[Abstract:0182] *Genetic psychiatry*

No association of DRD3 and CNR1 polymorphisms in premenstrual dysphoric disorder

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INTRODUCTION: Premenstrual syndrome (PMS) is characterized by recurrent psychological and/or somatic symptoms occurring specifically during the luteal phase of the menstrual cycle and resolving during menstruation. Premenstrual dysphoric disorder (PMDD) is the extreme, predominantly psychological end of the PMS spectrum, and it is estimated that 5-10% of regularly ovulating women experience PMDD. The cause of PMDD is unknown. Studies attempting to elucidate the pathophysiology of the syndrome concentrate on the hypothalamic–pituitary–adrenal (HPA) axis, the γ-aminobutyric acid (GABA) system, the serotonergic system, and the opioid system. The dopamine D(3) receptor gene (DRD3) is a candidate for a number of psychiatric conditions. Rs6280, also known as Ser9Gly, is a SNP in the dopamine receptor D3 DRD3 gene. Polymorphisms in the DRD3 gene have associations with schizophrenia, depression, and nicotine dependence. DRD3Ser9Gly polymorphism affected response to antidepressant treatment in major depressive disorder[2]. The endocannabinoid system is widely distributed throughout the brain and involved in mood and related disorders. Genetic polymorphisms of the endocannabinoid system have been explored in mental disorders[3]. There are currently two known subtypes of endocannabinoid receptors, termed CB1 and CB2. CNR1 polymorphisms were found to be associated with substance use disorders, depression, and anxiety disorders[4]. Rs1049353 and rs12720071 are common variants of the CNR1 gene. Carriers of an rs1049353(G) allele were less likely to respond favorably to antidepressant treatment, particularly if they were females with comorbid anxiety[5]. Since polymorphisms in DRD3Ser9Gly and CB1 receptors seemed to be associated with anxiety and depressive disorders, and as it is known that PMDD shares a range of characteristics with depressive and anxiety disorders, our aim was to investigate whether DRD3Ser9Gly and CB1 receptor polymorphisms are related to PMDD.

METHODS:

Study Population: Patients were recruited from consecutive applications to the Harran University Research Hospital obstetrics and gynecology outpatient clinic. The control group was selected from the staff of the Faculty of Medicine. Fifty-one patients with PMDD and 51 healthy control subjects between the ages of 18 and 45 were included in this cross-sectional study. Anyone having an existing Axis I psychiatric disorder according to the DSM-4 criteria was excluded from the study. Clinical diagnosis was determined according to DSM-IV. Control subjects reported no significant premenstrual symptoms. All subjects were evaluated with a semi-structured interview form to determine their sociodemographic features. This form also evaluates the symptoms of Premenstrual Syndrome (PMS), family history of PMS, and nicotine use. Clinical categorization of PMDD patients and control subjects was determined by prospective symptom rating with the use of the Daily Record of Severity of Problems (DRSP) scale-short form.

Procedures: Venous blood samples were collected in EDTA-containing tubes. DNA was extracted from peripheral blood leukocytes by salting-out procedure.

Genotypic Analysis of the Dopamine Receptor D3 (DRD3) Gene Ser9Gly(rs6280) Polymorphism: Genotypes were determined using a TaqMan™ fluorogenic 5'-nuclease assay with TaqMan Probes. All reactions were carried out following the manufacturer's protocol.

Genotypic Analysis of CNR1 1359 G>A (codon Thr453Thr, rs1049353) Polymorphisms: The genotyping of CNR1 1359 G>A (codon Thr453Thr, rs1049353) polymorphisms was performed using predesigned TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA). The Assays-on-Demand SNP genotyping kit was used for the polymerase chain reaction (Applied Biosystems). Single nucleotide polymorphism amplification assays were performed according to the manufacturer's instructions. All procedures were conducted in a manner blind to the case status and other characteristics of the participants

Statistical Analysis: $\chi 2$ tests were performed to assess conformity to Hardy-Weinberg equilibrium and to detect any association between each genotype distribution and clinical category. Statistical significance was considered at exact probability values of $p<0.05$.

RESULTS: Fifty-one patients with PMDD (age range: 20-46; mean: 30.2) and 51 healthy control subjects (age range: 15-44; mean: 28.0) were included in the study. There was no significant difference in age, BMI, height, weight, or number of children between PMDD group and controls except for marriage rates. 5.9% of PMDD patients were single, against 29.4% of the controls. Allele and genotype frequencies were not different between PMDD patients and controls in DRD3Ser9Gly polymorphism ($\chi 2$: 0.356 and p: 0.837). Allele and genotype frequencies were not different between PMDD patients and controls in CNR1 polymorphism. Genotypes have Hardy-Weinberg equilibrium in DRD3Ser9Gly in the PMDD group (chi-square value=1.65 with 1 DF) but the other genotypes are not in Hardy-Weinberg

equilibrium. There was no significant difference of DRD3Ser9Gly polymorphism between PMDD patients and controls. Similarly, there was no significant difference of CNR1 polymorphism between PMDD patients and controls.

DISCUSSION: We genotyped the DRD3Ser9Gly (rs6280) and CNR1 polymorphisms in two groups of regularly ovulating women, one group with clinically diagnosed premenstrual dysphoric disorder and one group of normal healthy controls with no symptoms of premenstrual dysphoria. We found no association of DRD3Ser9Gly (rs6280) polymorphisms in PMDD. The D3 receptor is a candidate for being involved in mental disorders. Polymorphisms in the DRD3 gene have been studied in various psychiatric disorders. In a study of 88 patients being treated for schizophrenia with olanzapine, those who were rs6280(C;C) homozygote had greater positive symptom remission as compared with (C;T) or (T;T) genotypes. The ser9gly polymorphism has been associated with depression in different studies. A preliminary study showed that DRD3Ser9Gly polymorphism affected response to antidepressant treatment in major depressive disorder. Pharmacogenetic studies have reported that DRD3ser9gly polymorphism influenced antidepressant response in bipolar disorder patients treated with a combination of olanzapine and fluoxetine. Our first finding is the lack of an association of DRD3ser9Gly polymorphism in PMDD, and there is no other study looking for this association. As the etiology of PMDD is multifactorial, dopaminergic pathways may not be solely responsible in the pathophysiology of PMDD. Our second finding is a lack of association between CNR1 polymorphism and PMDD. The endocannabinoid system has been implicated in the pathogenesis of depression and anxiety.

Patients with depression are found to have reduced levels of circulating endocannabinoids, and an up-regulation of CB1R was observed in the prefrontal cortex of subjects with major depression who died by suicide. Since While polymorphism (rs1049353) is associated with depression and anxiety, we did not find an association between CNR1 polymorphism (rs1049353) and PMDD. The endocannabinoid system may not be the sole responsible in the pathophysiology of PMDD.

Previous genetic studies in premenstrual dysphoric disorder were mostly concerned with the serotonergic and noradrenergic systems. To our knowledge, this study is the first reported genotypic analysis of DRD3Ser9Gly (rs6280) and CNR1 polymorphisms in premenstrual dysphoric disorder. There may be several explanations for our negative findings. First, clinical categorization of patients with PMDD can be difficult because of the subjective nature of symptom interpretation. A second limitation is the possibility of population stratification. In studies comprising subjects taken primarily from a localized community, it is important to include healthy controls to determine typical genotype and allelic frequencies, although these may not be representative of the wider population.

Third, the lack of association between the DRD3Ser9Gly (rs6280) and CNR1 polymorphisms and PMDD may be affected by sample size. We were unable to identify either a single genetic marker or a combined polymorphic profile for susceptibility to PMDD. However, it is the first study evaluating DRD3Ser9Glyand CNR1 polymorphisms in PMDD. It is not plausible to expect a single polymorphism to be the sole factor responsible for PMDD. It is likely that PMDD is a polygenic disorder, but the relative contributions of the various implicated genes are unknown. Cautious interpretation of the present study is warranted, both by the preliminary nature of these findings and by their basis in simple association analysis. The polymorphisms that were studied here do not represent major risk factors for PMDD. Confirmation of our findings will require independent validation in a larger group of subjects.

Keywords: cannabinoid receptor, dopamine D3 receptor, premenstrual syndrome, genetic polymorphism

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