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 1Dβ 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-HT1B agonist CP-94,253 into the parabrachial nucleus reduced food intake.1 The human 5-HT1Dβ is encoded by a 1179-bp intronless gene located at chromosome 6q14.1.2 This gene was initially cloned in the rat (HTR1B), and comparison of amino-acid sequences with the human HTR1B gene showed 93% identity;3 indeed, human HTR1B shares more homology with rodent HTR1B than with the human 5-HT1Dα 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-HT1Dβ. 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.4

Studies have demonstrated a possible association of HTR1B with AN.5 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.6 The GG genotype has also been associated with severity of obsessive-compulsive symptoms in patients with BN.7

In addition, serotonin neurotransmission disturbances in patients with ED could be associated with the secondary effects of their nutritional status.8 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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other features associated with low serotoninergic tone. 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).

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 A1180G (rs6297) polymorphisms of the G861C, A-161 in the overall sample (z = 3.23, p = 0.0012). However, we did not observe transmission disequilibrium after ethidium bromide staining.

Analysis of the G861C and A1180G variants (Table 1). Considering three HTR1B gene polymorphisms corrected at p ≤ 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.

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. For qualitative 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 ≤ 0.016.

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
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, depres-
sion, 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 trans-
mision 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 tran-
scription 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.12 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,6
as well as a modulatory effect on OCD syndrome severity
in patients with BN.7 Our group previously reported an
association between the G861 allele and severity of
obsession in obsessive-compulsive disorder12; 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.13 Interest-
 ingly, 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.14
In addition, anxious behavior is associated with low BMI in
women with AN.15 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 receptors in appetitive

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

<table>
<thead>
<tr>
<th>Group/SNP/allele</th>
<th>Frequency</th>
<th>Informative families</th>
<th>z</th>
<th>p-value</th>
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<tr>
<td><strong>ED (overall)</strong></td>
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<td>A-161T</td>
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</tr>
<tr>
<td>A</td>
<td>0.692</td>
<td>72</td>
<td>3.232</td>
<td>0.001*</td>
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<tr>
<td>T</td>
<td>0.308</td>
<td>72</td>
<td>-3.232</td>
<td>0.001</td>
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<tr>
<td>G861C</td>
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<td></td>
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<tr>
<td>G</td>
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<tr>
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<td>47</td>
<td>1.723</td>
<td>0.084</td>
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</table>
| G                | 0.252     | 47                   | -1.723| 0.084   

AN = anorexia nervosa; BN = bulimia nervosa; ED = eating disorders; HTR1B = serotonin receptor 1Dβ gene; SNP = single-nucleotide polymorphism.
* Bonferroni's correction: p < 0.016.
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.

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Disclosure

The authors report no conflicts of interest.

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