GENETICS

Analysis of the serotonin transporter promoter rs25531 polymorphism in premenstrual dysphoric disorder

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OBJECTIVE: The objective of this study was to investigate whether the functional rs25531 promoter polymorphism in the serotonin transporter gene is associated with premenstrual dysphoric disorder.

STUDY DESIGN: The study sample comprised 53 women with clinically diagnosed premenstrual dysphoric disorder (age range, 27–46 years; mean, 37.7 years) and 52 healthy control subjects (age range, 22–48 years; mean, 36.2 years). The rs25531 polymorphism was genotyped in both groups. Because of its close proximity to rs25531, the 5-HTTLPR promoter polymorphism was also genotyped. Genotype and allele frequencies for rs25531 and for the composite 5-HTTLPR/rs25531 marker were analyzed by χ^2 test.

RESULTS: There was no significant association between any genotype and clinical category and no significant allele distribution profiles for rs25531 or 5-HTTLPR/rs25531 in either the premenstrual dysphoric disorder or the control groups.

CONCLUSION: These findings do not support a major role for rs25531, either in isolation or combined with 5-HTTLPR, in contributing to susceptibility to premenstrual dysphoria.

Key words: 5-HTTLPR, premenstrual dysphoric disorder, rs25531, serotonin transporter, triallelic polymorphism

Cite this article as: Magnay JL, El-Shourbagy M, Fryer AA, et al. Analysis of the serotonin transporter promoter rs25531 polymorphism in premenstrual dysphoric disorder. Am J Obstet Gynecol 2010;203:181.e1-5.

Premenstrual syndrome (PMS) is characterized by recurrent psychologic and/or somatic symptoms that occur specifically during the luteal phase of the menstrual cycle and that resolve by menstruation. Premenstrual dysphoric disorder (PMDD) is the extreme, predominantly psychologic end of the PMS spectrum and is estimated to occur in 3–8% of women with PMS.[1] The precise cause of PMDD is unknown. However, several lines of evidence implicate an underlying dysregulation of serotonin (5-hydroxytryptamine, 5-HT) neurotransmission, with the normal fluctuations in circulating ovarian hormones providing the cyclical trigger in susceptible individuals.[2-8]

Evidence from family and twin studies suggests that genetic factors contribute to PMDD.[9-11] Because of the putative involvement of the serotonin system, a number of studies have targeted polymorphisms of genes that regulate the serotonin pathway.[12-16] Most investigations have focused on the serotonin transporter gene (SLC6A4), which encodes a membrane-bound protein localized on the presynaptic terminal of serotonergic neurons. The serotonin transporter (5-HTT) regulates serotonergic neurotransmission by an active reuptake system that transports serotonin from the synaptic cleft into the presynaptic neuron. It is also the target of selective serotonin reuptake inhibitors (SSRIs). These specifically block serotonin reuptake and have been shown to rapidly control somatic and psychologic symptoms of PMDD in up to 70% of cases.[17]

The most frequently studied polymorphism of SLC6A4 is 5-HTTLPR, which is located in the promoter region and comprises a complex of 20–23 base-pair (bp) repeat elements. The 2 most common 5-HTTLPR variants arise from a 43-bp insertion/deletion that produces a long (L) or a short (S) allele. The S variant has been associated with lower basal and transcriptional efficiency of SLC6A4.[18] It has also been linked to major depressive disorders and SSRI response in white subjects, although these findings have not been consistently replicated.[19] This may be partly because of technical problems with certain 5-HTTLPR genotyping protocols, which have caused a marked bias toward S allele identification.[20-22]

Another explanation may be the confounding effect of a functional A/G single nucleotide polymorphism (SNP rs25531) that is located immediately upstream of the 43-bp insertion/deletion site.[23,24] The comparatively rare G variant creates an additional AP2 transcription site and has been shown to lower 5-HTT expression in vitro compared with the more common A allele.[25] Because of their close proximity, 5-HTTLPR and rs25531 are strongly linked. The SNP can be found in the context of both L and S alleles.[23,24,26,27] However, the combination S_G is rare; thus, many studies currently consider 5-HTTLPR as a triallelic marker:

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Received Aug. 4, 2009; revised Nov. 16, 2009; accepted Feb. 16, 2010.

Reprints not available from the authors.

This study was supported by a research grant from the Egyptian Cultural Bureau, London, UK.

0002-9378/$36.00
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doi: 10.1016/j.ajog.2010.02.043

L_A, L_G, and $S_{(A\ or\ G)}$. Functionally, the allelic composites L_G and $S_{(A\ or\ G)}$ are associated with low, nearly equivalent expression of the transporter protein relative to L_A.[25]

In previous studies, we and other groups failed to demonstrate significant 5-HTTLPR genotype and allele differences between women with PMDD and control subjects.[12,13,16] In this study, we determined the rs25531 genotype and allele frequency in our original cohort of regularly ovulating female subjects: a case group with clinically established PMDD and a healthy control group with negligible premenstrual symptoms. We also analyzed the data based on composite 5-HTTLPR/rs25531 functional activity. The purpose of this study was 2-fold. First, to investigate whether rs25531 per se is a risk factor for premenstrual dysphoric disorder. Second, to determine whether reclassification of the 5-HTTLPR L allele into the high-activity L_A and low-activity L_G variants results in a significant association of genotype or haplotype with PMDD. This study represents a natural progression of our previous work using the same gene (SLC6A4) and clinical samples.

MATERIALS AND METHODS

Study population

The study was originally approved by the Staffordshire and Shropshire Ethics Committee and informed written consent was obtained from each participant. Women were recruited from a specialized PMDD clinic, from general gynecology clinics, or after advertisement on the hospital intranet system. One hundred five white European women between the ages of 18–48 years were enrolled from the local population and were categorized into 2 groups: women with PMDD and control subjects. All of the participants reported regular menstrual cycles (28 ± 4 days) and none of the women was taking hormone replacement therapy, oral contraceptives, or psychotropic drugs. Any woman known to have an existing or previous psychiatric disorder was excluded.

In accordance with precise criteria that are outlined in the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV), clinical categorization of PMDD or control subjects was determined by prospective symptom rating with the use of the Daily Record of Severity of Problems (DRSP) scale, based on self-assessment reports that spanned 2 consecutive menstrual cycles.[28] DSM-IV criteria for PMDD require the presence of 5 of 11 specific diagnostic symptoms that are limited to the late-luteal phase of the menstrual cycle. These symptoms must include at least 1 of the following negative mood parameters: marked depression, anxiety/tension, affective lability, and irritability. Day 1 of each cycle was identified by the first day of menses. Symptom ratings of days 6–12 and the 7 days immediately preceding the next menstrual period were used to calculate the mean follicular and mean luteal scores, respectively. Women were diagnosed with PMDD if there was a ≥200% increase in severity of ≥1 or a ≥100% increase of ≥2 PMDD-defining symptoms during the luteal phase compared with the follicular phase in both menstrual cycles. The control group was recruited and diagnosed concurrently with the PMDD group. Control subjects comprised women who reported no significant premenstrual symptoms; the difference between their mean luteal and mean follicular scores was <100% for any of the DRSP-rated symptoms in the 2 monitored cycles.

Procedures

DNA was extracted from leukocytes in ethylenediaminetetraacetic acid-anticoagulated blood with the use of standard techniques. A genomic region of SLC6A4 that encompassed both 5-HTTLPR and rs25531 was amplified in duplicate by polymerase chain reaction (PCR) with the use of the Qiagen HotStar PCR kit (Qiagen, Crawley, West Sussex, UK), as previously described,[12] except that the initial denaturation step was reduced to 5 minutes, and 25 pmol of each primer was used per 25 μL reaction. To genotype 5-HTTLPR into L and S alleles, aliquots of the PCR products were electrophoresed on 2% agarose gels that contained 0.5 μg/mL ethidium bromide, and the bands were viewed under ultraviolet light.

To genotype rs25531 into A and G alleles, PCR products were digested in duplicate with the restriction enzyme *Msp*1, which recognizes and cuts the G variant only. Digests were incubated for 4 hours at 37°C in a total reaction volume of 20 μL that contained 5 μL PCR product, 1X reaction buffer, and 10 units of *Msp*1. Digest products were separated on 2% agarose gels.

The nucleotide sequence of selected genotypes was determined (Geneservice Ltd, Cambridge, UK) to confirm the 5-HTTLPR amplicon size (L = 529 bp, S = 486 bp), the location of the 43-bp insertion/deletion, and the position of the rs25531 A/G single nucleotide polymorphism. All genotypes were independently established by 2 researchers, without prior knowledge of clinical group status.

The genotype and allele frequencies of 5-HTTLPR and rs25531 were recorded separately. Data were then combined to produce a composite 5-HTTLPR/rs25531 classification for the PMDD and control groups. Although distinguishable by restriction digest, any S_A or S_G haplotypes were simply categorized as S, because both are associated with low promoter activity. Thus, the possible 5-HTTLPR/rs25531 genotype combinations were L_A/L_A, L_A/L_G, L_G/L_G, S/S, L_A/S, and L_G/S. Using the convention described by Parsey et al,[29] genotypes were further reclassified according to functional activity: S/S, L_G/S, and L_G/L_G were designated as S′/S′(low), L_A/S and L_A/L_G were designated as L′/S′(intermediate), and L_A/L_A was designated as L′/L′(high).

Statistical analysis

The χ^2 tests were performed to assess conformity to Hardy-Weinberg equilibrium and to detect any association between each genotype distribution and clinical category. To account for the low frequencies in some groups, Fisher's exact probability tests were used to compare genotype distribution between the PMDD and control group with the use of StatXact version 4.0.1 (Cytel Corp, Cambridge, MA). Statistical significance was considered at exact probability values of <.05.

RESULTS

One hundred five European white women were clinically categorized into 2

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