

No evidence of association between the serotonin 2A receptor – 1438G/A promoter polymorphism and childhood obesity in a Spanish population: A case-parent study and a matched case-control study

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Abstract

Serotonin has been related to feeding behaviour and body weight control through its suppressive effect on appetite. Conflicting results have been published in the literature regarding the association between the – 1438 G/A promoter polymorphism of the 5HT2A gene with obesity-related variables. The aim of this study was to assess the association between the – 1438 G/A polymorphism of the 5HT2A gene with childhood obesity in a Spanish population. A total of 136 cases aged 6–16 years with BMI above the 97th percentile of the Spanish BMI reference data for age and gender were matched by gender and age (\pm 6 months) with 136 controls. Additionally, 43 obese children and their parents were selected for a family-based association study (case-parent study). Genotyping was carried out by polymerase chain reaction and restriction enzyme analysis. Conditional logistic regression and transmission/disequilibrium test were used to assess genotype-obesity association. In the matched case-control study, the crude and adjusted odds ratios for the association between 5HT2A – 1438 G/A genotypes were non-significant. Likewise, no association is suggested by the case-parent study. In conclusion, it is unlikely that the – 1438 G/A polymorphism of 5HT2A gene may influence obesity in a Spanish children population.

Keywords: Genetic, obesity, polymorphism, serotonin, receptor

Introduction

Serotonin (5-hydroxy-tryptamine, 5-HT) has been related to feeding behaviour and body weight control through its suppressive effect on appetite, preferentially inhibiting the intake of carbohydrates rather than fat intake or protein intake (Leibowitz and Alexander 1998). The rate of serotonin synthesis depends on the plasma concentration of its precursor tryptophan in relation to the plasma concentration of other large neutral amino acids that compete with tryptophan for uptake across the blood–brain barrier (Wurtman et al. 2003).

The effect of serotonin on food intake is mediated by the serotonin receptors (Vickers and Dourish 2004). A number of studies have evaluated the contribution of genetic polymorphisms of 5-HT receptors and other proteins related to the serotonin pathway on obesity and feeding behaviour (Lentes et al. 1997; Sargent et al. 1997; Yuan et al. 2000; Durand et al. 2004; Pooley et al. 2004). In this context, conflicting results have been published in the literature regarding the association between the – 1438 G/A promoter polymorphism of the 5HT2A gene with obesity and obesity-related phenotypes

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(Hinney et al. 1997; Aubert et al. 2000; Ricca et al. 2002; Rosmond et al. 2002a, 2002b).

The aim of this study was to assess the possible association between the -1438G/A polymorphism of the 5HT2A gene promoter region and childhood obesity in a Spanish population.

Subjects and methods

Subjects

In the matched case-control study, cases and controls were recruited from the Paediatrics Departments at the Virgen del Camino Hospital (Pamplona, Navarra, Spain), the University Hospital and three primary Health Centres, comprising 272 Spanish children and adolescents (136 obese and 136 control subjects), aged 6–16 years. Obesity was defined according to Spanish BMI reference data. Therefore, cases were children with BMI above the 97th percentile of the Spanish BMI reference data for age and gender (Sobradillo et al. 2004), while controls were children with a BMI below the 90th percentile (range of age/gender specific population percentiles in controls: 10–90th). Cases and control subjects were enrolled from a relatively homogenous population of Navarra (Northern of Spain). Controls were matched to the obese subjects on a 1:1 basis on sex and age (\pm 6 months) and attended at the same health centres that the cases. Exclusion criteria were exposure to hormonal treatment, presence of endocrine diseases or serious intercurrent illness.

In the case-parent study, a group of 43 obese children (16 boys and 27 girls with BMI above the 97th percentile of the Spanish BMI reference data for age and gender) and their parents were selected for the case-parent study. Family trios were recruited from the Paediatrics Department of the University Hospital (Pamplona, Navarra, Spain). It is worth mentioning that obese children included in the case-parent study are different than the ones included in the matched case-control study (both studies are based in independent datasets). The case-parent study has the advantage that avoids the confounding effect of population stratification by ethnicity.

This association study was approved by the Ethics Committee of the University of Navarra and all parents and subjects above 12 years old provided written informed consent. Children below 12 years old acquiesced to participate in the study.

Measurement of obesity-related variables

Height and weight were measured in light clothing using standard procedures. Body Mass Index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Body fat mass was measured by bioelectrical impedance using a TBF-410 device

(TANITA[®], Tokyo, Japan) according to manufacturer's instructions. The measurement of fat mass with this device showed an intra-individual variation of 3.5% calculated during a trial conducted in our department (Pérez et al. in press). This study found a high correlation of bioelectrical impedance measurements with BMI and triceps skinfold values. Parental family history of obesity was defined as having at least one obese parent (BMI \geq 30) at the time of the examination. Plasma leptin levels were measured using a specific RIA kit (Linco Research, MO, USA). A physical activity questionnaire was applied as described elsewhere (Ochoa et al. 2004) to calculate Metabolic Equivalents (MET's) hours per week.

Genotyping methods

For the assessment of the -1438G/A variants, Polymerase Chain Reaction (PCR) assays were carried out in a final volume of 25 μ l containing 150–250 ng of genomic DNA, 0.5 μ M of each primer, 200 μ M of each dNTP, 1.5 mM of MgCl₂ and 1 U of Taq polymerase. All the reagents for this technique were supplied by Bionline (London, UK). All PCR assays were performed in a Perkin-Elmer GeneAmp 2400 thermocycler. A 352 base pair DNA fragment was amplified using the primers 5'-ACTGCGAAACCAACTTATTTCC-3' (forward primer) and 5'-TTGTGCAGATTCCCATTAAGG-3' (reverse primer). PCR was initiated with a denaturation step at 94° for 5 min, followed by 30 cycles (denaturation at 94°C for 30 s, annealing at 61°C for 30 s and extension at 72°C for 30 s) with a final extension at 72°C for 5 min. A total of 10 μ l of the PCR product were incubated at 37°C for 3 h with MspI restriction enzyme and electrophoresed through 2% agarose gels stained with ethidium bromide. Homozygous genotype for the A allele yielded an undigested fragment of 352 base pairs. Homozygous genotype for the G allele generated fragments of 232 and 120 base pairs (Mata et al. 2004).

Statistical methods

Genotype frequencies were tested for Hardy-Weinberg equilibrium after the estimation of allele and genotype frequencies. Multivariate conditional logistic regression techniques were fitted to estimate crude and adjusted odds ratios (OR's) in the matched case-control study. The transmission-disequilibrium test (TDT) was used to assess the differential pattern of excess transmission of alleles from heterozygous parents to diseased children. By sampling family trios through and affected child (proband), the association between 5HT2A alleles and obesity would cause transmission to appear different from the expected probability of 0.5. When cases are unrelated probands, TDT represents a valid test of association even if population stratification is present (Schaid 1999).

Results

Table I shows the characteristics of the subjects participating in the matched case-control study. Apart from BMI and percent of body fat, obesity-related variables such as plasma leptin levels, family history of obesity and physical activity levels were different in cases versus age-gender matched controls as expected. Genotype frequencies were concordant with Hardy-Weinberg expectations both in controls (exact p value = 0.31) and cases (exact p value = 0.84). When cases and controls were assessed separately, no significant differences were found in BMI averages by genotype after adjusting by gender and age. Table II shows the main results of the matched case-control study in terms of gene-obesity associations. Crude and adjusted OR's (including physical activity levels as a covariate in the conditional logistic model) do not support the existence of an association between the -1438G/A polymorphism and childhood obesity.

Among the 43 case-parent trios, a total of 38 heterozygote parents (informative) for allelic transmission were found. The probability of transmission of the A allele was 50% (19 transmitted versus 19 non-transmitted) leading to a complete concordance with the expected value predicted by the null hypothesis of no association (p value = 1).

Discussion

The biological action of serotonin on appetite is mediated by the serotonin receptors and it seems to affect the control of temporal aspects of feeding as well as the intake of macronutrients (Blundell 1986). There is a number of studies that have evaluated the relation between genes participating in the serotonin pathway (serotonin transporters, serotonin receptors or genes participating in serotonin synthesis) in

pathologies related with eating disorders as anorexia nervosa or bulimia nervosa (Ricca et al. 2002). In common polygenic obesity, the evaluation of feeding behaviour is a complex issue that has been frequently dealt with instruments such as the three-factor eating questionnaire (TFEQ) (Bouchard et al. 2004). In this context, a significant association has been reported between polymorphisms of an amino acid transporter gene potentially related to tryptophan transport (which is the precursor of serotonin) with the TFEQ scores and obesity (Suviolahti et al. 2003; Durand et al. 2004). Therefore, the effect of polymorphisms of genes related to serotonin on feeding behaviour seems to be an interesting research area to explore in common obesity of our population.

The present epidemiological study was conducted using two different matching strategies. The first one was to match controls to cases by gender and age and the second one was a case-parent study that can be considered as a special type of a case-control study where cases (unrelated probands) and the parental non-transmitted alleles are matched by ethnic origin. The advantage of the matched case-control study is that it considers the body fluctuations that occur during childhood growth while the advantage of the case-parent study is that it avoids potential spurious results as a consequence of population stratification (Schaid 1999). The magnitude of the association can be assessed through odds ratios in the matched case-control study while the deviations of the transmission probabilities from the expected value under the null hypothesis of no-association (50%) are related with the genotype relative risk in the case-parents study (Schaid 1999). Although the sample size of our study is relatively small, the close proximity of either point estimates of odds ratios or the estimates of transmission probabilities in the case-parent study to the expected values under the null hypothesis lead us to discard a major role for the -1438G/A polymorphism

Table I. Characteristics of participants of the matched case-control study.

	Obese ($n = 136$)	Controls ($n = 136$)
% Male	55.5%	55.5%
Age (years)	11.5 (2.5)	11.6 (2.6)
BMI (kg/m^2)	28.0 (4.8)	19.0 (2.6)
Percentage body fat	35.5 (7.4)	18.1 (8.5)
Leptin (ng/mL)	38.8 (20.6)	10.5 (10.5)
Physical activity (METs-h/week)	20.2 (12.9)	37.5 (22.3)
Parental family history of obesity	44.5%	13.9%
Genotypes -1438 5HT2A G/A		
% AA	17.7%	15.4%
% AG	52.2%	55.9%
% GG	30.2%	28.7%
Allele Frequencies		
% A	43.8%	43.4%
% G	56.2%	56.6%

Cases and controls were matched by gender and age (± 6 months). Continuous variables are expressed as means (standard deviations).

Table II. Crude and adjusted odds ratios for the association between 5HT2A -1438A/G polymorphism and childhood obesity in the matched case-control study.

Genotypes	Crude Odds Ratios* (95% C.I.)	Adjusted Odds Ratios† (95% C.I.)
GG	1 (Ref.)	1 (Ref.)
AG	0.85 (0.50–1.46)	0.88 (0.43–1.79)
AA	1.11 (0.51–2.40)	1.57 (0.59–4.18)

* Cases and controls were matched by gender and age (\pm 6 months).

† Calculated including physical activity (MET's hours per week) as a covariate in the logistic model.

in childhood obesity in the population of Navarra (Spain).

Several studies have evaluated the association between the -1438G/A 5HT2A polymorphism and obesity using different study designs. Rosmond et al. (2002a) observed that Swedish adult homozygotes for the -1438G allele have higher average body mass index and waist-to-hip ratio values than the other genotypes. Using three different 5HT2A simple polymorphisms, Hinney et al. (1997) reported no association between genotypes and obesity when comparing a sample of German obese children and adolescents with a sample of underweight students. Frequencies for the G allele are roughly similar in our study (0.56–0.57) and in the German study (0.57–0.58) (Hinney et al. 1997) or in the Swedish study (0.61) (Rosmond et al. 2002). On the other hand, Ricca et al. (2002) observed a higher frequency of the G allele in controls (0.64) compared with obese subjects (0.49), while Aubert et al. (2000) reported that French overweight or obese subjects with the G/G genotype have a higher calorie intake than carriers of the A allele, without differences in BMI. The frequency of the G allele in the French study (recalculated from the published information) was 0.55 which is also similar to the allele frequency calculated in the present study. As reported by Hinney et al. (1997), we have also reported no significant association between the -1438G/A 5HT2A polymorphism using the case-parent design.

As in other observational studies, we have found many difficulties in assessing the energy intake in study subjects (Jakes et al. 2004) partially due to the differential underreporting of energy intake in obese versus normal-weight children (Ochoa et al. 2004). For this reason, we have not adjusted odds ratios by energy intake in Table II since we consider that information of energy intake might be inaccurate and biased in the study subjects. The effect of other confounding variables such as gender and age were considered in the design of the study by individually matching cases and controls. On the other hand, the case-parent study does not use real controls and consequently, adjustments for gender, age, physical

activity or energy intake are not easily accounted for. In any case, the total concordance of transmission probabilities with the null hypothesis of no association found in our study seems to be sufficiently evident to discard a relation between the -1438G/A 5HT2A polymorphism and childhood obesity, even assuming a possible existence of confounding or effect modification by additional covariates.

In conclusion, both the matched case-control study and the case-parent study lead us to conclude that it is unlikely that the polymorphism -1438 G/A of the 5HT2A gene may have an important influence on childhood obesity in the population of Navarra (Spain).

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