

Association of the Muscarinic Cholinergic 2 Receptor (*CHRM2*) Gene With Major Depression in Women

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Cholinergic neurons have been implicated in depression and in the disorders of REM sleep in depression. We examined a common A->T 1890 polymorphism in the 3' UTR of the cholinergic muscarinic receptor 2 (*CHRM2*) gene. There was a significant increase in the frequency of 11 homozygotes in 126 women with major depression (43.7%) compared to 304 women without major depression (25.7%), $P = .001$. There was no increase in the frequency of 11 homozygotes in 52 men with depression (26.9%) compared to 278 men without depression (27.7%). Regression analysis, scoring subjects with the 11 genotype as 1, and those with other genotypes as 0, showed that in women $r^2 = .030$, $F = 13.37$, $P = .0003$. By contrast, in men $r^2 = .00001$, $F = 0.002$, $P = .96$. These results are consistent with a gender-specific role of the *CHRM2* gene in depression in women.

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INTRODUCTION

The lifetime frequency of major depression is twice as high in women as in men [Weisman, 1991]. Twin studies have shown a significant genetic contribution to major depression (MD) in women with heritabilities ranging from .33 to .45 and higher [Kendler et al.,

1993a,b; Bierut et al., 1999; Kendler & Prescott, 1999], and an important role of stress [Kendler et al., 1995, 2000]. Some twin studies have suggested a comparable heritability for men and women [Kendler & Prescott, 1999], while others have suggested a greater heritability for women [Bierut et al., 1999]. The muscarinic cholinergic receptors belong to a larger family of G protein-coupled receptors. The binding of acetylcholine to these receptors leads to further intracellular and intranuclear events in the signal transduction pathway. The cholinergic muscarinic₂ receptor is present throughout the central nervous system. In 1972, 1981, and again in 1994, Janowsky reviewed the evidence for a role of cholinergic hypersensitivity in depression [Janowsky et al., 1972, 1994; Risch et al., 1981]. Both REM and non-REM sleep is regulated by cholinergic, serotonergic, and noradrenergic neurons in the brain stem [Riemann et al., 1994]. The early onset of REM sleep, increased REM density, and exaggerated REM response to cholinergic stimulation, are consistent with CNS cholinergic overactivity or muscarinic supersensitivity in depression [Riemann et al., 1994]. Cholinergic mechanisms have also been implicated in stress. While the HPA axis has been emphasized in the response to stress, recent studies of Kaufer et al. [1998] have identified an important alternative cholinergic pathway. They showed that acute stress resulted in an immediate increase in synaptosomal acetylcholine with neuronal excitability, with a delayed phase response of increased expression of acetylcholinesterase, decreased choline acetyl transferase and vesicular acetylcholine transporter (CHAT) activity, and a resulting decrease in neuronal excitability [Kaufer et al., 1998; Sapolsky, 1998]. Others have also emphasized the important role of stress in activating muscarinic systems [Dilsaver, 1988].

We have recently identified a common single A->T nucleotide polymorphism in the cholinergic muscarinic receptor 2 (*CHRM2*) gene. The above literature on the potential role of defects in muscarinic cholinergic tone in MD led us to examine the possible association between this polymorphism and MD, especially in women.

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METHODS

Subjects

The sample consisted of 760 non-Hispanic Caucasian adults from the Minnesota Twin and Family Study (MTFS) [Iacono et al., 1999]. The MTFS is a large, multi-discipline, multi-year study to examine the interaction between genetic and environmental risk factors in the development of adolescent and adult alcoholism and drug abuse. The advantage of the study is that it uses a population based twin ascertainment in which all same sex twins born in the state of Minnesota are identified by public birth records. The recruitment targets 11- and 17-year-old twins. Of the eligible families only 17% declined invitations to participate. The present study was restricted to the parents of the twins. They were administered the parent version of the Diagnostic Interview for Children and Adolescents (DICA-R) [Welner et al., 1987] and the Structured Clinical Interview for DSM-III-R (SCID-R) [Spitzer et al., 1987]. Interviews were administered by individuals who have a bachelor's or master's degree in psychology or a related field. Interviewers also complete an intensive course of training that includes didactic instruction, practice interviews, mentoring by an experienced clinical interviewer, and a written examination covering the DSM disorders assessed. All interviews are tape-recorded. Complete interviews are reviewed in a consensus conference by at least two advanced clinical psychology graduate students. Individual symptoms are reviewed, including listening to the audiotapes as needed, to determine whether the behaviors reported by the interviewees were frequent and severe enough to count as a symptom under DSM. In a study of the reliability of the diagnostic and consensus procedures that involved review of clinical material by two independent teams for clinicians, the kappa coefficient for the depression diagnosis was estimated at .82 [Iacono et al., 1999]. All patients met criteria for major depression of one or more major depressive episodes of at least two weeks of depressed mood or loss of interest accompanied by at least four additional symptoms of depression. None of the subjects met criteria for bipolar disorder. We genotyped 430 women and 330 men. Of these, 126 of the women and 52 of the men had a DSM-III-R diagnosis of definite lifetime major depression (MD).

Genotyping

Using the SSCP technique [Orita et al., 1989], we identified a common single nucleotide polymorphism, A->T 1890 in the 3' UTR of the *CHRM2* gene, based on accession #M16404. The upstream primer was 5'-ACAAAACGTGCAATTCAGGAG-3'. The downstream primer was 5'-CAGAGACTGATAAAAATTGTAG-3'. The PCR reaction consisted of QIAGEN 10 × PCR buffer 2.0 ul, 0.4 ul of 10 mM dNTP, 0.4 ul of 10 uM each primer, 0.5 units of *Taq* polymerase, ddH₂O 16.1 ul, and 50 ng DNA in total volume of 20 ul. Amplification consisted of 30 cycles with denaturation at 95°C for 30 sec, annealing temperature 54°C for 1 min, and extension at 72°C for 1 min. This produced a 208 bp product. This was digested with *Dpn* II restriction endonuclease using 1 ul 10 × New England Biological (NEB) enzyme buffer, 0.3 ul of *Dpn* II, 3.7 ul of double distilled H₂O, and 5 ul PCR product incubated at 37°C for 3 hr. The products were visualized by electrophoresis in 10% acrylamide gel. The A allele gave 81 and 127 bp products. The T allele gave 58 + 23 and 127 bp products.

Statistics

The frequency of the 11, 12, and 22 genotypes in male and female subjects with and without a diagnosis of MD was compared by Chi square analysis. A Bonferroni corrected α of .025 was used. To determine the percent of the variance of MD, accounted for by the *CHMR2* gene, we scored those with the 11 genotype as 1, and those with the remaining two genotypes as 0. Those without MD were scored as 0, and those with MD as 1. The presence or absence of MD, the dependent variable, and the gene score as the independent variable in a linear regression analysis. The SPSS statistical package was used (SPSS, Inc, Chicago, IL).

RESULTS

The results are shown in Table I. For the women without MD, 25.7% carried the 11 genotype compared to 43.7% for with MD. There was a proportionate decrease in the frequency of the other two genotypes ($\chi^2 = 13.53$, d.f. = 1., $P = .001$). By contrast for men, while the frequency of the 11 genotype in those without MD was similar to that for the women (27.7%), there

TABLE I. Association of the *CHRM2* Gene With Major Depression in Women and Men in the Minnesota Twin and Family Study Database

	N	<i>CHRM2</i> genotypes			χ^2	P
		11	12	22		
Women						
No MD	304	78 (25.7)	159 (52.3)	67 (22.0)		
Definite MD	126	55 (43.7)	49 (38.9)	22 (17.5)	13.53	.001
Total	430					
Men						
No MD	278	77 (27.7)	122 (43.9)	79 (28.4)		
Definite MD	52	14 (26.9)	27 (51.9)	11 (21.2)	1.48	.47
Total	330					

was no increase in frequency for the 11 genotype for those with MD (26.9%, $\chi^2 = 1.48$, d.f. = 1., $P = .47$). The *CHRM2* alleles were in Hardy-Weinberg equilibrium for those without MD. Regression analysis showed that in women, the fraction of the variance of major depression explained by the *CHRM2* gene, r^2 , was .030, $F = 13.37$, $P = .0003$. By contrast, in men $r^2 = .00001$, $F = 0.002$, $P = .96$.

DISCUSSION

While requiring replication in independent samples, these results suggest that *CHRM2* may be a gene associated with MD in women but not in men. This association with MD is consistent with the postulated role of enhanced or hypersensitive cholinergic systems in depression [Janowsky et al., 1972, 1994]. The association of cholinergic systems with REM sleep [Gillin et al., 1979; Riemann et al., 1994], and the disturbance of REM sleep in individuals with depression are consistent with the presence of genetic defects in the cholinergic system in MD.

In women, the *CHRM2* gene accounted for 3% of the variance of MD while in men it accounted for virtually none of the variance. The increased frequency of MD in females and some twin studies [Bierut et al., 1999], suggesting a greater heritability of MD in women, raises the possibility that the *CHRM2* is a gender-specific gene for MD. While the mechanism by which a non-X-linked gene would have this effect is not known, there are several possibilities. These include hormone responsive promoters or enhancers, affecting either *CHRM2* or other genes that interact with *CHRM2*. A second possibility is that if women are more sensitive to stress expressed through the cholinergic stress pathway [Dilsaver, 1988; Kaufert et al., 1998; Sapolsky, 1998], they would also be more likely to show an association between the *CHRM2* gene and depression.

Since the A/T polymorphism was in the 3' region of the *CHRM2* gene we assume it was in linkage disequilibrium with alleles affecting gene function, possibly microsatellites [Comings, 1998]. Although the *CHRM2* gene accounted for only 3% of the variance of MD in women, this association was significant. This is an expected and characteristic aspect of a complex polygenic disorder where multiple genes are involved and each gene accounts for only a small percent of the total variance. In our experience, 3% is a higher than the average r^2 for most gene—phenotype associations for behavioral disorders [Comings et al., 2000a,b,c; Comings et al., 2001].

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