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Association study of CREB1 with Major Depressive Disorder and related phenotypes†,‡

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Abstract

Cyclic AMP response element binding protein (CREB) has been implicated in behavioral models of anxiety and depression, antidepressant response in humans, and suicide. One group reported a female-specific association of the *CREB1* gene in early-onset Major Depressive Disorder (MDD), while another found no evidence of association with this phenotype. In this study, we sought to examine the evidence for association of the *CREB1* gene to MDD and related phenotypes. We used multivariate structural equation modeling to identify and select twin pairs that scored at the extremes of a latent genetic risk factor shared by MDD, neuroticism, and several anxiety disorders from the Virginia Twin Registry. Using one member from each of these pairs, the resulting sample of 589 cases (including 473 subjects with lifetime MDD) and 539 controls were entered into a 2-stage association study in which genetic markers were screened in stage 1, the positive results of which were tested for replication in stage 2. Eight SNP markers selected to capture the major allelic variation across the haplotype block containing *CREB1* were analyzed for differences between cases and controls. Several markers showed criterion differences between cases and controls in the stage 1 sample with some evidence of sex specific effects. However, none of these markers were significant in stage 2 in either sex individually or combined. Our data suggests that common variations in the *CREB1* gene do not appear to increase susceptibility for MDD or related phenotypes.

Keywords

CREB; depression; genetic association

Cyclic AMP response element binding protein (CREB) plays a central role in intracellular signal transduction. It has been implicated in behavioral models of anxiety and depression [Hebda-Bauer et al., [2004]; Wallace et al., [2004]; Blundell and Adamec, [2006]],

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antidepressant response [Chen et al., [2001]; Lai et al., [2003]; Blendy, [2006]; Iga et al., [2007]; Wilkie et al., [2007]], and suicide [Dwivedi et al., [2003]]. Several groups have attempted to investigate the potential role of *CREB1*, the human gene coding for CREB, in the etiology of Major Depressive Disorder (MDD). Zubenko et al. [2002] reported female-specific linkage to *CREB1* in families with recurrent, early-onset MDD, with follow-up analyses that implicated variations in *CREB1* promoter polymorphism [Zubenko et al., [2003]]. However, no association was found with *CREB1* variants in another study using two samples of subjects with childhood-onset mood disorders [Burcescu et al., [2005]]. Within subjects with MDD, Perlis et al. recently reported male-specific associations of *CREB1* with anger expression [Perlis et al., [2007a]] and the emergence of suicidal ideation during antidepressant treatment [Perlis et al., [2007b]].

In this study, we sought to investigate the potential role of *CREB1* in susceptibility to MDD and related phenotypes. Given that no specific polymorphisms in this gene have displayed consistent relationships with MDD liability, we included several SNPs characterizing the major allelic variation across the *CREB1* locus. Further, we performed planned sex-specific analyses in response to prior reports of significant sex differences.

The subjects in this study derive from the longitudinal population-based Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (VATSPSUD) [Kendler and Prescott, [1999],[2006]] All subjects were Caucasian (by self-report) and born in Virginia. Their age (mean, SD, range) at time of last interview was (37, 9, 20-58) for males and (36, 8, 21-62) for females. Approval of the local Institutional Review Board was obtained prior to the study and informed consent was obtained from all subjects prior to data collection.

We obtained lifetime psychiatric diagnoses via face-to-face or telephone structured psychiatric interview based on the Structured Clinical Interview for DSM-III-R (SCID) [Spitzer and Williams, [1985]]. We used DSM-III-R [American Psychiatric Association, [1987]] diagnostic criteria to assess lifetime MDD, modified DSM-III-R criteria for lifetime generalized anxiety disorder (GAD) and panic disorder [Hettema et al., [2001]; Kendler et al., [2001a]], and an adaptation of DSM-III criteria for phobias [American Psychiatric Association, [1980]; Kendler et al., [2001b]]. Neuroticism was assessed using the 12 items from the short form of the Eysenck Personality Questionnaire (EPQ) [Eysenck and Eysenck, [1975]] via self-report questionnaire.

The phenotypic characterization and subject selection scheme, as previously described for this sample [Hettema et al., [2006a]], utilizes several potentially powerful strategies for detecting genes related to psychiatric phenotypes. First, it takes advantage of the genetic information inherent in a large, population-based twin sample to select subjects for genotyping based upon their genetic risk for the phenotype of interest rather than the measured phenotype itself. For example, individuals may meet diagnostic criteria for MDD due to non-genetic risk factors, thus reducing the power of a typical case-control study aiming to detect associations with liability genes. Second, the extant literature suggests moderate overlap in genetic susceptibility between MDD, some anxiety disorders, and neuroticism [Jardine et al., [1984]; Scherrer et al., [2000]; Middeldorp et al., [2005]; Hettema et al., [2006b]]. Combining phenotypic information across these internalizing phenotypes in a genetically informative manner may provide a better target for association analyses than a single, clinically defined disorder. Starting with a total of 9,270 twin subjects, we used multivariate structural equation modeling to estimate a latent genetic factor for neuroticism that is highly correlated with genetic susceptibility to MDD and several anxiety disorders [see Hettema et al., [2006b] for details]. One member from each twin pair for whom DNA was available was selected as a case or control based upon the pair scoring above the 80th or below the 20th percentile, respectively, of the genetic factor extracted from the above analysis. Thus, subjects selected for genotyping and their co-twins were determined to be high (cases) or low (controls) on genetic susceptibility for several highly related

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internalizing phenotypes. This produced a total sample of 1,128 independent subjects for genotyping, consisting of 589 cases (350 males, 239 females) and 539 controls (343 males, 196 females). Overall, the cases had a mean raw neuroticism score of 6.3 (z-score = 1.04) and had the following frequencies of the target psychiatric conditions: MDD (80.3%), GAD (53.8%), panic disorder (20.5%), agoraphobia (14.1%), and social phobia (17.5%). Specifically relevant for this study, the cases included 473 subjects with lifetime history of MDD. The controls were free of these five disorders and had a mean raw neuroticism score of 0.55 (z-score = − 0.89).

As also described previously, we used a 2-stage association design in which candidate loci were initially screened in the stage 1 sample [Hettema et al., [2006a]]. If any of the markers genotyped in stage 1 met the threshold P-value of 0.1 or less (chosen to balance the proportion of Type 1 and Type 2 errors), they were then also tested for confirmation of association in the stage 2 sample. Of the 1,128 twin subjects we selected for genotyping, 376 (196 males, 180 females) and 752 (497 males, 255 females) were used in stage 1 and stage 2 respectively. The parameters for this design were calculated using the LGA972 program [Robles and van den Oord, [2004]] to achieve 80% overall power to detect markers that explained 1-2% of the variance of the liability distribution while controlling the false discovery rate at 10% [van den Oord and Sullivan, [2003]]. The subjects' neuroticism scores and rates of specific psychiatric disorders were similar across the two stages. We used Pearson's Chi-squared tests to test for allelic or genotypic differences by marker between cases and controls, separately by stage in order to check for consistency of results across the two stages. We used the program PEDSTATS [Wigginton and Abecasis, [2005]] to test for Hardy-Weinberg equilibrium (HWE) violations and HAPLOVIEW 3.2 [Barrett et al., [2005]] to characterize linkage disequilibrium (LD) between the markers in our sample.

CREB1 spans a 68.9 kb interval on human chromosomal region 2q33.3 and is contained in a 122 kb haplotype block together with the uncharacterized locus LOC15119A. We selected SNP markers in this block, including the putative *CREB1* promoter region, with the aim to tag the major haplotypes observed in the Caucasian panel from the International HapMap Project [2003]. We used the Tagger module of HAPLOVIEW 3.2 [Barrett et al., [2005]] with HapMap Phase II data, specifying pair-wise tagging and a threshold of $r^2 = 0.8$. We selected a total of 8 intronic SNPs that captured the 52 HapMap alleles with MAF >0.05 in that interval (mean $r^2 = 0.98$).

DNA was extracted from buccal epithelial cells obtained via cytology brushes [Straub et al., [1999]]. SNPs were genotyped by the 5′ nuclease cleavage assay (TaqMan method) [Livak, [1999]]. Reactions were performed in 384-well plates with 5 μl reaction volume containing 0.25 μl of 20x Assays-on-Demand™ SNP assay mix, 2.5 μl of TaqMan universal PCR master mix, and 5 ng of genomic DNA. Each 384-well plate contains intercalated 96-well sections of cases and controls to reduce the risk of batch effects differentially affecting these two groups. The conditions for PCR were initial denaturizing at 95°C for 10 min, followed by 40 cycles of 92°C for 15 sec and 60°C for 1 min. After the reaction, fluorescence intensities for reporter 1 (VIC, excitation = 520 ± 10 nm, emission = 550 ± 10 nm) and reporter 2 (FAM, excitation = 490 ± 10 nm, emission = 510 ± 10 nm) were read by the Analyst fluorescence plate reader (LJL Biosytems, Sunnyvale, CA). We performed duplicate genotyping on a subset of plates as a quality control check and for any assays that did not perform optimally. Our average genotyping success and replication rates were 96% and 99%, respectively.

The genotype and allele frequencies for these markers in our stage 1 sample and results of chi-squared association tests are listed in Table I. In order to conserve space and simplify the table, the genotype and allele frequencies are only shown for the combination of males and females (the values broken down by sex are available upon request). P-values are shown for the entire

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stage 1 sample and broken down by sex to indicate from which group significance may derive. The observed LD structure was generally consistent with that found in the HapMap samples (available upon request).

Markers 4 and 8 met threshold criteria of allelic *P*-value <0.1 in our entire stage 1 sample, with the association signal deriving from the male subjects. Additionally, markers 1 and 3 achieved $P < 0.1$ for male subjects and marker 5 achieved $P < 0.1$ for female subjects, so we genotyped these five markers in stage 2. However, none of these markers were significantly associated with case status in either the entire stage 2 sample or when the analysis was limited by sex (Supplementary Table). Further, two- and three-marker sliding window haplotype analyses using these markers showed no evidence of association in the stage 2 sample (results available upon request). In order to test for specific association with the MDD phenotype, we performed a pooled analysis across both stages including only the subjects with MDD as cases (N = 473) versus controls (N = 539). No association was detected with any of these five markers, including analyses performed separately by sex.

Our inability to detect an association between common variants in the *CREB1* gene and MDD is consistent with the null findings reported in the single previously published association study of *CREB1* and liability to mood disorders [Burcescu et al., [2005]]. Our results need to be considered within the limitations of the present study. First, although this study contains the largest sample of MDD cases thus far used to examine the role of *CREB1* in its etiology, it may still have been underpowered to detect association with alleles of very small effect. Second, we chose common SNPs (MAF > 0.05) across the *CREB1* gene using a tagging strategy rather than genotyping specific functional variants or polymorphisms tested in prior studies. This scheme could miss the effects of rare variants or SNPS not in strong LD with those chosen. For example, Zubenko et al. [2003] observed that their strongest signal derived from a rare variant in the promoter region of *CREB1* that contributed to linkage in only two of their multiplex families segregating recurrent MDD. This marker was found to be monomorphic in the subjects of the study of childhood-onset mood disorders by Burcescu et al. [2005], making it a poor candidate to select for a population-based sample such as that used in the current study. Third, the subjects were not originally selected for presence or absence of MDD but, rather, for a genetic liability to internalizing phenotypes including MDD. While this resulted in most of the cases being affected with lifetime MDD and all of the controls unaffected, most likely for genetic reasons, this differs from the design of prior studies. Fourth, our analyses tested only for main effects of the *CREB1* variants and accounted only for differences based upon sex without considering other potential sources of heterogeneity. Within these potential limitations in mind, although extant research implicates CREB in several depression-related phenotypes, this study suggests that common variations in the *CREB1* gene do not increase susceptibility for MDD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table I

CREB1 Single Marker Association Results for Stage 1 (N = 188 Cases, 188 Controls) for Males [M] (N = 196), Females [F] (N = 180), and Together [All]

				Genotypes (%)			Genotypic *P*-value			Alleles (%)		Allelic *P*-valued		
Marker	**Marker ID (dbSNP)**	**Alleles (major)**	**Group**	**A1/A1**	**A1/A2**	**A2/A2**	**All**	**M**	**F**	**A1**	**A2**	**All**	**M**	**F**
1	rs2253206	A/G (G)	Cases	25.4	53.0	21.6	0.35	0.082[a]	0.55	51.9	48.1	0.19	0.026[a]	0.68
			Controls	32.1	49.2	18.7				56.7	43.3			
2	rs2709356	C/T (C)	Cases	63.6	31.0	5.4	0.27	0.79	0.25	79.1	20.9	0.26	0.68	0.23
			Controls	67.0	30.8	2.2				82.4	17.6			
3	rs11904814	G/T (T)	Cases	45.7	45.7	8.6	0.17	0.22	0.33	68.5	31.5	0.12	0.082[a]	0.68
			Controls	55.3	36.7	8.0				73.7	26.3			
4	rs6740584	C/T (T)	Cases	31.0	49.2	19.8	0.078[a]	0.19	0.25	55.6	44.4	0.023[a]	0.085[a]	0.14
			Controls	22.5	49.7	27.8				47.3	52.7			
5	rs17811997	C/G (G)	Cases	86.2	13.3	0.5	0.35	0.78	0.17	92.8	7.2	0.18	0.79	0.093[a]
			Controls	80.7	18.8	0.5				90.0	10.0			
6	rs13029936	C/T (C)	Cases	65.2	30.4	4.4	0.83	0.79	0.89	80.4	19.6	0.90	0.80	0.65
			Controls	64.9	31.9	3.2				80.8	19.2			
7	rs1806584	A/G (A)	Cases	64.0	30.1	5.9	0.18	0.53	0.26	79.0	21.0	0.37	0.76	0.32
			Controls	65.4	32.4	2.2				81.6	18.4			
8	rs2551941	A/T (T)	Cases	39.8	48.9	11.3	0.034[a]	0.028[a]	0.62	64.3	35.7	0.022[a]	0.019[a]	0.40
			Controls	53.2	37.6	9.4				72.0	28.0			

[a] *P*-values that met the stage 1 screening threshold $P < 0.1$.