



## BRIEF COMMUNICATION

# A family-based association study of the *HTR1B* gene in eating disorders

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**Objective:** To explore the association of three polymorphisms of the serotonin receptor 1D $\beta$  gene (*HTR1B*) in the etiology of eating disorders and their relationship with clinical characteristics.

**Methods:** We analyzed the G861C, A-161T, and A1180G polymorphisms of the *HTR1B* gene through a family-based association test (FBAT) in 245 nuclear families. The sample was stratified into anorexia nervosa (AN) spectrum and bulimia nervosa (BN) spectrum. In addition, we performed a quantitative FBAT analysis of anxiety severity, depression severity, and Yale-Brown-Cornell Eating Disorders Scale (YBC-EDS) in the AN and BN-spectrum groups.

**Results:** FBAT analysis of the A-161T polymorphism found preferential transmission of allele A-161 in the overall sample. This association was stronger when the sample was stratified by spectrums, showing transmission disequilibrium between the A-161 allele and BN spectrum ( $z = 2.871$ ,  $p = 0.004$ ). Quantitative trait analysis showed an association between severity of anxiety symptoms and the C861 allele in AN-spectrum participants ( $z = 2.871$ ,  $p = 0.004$ ). We found no associations on analysis of depression severity or preoccupation and ritual scores in AN or BN-spectrum participants.

**Conclusions:** Our preliminary findings suggest a role of the *HTR1B* gene in susceptibility to development of BN subtypes. Furthermore, this gene might have an impact on the severity of anxiety in AN-spectrum patients.

**Keywords:** Anorexia nervosa; bulimia nervosa; serotonin receptor; association; anxiety

## Introduction

Eating disorders (ED) are defined by maladaptive attitudes and behaviors around eating, weight, and shape. According to DSM-IV criteria, used in this study, EDs are classified into three major types: anorexia nervosa (AN), bulimia nervosa (BN), and eating disorders not otherwise specified (EDNOS). The etiology of ED is unknown, but the influence of genetic factors has been demonstrated in family and twin studies.

Evidence supports a role for altered serotonin neurotransmission in the pathophysiology of ED, based on animal and human data accumulated over recent years. The serotonin receptors, particularly, are implicated in the modulation of food intake. In rats, infusion of the 5-HT<sub>1B</sub> agonist CP-94,253 into the parabrachial nucleus reduced food intake.<sup>1</sup> The human 5-HT<sub>1D $\beta$</sub>  is encoded by a 1179-bp intronless gene located at chromosome 6q14.1.<sup>2</sup> This gene was initially cloned in the rat (*HTR1B*), and comparison of amino-acid sequences with the human *HTR1B* gene showed 93% identity<sup>3</sup>; indeed, human *HTR1B*

shares more homology with rodent *HTR1B* than with the human 5-HT<sub>1D $\alpha$</sub>  receptor gene (*HTR1A*). A synonymous polymorphism, G861C, could be in linkage disequilibrium with an entirely different gene in the same chromosomal region that may alter expression of 5-HT<sub>1D $\beta$</sub> . Studies in transfected cells using an in vitro reporter gene expression assay showed that the haplotype -261G/-182INS/A-161 increases binding of transcription factors in the promoter region, producing a 2.3-fold increase in gene transcription.<sup>4</sup>

Studies have demonstrated a possible association of *HTR1B* with AN.<sup>5</sup> Furthermore, genetic studies exploring the association between the G861C polymorphism and phenotypic traits in BN patients showed that C carriers had a significantly lower minimum lifetime body mass index (BMI) than GG patients.<sup>6</sup> The GG genotype has also been associated with severity of obsessive-compulsive symptoms in patients with BN.<sup>7</sup>

In addition, serotonin neurotransmission disturbances in patients with ED could be associated with the secondary effects of their nutritional status.<sup>8</sup> This leads to the expectation that syndromes with a restrictive form should be characterized by anxiety, depression, and obsessions (traits related with increased serotonergic tone), whereas syndromes characterized by binge eating (such as BN or the binge-purge type of AN) would correspond to increased impulsivity, disinhibition of eating behavior, and

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Submitted Feb 09 2016, accepted Mar 10 2016.

other features associated with low serotonergic tone.<sup>9</sup> The identification of clinical characteristics and personality traits in the EDs has proven to be an interesting strategy for the definition of alternative phenotypes.

The aim of the present study was to analyze the role of the G861C (rs6296), A-161T (rs130058), and T1180C (rs6297) polymorphisms of the *HTR1B* gene in patients with ED, and their potential associations with anxiety severity, depression severity, and preoccupations and rituals, using a family-based association methodology.

## Method

### Subjects

The sample consisted of consecutive, consenting, unrelated patients with ED (227 females and 18 males) and their parents, from a family background of three generations born in Mexico, recruited from the Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Mexico. This sample comprised 245 nuclear families. The local institutional Ethics Committee approved the study protocol, and all individuals gave written informed consent for participation at the time of recruitment.

### Assessment

Patients were diagnosed according to DSM-IV criteria for ED using the Structured Clinical Interview for Mental Disorders v.2.0 (SCID-I). We measured the severity of anxiety and depression using the Hamilton Scales for Anxiety and Depression (HAM-A and HAM-D), and the severity of preoccupations and rituals using the Spanish-language version of the Yale Brown-Cornell Eating Disorders Scale (YBC-EDS).<sup>10</sup>

### Genotyping

Peripheral blood was collected and genomic DNA was extracted by a standard procedure. Single-nucleotide polymorphisms (SNPs) were selected based on location: the -161A/T (rs130058) variant is located in the 5' untranslated (UTR) region; G861C (rs6296) in intracellular loop III; and A1180G (rs6297) in the 3'-UTR region. Linkage disequilibrium has been reported between -261T/G and G861C, variants located in the 5'-UTR region; therefore, based on location and previous studies in patients with ED, we selected the G861C variant for analysis in the present study.

Analysis of the G861C polymorphism was performed with the polymerase chain reaction (PCR)-restriction fragment length polymorphism. PCR amplification was carried out in a total volume of 13  $\mu$ L containing 2 mM MgCl<sub>2</sub>, 200  $\mu$ M each of dATP, dCTP, dGTP, dTTP, 0.2 U *Taq* polymerase, 0.4  $\mu$ M primers (5'-CGTCGGACATCACTTGTTG-3' and 5'-TGGAACCAGCAGGCATCTT-3'), and 50 ng of genomic DNA. After an initial 10-minute denaturation step at 94 °C, 36 cycles were performed under the following conditions: 60 s at 94 °C, 60 s at 65 °C, and 45 s at 72 °C, followed by a final step at 72 °C. PCR products were digested with *HincII* (New England

BioLabs, Herts, UK) at 37 °C overnight, and the fragments resolved on a 2.5% agarose gel and visualized by UV after ethidium bromide staining.

Genotyping of -A161T and A1180G was performed using a 5'-exonuclease TaqMan SNP Genotyping Assay-by-Design (C\_2248101\_10 and C\_2523535\_20 respectively). The reaction was performed in a total volume of 20 ng of genomic DNA, 2.5  $\mu$ L of TaqMan Master Mix, and 0.125  $\mu$ L of genotyping assay, in 96-well plates, using the TaqMan SNP Genotyping Assay Protocol. The intensity was measured with a 7500 Fast Real Time PCR System (Foster City, CA), and genotypes were scored using software supplied by the manufacturer.

### Statistical analysis

Hardy-Weinberg equilibrium analysis was performed with the HWE free software (www.tufts.edu). Analysis of *HTR1B* polymorphisms was performed with the Family-Based Association Test (FBAT) suite (<http://www.biostat.harvard.edu/wfbat/fbat.htm>), version 2.0.4.<sup>11</sup> For quantitative analysis, we used the mean-centered variables of the anxiety, depression, and preoccupations and rituals scores. FBAT analysis was carried out under an additive model using bi-allelic mode. The power of the sample was calculated with Quanto version 1.2 (<http://biostats.usc.edu/software>). Finally, Bonferroni's correction for multiple testing was applied, considering three *HTR1B* gene polymorphisms corrected at  $p \leq 0.016$ .

## Results

Of the study participants with ED, 10% (n=26) met DSM criteria for AN restricting subtype (AN-R), 11% (n=26) for AN binge-eating/purging subtype (AN-BP), 37% (n=92) for BN purging subtype (BN-P), 4% (n=9) for BN non-purging subtype (BN-NP), 15% (n=36) for AN-spectrum EDNOS, and 23% (n=56) for BN-EDNOS. For the purposes of the study, we divided the sample into AN-spectrum (AN-R and EDNOS-AN) and BN-spectrum (AN-BP, BN-P, BN-NP, and EDNOS-BN) groups. The mean age of the ED patients was 18.2 years (standard deviation [SD] = 4.4), mean age of onset was 14.4 (SD = 2.6) years, and median disease duration was 231.7 (SD = 215.1) weeks.

Genotype distribution was in Hardy-Weinberg equilibrium on analysis of the three polymorphisms. Considering the A-161 allele as the risk allele with an additive model and a disease prevalence of 0.01 conferred a statistical power of 0.99 for a significance level of 0.05. There was no evidence of linkage disequilibrium between the three regions ( $D' < 0.013$ ); therefore, we performed a single SNP FBAT analysis.

FBAT analysis of the A-161T polymorphism in 72 heterozygous parents found a preferential transmission of allele A-161 in the overall sample ( $z = 3.23$ ,  $p = 0.0012$ ). However, we did not observe transmission disequilibrium on analysis of the G861C and A1180G variants (Table 1). We performed an additional analysis in the female probands (227 families), which also showed preferential

**Table 1** Family-based association test of *HTR1B* gene polymorphisms

Group/SNP/allele	Frequency	Informative families	z	p-value
ED (overall)				
A-161T				
A	0.692	72	3.232	0.001*
T	0.308	72	-3.232	0.001
G861C				
G	0.608	65	-0.216	0.829
C	0.392	65	0.216	0.829
A1180G				
A	0.745	64	1.897	0.057
G	0.255	64	-1.897	0.057
AN spectrum				
A-161T				
A	0.735	13	1.414	0.157
T	0.265	13	-1.414	0.157
G861C				
G	0.547	14	-0.728	0.466
C	0.453	14	0.728	0.466
A1180G				
A	0.744	17	0.600	0.548
G	0.256	17	-0.600	0.548
BN spectrum				
A-161T				
A	0.677	59	2.871	0.004*
T	0.323	59	-2.871	0.004
G861C				
G	0.623	50	0.000	1.000
C	0.377	50	0.000	1.000
A1180G				
A	0.748	47	1.723	0.084
G	0.252	47	-1.723	0.084

AN = anorexia nervosa; BN = bulimia nervosa; ED = eating disorders; *HTR1B* = serotonin receptor 1D $\beta$  gene; SNP = single-nucleotide polymorphism.

\* Bonferroni's correction:  $p \leq 0.016$ .

transmission of the A-161 allele in 69 informative families ( $z = 3.09$ ,  $p = 0.0019$ ).

In addition, we analyzed allele transmission in the AN-spectrum and BN-spectrum groups. Interestingly, we observed preferential transmission of the A-161 allele in the BN-spectrum group ( $z = 2.87$ ,  $p = 0.004$ ), as well as in analysis of female proband families ( $z = 2.71$ ,  $p = 0.0065$ ), after Bonferroni's correction (Table 1).

Finally, FBAT analysis considering anxiety, depression, and YBC-EDS preoccupations and rituals scores as quantitative traits detected an association between the C861 allele and anxiety in AN-spectrum participants ( $z = 2.453$ ,  $p = 0.014$ ). We did not find any associations on analysis of depression or YBC-EDS preoccupations and ritual scores in the AN-spectrum and BN-spectrum groups (data not shown).

## Discussion

We investigated the role of three SNPs of the *HTR1B* gene in a Mexican population. The results showed transmission disequilibrium between the A-161 allele of the *HTR1B* gene in the overall ED sample and on analysis of the families of female probands. ED is a heterogeneous phenotype; therefore, we decided to stratify the sample into spectrums, and found that differences in A-161 transmission were only observed in the BN spectrum.

Studies using reporter gene assays demonstrated that the A-161 and -261G alleles increase binding of transcription factors in the 5'-UTR region. It has been suggested that inherited allelic variations related with gene expression levels may result in a predisposition to severe diseases.<sup>12</sup> Therefore, our findings might suggest that the A-161 allele in linkage disequilibrium with other variants is implicated in the development of BN subtypes.

Previous studies have found associations between the G861C polymorphism and minimum lifetime BMI,<sup>6</sup> as well as a modulatory effect on OCD syndrome severity in patients with BN.<sup>7</sup> Our group previously reported an association between the G861 allele and severity of obsession in obsessive-compulsive disorder<sup>12</sup>; however, analysis of YBC-EDS scores did not show transmission disequilibrium in the anorexia and bulimia spectrums. Anxiety has been considered a risk trait in anorexia subtypes, suggesting that malnutrition states tends to exaggerate some comorbid behavioral traits.<sup>13</sup> Interestingly, we observed an association between severity of anxiety and the C861 allele in AN-spectrum patients. High levels of anxiety have been reported in patients with ED.<sup>14</sup> In addition, anxious behavior is associated with low BMI in women with AN.<sup>15</sup> Further genetic studies in AN should provide information to clarify the role of the G861C polymorphism in low BMI, providing additional evidence of the involvement of 5-HT1D $\beta$  receptors in appetitive

behavior, and explore the effect of this genetic variant in psychopathological traits, such as anxiety.

In conclusion, the association observed in the present study provides evidence that *HTR1B* may be implicated in the etiology of the BN spectrum, and that a polymorphic variant of this gene appears to be associated with the severity of anxiety in patients on the AN spectrum. Further studies in a larger sample using alternative phenotypes should further elucidate the role of this gene in EDs.

### Acknowledgements

The authors are grateful to all participants. This study was supported by Pfizer Scientific Institute (PSI; grant 2006); Consejo Nacional de Ciencia y Tecnología (CONACyT; grant 52272); Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz; and Instituto de Ciencia y Tecnología del Distrito Federal (ICyTDF; grant DGC-279-2008).

### Disclosure

The authors report no conflicts of interest.

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