



ORIGINAL RESEARCH ARTICLE

Lack of linkage between the corticotropin-releasing hormone (CRH) gene and bipolar affective disorder

CA Stratakis^{1,5}, NJ Sarlis², WH Berrettini⁴, JA Badner³, GP Chrousos¹, ES Gershon³ and SD Detera-Wadleigh³

¹Developmental Endocrinology Branch (DEB), National Institute of Child Health and Human Development (NICHD);

²Laboratory of Molecular and Cellular Biology (LMCB), National Institute of Diabetes and Digestive and Kidney Diseases

(NIDDK); ³Clinical Neurogenetics Branch (CNB), National Institute of Mental Health (NIMH), National Institutes of Health

(NIH), Bethesda, MD 20892; ⁴Department of Psychiatry and Human Behavior, Thomas Jefferson Medical College,

Philadelphia, PA 19107; ⁵Genetics & Endocrinology, Georgetown University Children's Medical Center, Washington, DC 20007, USA

Corticotropin-releasing hormone (CRH) plays a key role in the regulation of the stress response. Abnormalities in CRH secretion have been documented in both the depression and manic phases of bipolar disorder (BPD). In the present study, we investigated genetic linkage between the CRH gene and BPD in 22 pedigrees. A highly informative, short tandem repeat (STR) polymorphism adjacent to the CRH gene on human chromosomal region 8q13 was used to examine linkage. Affected sibling pair (ASP) and the likelihood-based disequilibrium tests revealed nonsignificant values. We conclude that the CRH gene is not linked to BPD; if genes involved in the regulation of stress response are indeed linked to BPD, the search should be directed towards those that regulate CRH secretion or its effects on target tissues.

Keywords: corticotropin-releasing hormone (CRH) gene; linkage; bipolar disorder; stress; chromosome 8q13; dinucleotide repeat polymorphism

Introduction

Evidence from twin, family, and adoption studies has suggested that BPD is, in part, a heritable clinical syndrome, albeit with complex genetics, characterized by variable transmission, age of onset and penetrance.^{1–5} Systematic searches of the human genome have recently revealed susceptibility loci for BPD on chromosomes 18, 21 and possibly X.^{6–16} Due to the large number of genomic regions possibly involved in the expression of the disease, the construction of an exclusion map of the genome alongside that of the linked areas, has also been proposed.¹⁷ More recently, evidence was provided that chromosomal regions 6p24, 13q13 and 15q11 harbor susceptibility loci for BPD in the Old Order Amish population;¹⁶ a locus on chromosome 4p has also been reported to be linked strongly with BPD.¹⁰

Among the biologic markers most closely associated with BPD, are those that relate to the regulation of the stress response.^{18,19} During the depressive and manic episodes of active disease, patients with BPD experience changes in appetite, sleep, and sexual behavior, and exhibit a constellation of endocrine abnormalities

compatible with changes in the activity of the hypothalamic-pituitary-adrenal (HPA) axis.^{20–23} Corticotropin-releasing hormone (CRH) is a main regulator of the HPA axis; CRH-secreting neurons are present throughout the central nervous system (CNS) and its receptors are expressed not only in the pituitary and hypothalamus, but also elsewhere in the CNS.²⁴ CRH hypersecretion has been demonstrated to be associated with depression,^{25–27} and CRH administration to experimental animals reproduced the stress response;²¹ moreover, the number of CRH neurons in the CNS of depressed individuals is increased.²⁸ The possible mechanisms involving dysregulated CRH secretion in BPD are numerous.^{21,22} There could be an abnormality in the CRH gene itself, or the genes of its secretagogues. It could also be a defect in the cellular transduction mechanisms between any of these substances and CRH, or an abnormality of one of the CRH receptors and/or their signaling systems.

We undertook this study to investigate whether there is genetic linkage between the CRH gene and BPD. For this purpose, a highly informative, short tandem repeat (STR) polymorphism adjacent to the corticotropin-releasing hormone (CRH) gene on human chromosomal region 8q13 was used in a panel of 22 families with this condition.

Correspondence: Dr CA Stratakis, DEB, NICHD, National Institutes of Health, Bldg 10, Rm 10 N 262, 10 Center Drive, MSC 1862, Bethesda, Maryland 20892–1862, USA. E-mail: stratak@ccl.nichd.nih.gov

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Materials and methods

Pedigrees

We studied 22 medium-size pedigrees, each consisting of at least five individuals with BPD. A total of 395 informative individuals were tested; 167 subjects were affected, as previously described.⁸ Diagnoses were based on personal semi-structured interviews, medical records, and information from relatives.

Marker locus

A highly informative, dinucleotide [(CA)*n*] polymorphism adjacent to the CRH gene on human chromosome 8 (cytogenetic band 8q13) was used.²⁹ This short tandem sequence (STS) represents a 144-bp fragment amplified by the following primers: 5'-CCCAGTCCCCATGATATCAG-3' (CA strand) and 5'-AACTTTCCACCAGTAATGCC-3' (GT strand). The heterozygosity for this marker was 0.72 with nine observed alleles. Polymerase chain reaction (PCR) conditions were as previously described.²⁹

Genotyping

DNA was extracted from immortalized lymphoblastoid cell lines or from peripheral blood samples and was amplified by PCR. The samples were run on 6% polyacrylamide gels, which were dried and exposed to autoradiography, as described.^{8,30,31} Genotypes were read by two readers and matched by computer. Discrepancies were resolved by reference to a third reader. Readers had no knowledge of the diagnosis of a given individual.

Linkage analysis

Non-parametric methods were applied.³²⁻³⁶ We computed the affected sibling pair (ASP) statistics that test whether ASPs have a mean proportion of marker genes identical-by-descent (IBD) that is greater than 50%.³³ The sib-pair statistics were computed (using all the available data on the sibship and their parents) by using the SIBPAL program (version 2.5.1) of the SAGE package, as described before.^{8,30-34} A likelihood-based approach to testing for linkage disequilibrium [likelihood-ratio statistic (LRT)] was also applied to these data, as recently reported.³⁵

Results

We used LRT analysis to take into account potential heterogeneity on our data set. However, the test produced nonsignificant values (data not shown). Similarly, ASP analysis was nonsignificant.

Discussion

Although the hypothesis that there is a single major locus accounting for the majority of inherited BPD cases has been rejected, the candidate gene approach is still valid in the search of responsible genetic defects in complex diseases, if heterogeneity is taken into account.¹⁷ The best approach to candidate gene studies

in complex traits is probably to conduct association studies using intragenic polymorphisms. In the absence of the latter, polymorphisms that are proximal to the candidate genes can be used.

The components of the HPA axis have long been considered candidates for BPD, since this disease is associated with dysregulation of the HPA axis-controlled stress response.²¹⁻²³ Thus, hypercortisolemia and/or loss of the normal circadian rhythm, failure to suppress plasma cortisol after dexamethasone administration, enlarged adrenal glands, elevated cerebrospinal fluid CRH levels, and a blunted response to exogenous administration of CRH, are all findings in depression.^{18,19,21} Some components of the axis have already been tested for linkage. Thus, the glucocorticoid receptor gene, on chromosome 5, was excluded as a candidate for BPD, along with the genes for β_2 - and α_1 -adrenergic receptors, on the same chromosome based on parametric lod score analyses.³⁰ Similarly, parametric methods of analysis excluded the area of the proopiomelanocortin (POMC) gene on chromosome 2.³¹ Although chromosome 18 was linked to BPD,⁷⁻⁹ mutations in the gene for the adrenocorticotropin receptor (MC2R), located on chromosome 18p11.2,³⁷ have not been found in patients with this disorder (Detera-Wadleigh *et al*, unpublished data).

Chromosome 8 harbors the gene for CRH (band 8q13)³⁸ and this chromosome has not been studied extensively for linkage with BPD. The present study, using a dinucleotide repeat marker proximal to the CRH gene,²⁹ also failed to establish linkage of the CRH locus with BPD. Although a weak effect of the CRH gene in the expression of BPD can not be entirely excluded due to the modest sample size used in the study,³⁹ our findings suggest that genetic defects in the expression of other molecules (including those that participate in the function of the HPA axis) may be responsible for BPD.

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