf>. Accessed July 11, 2017.
8. Hernandez-Cordero S, Cuevas-Nasu L, Morales-Ruan MC, et al. Overweight and obesity in Mexican children and adolescents during the last 25 years. *Nutr Diabetes* 2017;7:e247.
9. Kopelman PG. Obesity as a medical problem. *Nature* 2000;404:635–643.
10. Zhao J, Grant SF. Genetics of childhood obesity. *J Obes* 2011;2011:845148.
11. El-Sayed Moustafa JS, Froguel P. From obesity genetics to the future of personalized obesity therapy. *Nat Rev Endocrinol* 2013;9:402–413.
12. Maes HH, Neale MC, Eaves LJ. Genetic and environmental factors in relative body weight and human adiposity. *Behav Genet* 1997;27:325–351.
13. Blakemore AI, Froguel P. Investigation of Mendelian forms of obesity holds out the prospect of personalized medicine. *Ann N Y Acad Sci* 2010;1214:180–189.
14. Speliotes EK, Willer CJ, Berndt SI, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet* 2010;42:937–948.
15. Chung WK, Leibel RL. Molecular physiology of syndromic obesities in humans. *Trends Endocrinol Metab* 2005;16:267–272.
16. Yates A, Akanni W, Amode MR, et al. Ensembl 2016. *Nucleic Acids Res* 2016;44:D710–D716.
17. Rosas-Vargas H, Martínez-Ezquerro JD, Bienvenu T. Brain-derived neurotrophic factor, food intake regulation, and obesity. *Arch Med Res* 2011;42:482–494.
18. Yamamoto M, Sobue G, Yamamoto K, et al. Expression of mRNAs for neurotrophic factors (NGF, BDNF, NT-3, and GDNF) and their receptors (p75NGFR, trkA, trkB, and trkC) in the adult human peripheral nervous system and nonneural tissues. *Neurochem Res* 1996;21:929–938.
19. Ernfors P, Ibanez CF, Ebendal T, et al. Molecular cloning and neurotrophic activities of a protein with structural similarities to nerve growth factor: developmental and topographical expression in the brain. *Proc Natl Acad Sci U S A* 1990;87:5454–5458.
20. Leibrock J, Lottspeich F, Hohn A, et al. Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* 1989;341:149–152.
21. Hofer M, Pagliusi SR, Hohn A, et al. Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. *EMBO J* 1990;9:2459–2464.
22. Tang S, Machaalani R, Waters KA. Immunolocalization of pro- and mature-brain derived neurotrophic factor (BDNF) and receptor TrkB in the human brainstem and hippocampus. *Brain Res* 2010;1354:1–14.
23. Webster MJ, Herman MM, Kleinman JE, et al. BDNF and trkB mRNA expression in the hippocampus and temporal cortex during the human lifespan. *Gene Expr Patterns* 2006;6:941–951.
24. Chao MV, Rajagopal R, Lee FS. Neurotrophin signalling in health and disease. *Clin Sci (Lond)* 2006;110:167–173.
25. Reichardt LF. Neurotrophin-regulated signalling pathways. *Philos Trans R Soc Lond B Biol Sci* 2006;361:1545–1564.
26. Takei N, Furukawa K, Hanyu O, et al. A possible link between BDNF and mTOR in control of food intake. *Front Psychol* 2014;5:1093.
27. Nakazato M, Hashimoto K, Shimizu E, et al. Possible involvement of brain-derived neurotrophic factor in eating disorders. *IUBMB Life* 2012;64:355–361.
28. Gratacos M, Gonzalez JR, Mercader JM, et al. Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. *Biol Psychiatry* 2007;61:911–922.
29. Chen ZY, Patel PD, Sant G, et al. Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J Neurosci* 2004;24:4401–4411.



30. Egan MF, Kojima M, Callicott JH, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 2003;112:257–269.
31. Akbarian SA, Salehi-Abargouei A, Pourmasoumi M, et al. Association of Brain-derived neurotrophic factor gene polymorphisms with body mass index: A systematic review and meta-analysis. *Adv Med Sci* 2017;63:43–56.
32. Mitchell JA, Hakonarson H, Rebbeck TR, et al. Obesity-susceptibility loci and the tails of the pediatric BMI distribution. *Obesity (Silver Spring)* 2013;21:1256–1260.
33. Zhang M, Zhao X, Xi B, et al. [Impact of obesity-related gene polymorphism on risk of obesity and metabolic disorder in childhood]. *Zhonghua Yu Fang Yi Xue Za Zhi* 2014;48:776–783.
34. Xi B, Cheng H, Shen Y, et al. Study of 11 BMI-associated loci identified in GWAS for associations with central obesity in the Chinese children. *PLoS One* 2013;8:e56472.
35. Wu L, Xi B, Zhang M, et al. Associations of six single nucleotide polymorphisms in obesity-related genes with BMI and risk of obesity in Chinese children. *Diabetes* 2010;59:3085–3089.
36. Zhao J, Bradfield JP, Li M, et al. The role of obesity-associated loci identified in genome-wide association studies in the determination of pediatric BMI. *Obesity (Silver Spring)* 2009;17:2254–2257.
37. Skledar M, Nikolac M, Dodig-Curkovic K, et al. Association between brain-derived neurotrophic factor Val66Met and obesity in children and adolescents. *Prog Neuropsychopharmacol Biol Psychiatry* 2012;36:136–140.
38. Arija V, Ferrer-Barcala M, Aranda N, et al. BDNF Val66Met polymorphism, energy intake and BMI: a follow-up study in schoolchildren at risk of eating disorders. *BMC Public Health* 2010;10:363.
39. Leon-Mimila P, Villamil-Ramirez H, Villalobos-Comparan M, et al. Contribution of common genetic variants to obesity and obesity-related traits in Mexican children and adults. *PLoS One* 2013;8:e70640.
40. Friedel S, Horro FF, Wermter AK, et al. Mutation screen of the brain derived neurotrophic factor gene (BDNF): identification of several genetic variants and association studies in patients with obesity, eating disorders, and attention-deficit/hyperactivity disorder. *Am J Med Genet B Neuropsychiatr Genet* 2005;132B:96–99.
41. Gray J, Yeo GS, Cox JJ, et al. Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. *Diabetes* 2006;55:3366–3371.
42. Xu B, Xie X. Neurotrophic factor control of satiety and body weight. *Nat Rev Neurosci* 2016;17:282–292.
43. Zhao X, Xi B, Shen Y, et al. An obesity genetic risk score is associated with metabolic syndrome in Chinese children. *Gene* 2014;535:299–302.
44. Akkermann K, Hiio K, Villa I, et al. Food restriction leads to binge eating dependent upon the effect of the brain-derived neurotrophic factor Val66Met polymorphism. *Psychiatry Res* 2011;185:39–43.
45. Xi B, Wang C, Wu L, et al. Influence of physical inactivity on associations between single nucleotide polymorphisms and genetic predisposition to childhood obesity. *Am J Epidemiol* 2011;173:1256–1262.
46. Tuyet LT, Nhung BT, Dao DTA, et al. The Brain-Derived Neurotrophic Factor Val66Met Polymorphism, Delivery Method, Birth Weight, and Night Sleep Duration as Determinants of Obesity in Vietnamese Children of Primary School Age. *Child Obes* 2017;13:392–399.
47. Kalenda A, Landgraf K, Löffler D, et al. The BDNF Val66Met polymorphism is associated with lower BMI, lower postprandial glucose levels and elevated carbohydrate intake in children and adolescents. *Pediatr Obes* 2017;13:159–167.
48. de OM, Onyango AW, Borghi E, et al. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ* 2007;85:660–667.
49. World Health Organization. WHO AnthroPlus Software. World Health Organization; 2017. Available at. <http://www.who.int/growthref/tools/en/>. Accessed January 9, 2017.
50. Sim MS, Mohamed Z, Hatim A, et al. Association of brain-derived neurotrophic factor (Val66Met) genetic polymorphism with methamphetamine dependence in a Malaysian population. *Brain Res* 2010;1357:91–96.
51. Lowry R. VassarStats: Website for statistical computation. Vassar College; 2004; Available at. <http://vassarstats.net/index.html>. Accessed February 25, 2017.
52. Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am J Epidemiol* 2009;169:505–514.
53. Storm TM, Wienker TF. Tests for deviation from Hardy-Weinberg equilibrium and tests for association. Institute of Human Genetics, Helmholtz Center Munich; 2008. Available at. <https://ihg.gsf.de/cgi-bin/hw/hwa1.pl>. Accessed June 12, 2017.
54. Meyer D, Zeileis A, Hornik K. vcd: Visualizing Categorical Data. R package version 1.4-3; 2016.
55. JASP Team. JASP (Version 0.8.3.1)[Computer software]; 2017.
56. Hall D, Dhilla A, Charalambous A, et al. Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. *Am J Hum Genet* 2003;73:370–376.
57. Lang UE, Hellweg R, Kalus P, et al. Association of a functional BDNF polymorphism and anxiety-related personality traits. *Psychopharmacology (Berl)* 2005;180:95–99.
58. Timpano KR, Schmidt NB, Wheaton MG, et al. Consideration of the BDNF gene in relation to two phenotypes: hoarding and obesity. *J Abnorm Psychol* 2011;120:700–707.
59. Martínez-Ezquerro JD, Michán L, Rosas-Vargas H. Bibliometric analysis of the BDNF Val66Met polymorphism based on Web of Science, Pubmed, and Scopus databases [version 1; not peer reviewed]. F1000Research 2016;5:2773. <https://doi.org/10.7490/f1000research.1113470.1>. (poster) [Spanish].
60. Flores-Dorantes T, Arellano-Campos O, Posadas-Sanchez R, et al. Association of R230C ABCA1 gene variant with low HDL-C levels and abnormal HDL subclass distribution in Mexican school-aged children. *Clin Chim Acta* 2010;411:1214–1217.
61. Petryshen TL, Sabeti PC, Aldinger KA, et al. Population genetic study of the brain-derived neurotrophic factor (BDNF) gene. *Mol Psychiatry* 2010;15:810–815.
62. Gonzalez-Casanova I, Sarmiento OL, Gazmararian JA, et al. Comparing three body mass index classification systems to assess overweight and obesity in children and adolescents. *Rev Panam Salud Publica* 2013;33:349–355.
63. Nascimento H, Catarino C, Mendonca D, et al. Comparison between CDC and WHO BMI z-score and their relation with metabolic risk markers in Northern Portuguese obese adolescents. *Diabetol Metab Syndr* 2015;7:32.
64. Oliveira GJ, Barbiero SM, Cesa CC, et al. Comparison of NCHS, CDC, and WHO curves in children with cardiovascular risk. *Rev Assoc Med Bras* 1992;2013(59):375–380.
65. Abadi A, Peralta-Romero J, Suarez F, et al. Assessing the effects of 35 European-derived BMI-associated SNPs in Mexican children. *Obesity (Silver Spring)* 2016;24:1989–1995.
66. Nikolac PM, Mustapic M, Pavlovic M, et al. Lack of association between brain-derived neurotrophic factor Val66Met polymorphism and body mass index change over time in healthy adults. *Neurosci Lett* 2013;545:127–131.

67. Gunstad J, Schofield P, Paul RH, et al. BDNF Val66Met polymorphism is associated with body mass index in healthy adults. *Neuropsychobiology* 2006;53:153–156.
68. Folch J, Pedros I, Patraca I, et al. Neuroprotective and anti-ageing role of leptin. *J Mol Endocrinol* 2012;49:R149–R156.
69. Harvey J. Leptin: a multifaceted hormone in the central nervous system. *Mol Neurobiol* 2003;28:245–258.
70. Tanaka M, Nagai K, Koshiba H, et al. Weight loss and homeostatic imbalance of leptin and ghrelin levels in lean older adults. *J Am Geriatr Soc* 2013;61:2234–2236.