ORIGINAL ARTICLE

Operation of the Schizophrenia Susceptibility Gene, Neuregulin 1, Across Traditional Diagnostic Boundaries to Increase Risk for Bipolar Disorder

Elaine K. Green, PhD; Rachel Raybould, BSc; Stuart Macgregor, PhD; Katherine Gordon-Smith, BSc; Jess Heron, BA; Sally Hyde, BSc; Detelina Grozeva, MSc; Marian Hamshere, PhD; Nigel Williams, PhD; Michael J. Owen, PhD, FRCPsych; Michael C. O'Donovan, PhD, FRCPsych; Lisa Jones, PhD; Ian Jones, PhD, MRCPsych; George Kirov, MD, MRCPsych; Nick Craddock, MB, PhD

Context: Family and twin data suggest that, in addition to susceptibility genes specific for bipolar disorder or schizophrenia, genes exist that contribute to susceptibility across the traditional kraepelinian divide. Several studies have provided evidence that variation at the neuregulin 1 (*NRG1*) gene on chromosome 8p12 influences susceptibility to schizophrenia. The most consistent finding has been that one particular haplotype (the "core" haplotype) is overrepresented in cases compared with control subjects.

Objective: To investigate the possible role of *NRG1* in bipolar disorder.

Design: Genetic case-control association analysis.

Setting: Subjects were unrelated and ascertained from general psychiatric inpatient and outpatient services.

Participants: Five hundred twenty-nine patients with *DSM-IV* bipolar I disorder and 1011 controls from the United Kingdom (100% white).

Methods: We genotyped the markers constituting the *NRG1* core haplotype in cases and controls and reanalyzed our existing data from 573 *DSM-IV* schizophrenia cases with this larger set of controls.

Results: We found a significant difference in haplotype distribution between bipolar cases and controls globally ($P=.003$) and specifically for the core haplotype. Frequencies were 10.2% for bipolar cases and 7.8% for controls (effect size, as measured by odds ratio [OR], 1.37; 95% confidence interval [CI], 1.03-1.80; $P=.04$). The effect size in our bipolar sample was similar to that in our schizophrenia sample (OR, 1.22; 95% CI, 0.92-1.61). In the bipolar cases with predominantly mood-incongruent psychotic features (n=193), the effect was greater (OR, 1.71; 95% CI, 1.29-2.59; $P=.009$), as was the case in the subset of schizophrenia cases (n=27) who had experienced mania (OR, 1.64; 95% CI, 0.54-5.01).

Conclusions: Our findings suggest that neuregulin 1 plays a role in influencing susceptibility to bipolar disorder and schizophrenia and that it may exert a specific effect in the subset of functional psychosis that has manic and mood-incongruent psychotic features.

Arch Gen Psychiatry. 2005;62:642-648

Author Affiliations:
Department of Psychological Medicine (Drs Green, Hamshere, Williams, Owen, O'Donovan, I. Jones, Kirov, and Craddock and Mss Raybould and Grozeva) and Biostatics and Bioinformatics Unit (Drs Macgregor and Hamshere), University of Wales College of Medicine, Cardiff; and Division of Neuroscience, Department of Psychiatry, University of Birmingham, Queen Elizabeth Psychiatric Hospital, Birmingham, England (Dr L. Jones and Mss Gordon-Smith, Heron, and Hyde).

BIPOLAR DISORDER (MANIC-depressive illness) has a lifetime prevalence of approximately 1% and is characterized by disturbances in mood ranging from extreme elation or mania to severe depression. Family, twin, and adoption studies provide evidence that genetic factors are important in determining susceptibility and that there is a range in phenotypic expression of this genetic predisposition.[1] Although single genes may play a major role in a few families, in the wider population the bipolar inheritance is consistent with the actions of multiple genes of small to moderate effect and environmental factors.[2]

Psychiatric research in general, and the search for predisposing genes in particular, has proceeded under the assumption that schizophrenia and bipolar disorder are separate disease entities with separate underlying etiologies (and treatments), ie, the kraepelinian dichotomy. This distinction has pervaded Western psychiatry since the influential nosologic writings of Kraepelin[3] and survives in current operational classification systems such as the *DSM-IV*,[4] *International Classification of Diseases, 10th Revision* (*ICD-10*),[5] and the Research Diagnostic Criteria (RDC).[6] However, the clinical reality is that many individuals with severe psychiatric illness have features that fall between these 2 extremes and have mood and psychotic features (often classified as schizoaffective disorder or some similar atypical diagnosis), which raises the possibility, per-

haps likelihood, that there is not a neat biological distinction between schizophrenia and bipolar disorder. Some workers, such as Crow,[7] have argued strongly in support of a continuum approach to psychosis. The likelihood of a genetic overlap across the kraepelinian divide finds support in several observations from genetic research. First, although family studies have shown substantial consistency in demonstrating that schizophrenia and bipolar disorder tend to "breed true,"[8-10] some studies have also shown statistically significant evidence that bipolar disorder occurs at increased frequency in the relatives of probands with schizophrenia[11] and that schizophrenia occurs at increased frequency in the relatives of bipolar probands.[12] Moreover, schizoaffective disorder has been shown to occur at an increased rate in the families of probands with schizophrenia[13] and bipolar disorder,[14] and schizophrenia and bipolar disorder have been shown to occur at an increased rate in the families of probands with schizoaffective disorder.[14] These family data argue for a more complex relationship between the psychoses than is reflected in the conventional dichotomous view. Second, a recent twin study that used an analysis unconstrained by the diagnostic hierarchy inherent in current classification systems (ie, the principle that schizophrenia trumps mood disorder in diagnosis) demonstrated an overlap in the genetic susceptibility to mania and schizophrenia.[15] A graphic illustration of the varied expression of the same set of susceptibility genes is provided by the Maudsley triplets—a set of genetically identical triplets, of whom 2 had an RDC lifetime diagnosis of schizophrenia and the third an RDC[6] lifetime diagnosis of bipolar I disorder.[16] Third, genetic linkage studies have identified some chromosomal regions that show convergent or overlapping regions of interest in both disorders, including regions of chromosome 13q, 22q, and 18.[17,18] Fourth, recent reports implicating variation at the gene encoding D-amino-acid oxidase activator (*DAOA*; formerly known as the *G72/G30* locus) on chromosome 13q in schizophrenia[19,20] and bipolar disorder[20-22] suggest that the substantial circumstantial and genetic evidence is starting to gain molecular genetic support.

Given this background, it is important that any gene found to influence susceptibility to schizophrenia should also be examined in bipolar disorder. Herein we report the first study, to our knowledge, of bipolar disorder with polymorphisms in the neuregulin 1 (*NRG1*) gene. Neuregulin 1 is highly expressed in the brain and is a member of a family of proteins that signal through tyrosine kinase receptors and thereby exert a key role in neurodevelopmental processes in the central nervous system that are thought to be relevant to the pathogenesis of schizophrenia, including (1) neuronal migration and specification, (2) hormonal controls of puberty, (3) regulation of acetylcholine, γ-aminobutyric acid, and glutamate, and (4) oligodendrocyte development.[23,24] The *NRG1* gene maps to chromosome 8p12, a region implicated in several genome scans for schizophrenia (locus *SCZD6* [Mendelian Inheritance in Man No. 603013]).[25-29] After their initial evidence for linkage of schizophrenia to 8p21-p12 in a large number of multigeneration families from Iceland, Stefansson and colleagues[25] performed case-control and family-based association analyses and obtained evidence for association between schizophrenia and several haplotypes at *NRG1*. The entire exonic sequence of *NRG1* was extensively screened for mutations in 184 patients with schizophrenia, but despite identifying a large number of variants, it was not possible to find any that were unambiguously associated with schizophrenia. The most important finding, however, was the identification of a single "core at-risk haplotype" (henceforth referred to as the core haplotype), which was significantly overrepresented in individuals with schizophrenia compared with controls (15.4% vs 7.5%; $P=6.7\times10^{-6}$). This has been replicated in a large case-control sample from Scotland[30] and our own sample from the United Kingdom[31] demonstrating that the risk haplotype is not specific to the Icelandic population. A study of Japanese schizophrenia cases and controls failed to find significant evidence for association with the core haplotype but, consistent with other studies, the core haplotype was more common in familial cases than controls (7.7% vs 5.1%).[32] Evidence for linkage disequilibrium between schizophrenia and different haplotypes that overlap the core haplotype has been reported in an Irish case-control sample,[33] 3 Han Chinese samples,[34-36] and a South African sample but not in a US sample.[37] A recent analysis of the Irish high-density schizophrenia families found no clear-cut evidence of linkage disequilibrium between schizophrenia and haplotypes at *NRG1*. The full-core haplotype itself was not examined in that study, but sliding window analysis of haplotypes across the core haplotype region showed modest nominally significant evidence for association with some haplotypes.[38] A schematic representation of the *NRG1* gene and previous findings is shown in **Figure 1**.

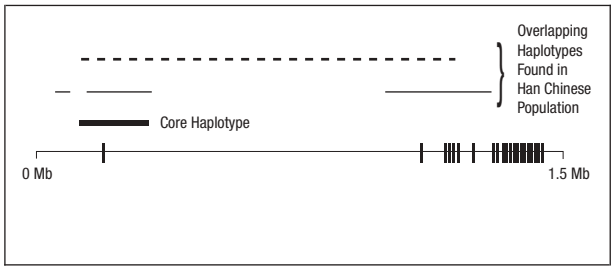

Figure 1. Schematic diagram (not to scale) of the neuregulin 1 gene structure showing the position of each exon (black bars) included in reported isoforms[39] and intervening intronic sequence. The position of the "core at-risk haplotype" (core haplotype) investigated in the present study is indicated by a thick horizontal bar and is defined by a minimal haplotype of 1 single nucleotide polymorphism (SNP) (SNP8NRG221533) and 2 microsatellites (478B14848 and 420M91395). There is evidence of association of this haplotype with schizophrenia in several case-control samples, mainly of European origin. The overlapping but more extended haplotypes identified in Chinese populations are shown in the thin bar[35] and dashed bar.[34] In our sample, the linkage disequilibrium (D′) between the SNP and microsatellites (in the order listed above) was D′=0.25 and D′=0.30, respectively; between the 2 microsatellites, D′=0.71. Mb indicates megabase.

Given that we found supportive evidence for the association with the core haplotype in our schizophrenia sample, we have used our similarly recruited bipolar sample to examine the hypothesis that the core haplotype is also associated with risk for bipolar disorder in general and a subset with phenotypic features related to

schizophrenia in particular. Within current classifications, 1 of the key features of schizophrenia is the presence of psychotic features that are mood incongruent, ie, they are not understandable within the context of mood state.[4,5] We therefore hypothesized that the subset of patients with bipolar disorder who experienced predominantly mood-incongruent psychotic features would be most likely to show evidence of an influence of susceptibility from *NRG1*.

METHODS

SUBJECTS

All of the subjects in these studies were white and of UK origin and provided written informed consent to participate in genetic studies. Protocols and procedures were approved by relevant ethical review panels including the UK West Midlands Multi-Center Research Ethics Committee, Birmingham, England.

BIPOLAR PROBANDS

Cases were recruited through mental health services in England and Wales and met *DSM-IV*[4] criteria for bipolar I disorder (n=529; 38% men; mean age, 48 years [SD, 13 years]; mean age at onset, 26 years [SD, 10 years]). The *ICD-10* diagnoses included bipolar disorder (manic) in 512 and schizoaffective disorder (bipolar) in 17; RDC diagnoses, bipolar I disorder in 481 and schizoaffective disorder (bipolar) in 48. A history of psychiatric illness in a first- or second-degree relative was present in 60%. Diagnoses were made by the consensus lifetime best-estimate method[40] on the basis of all available information, including a semistructured interview (Schedules for Clinical Assessment in Neuropsychiatry),[41] and review of psychiatric case records, and an OPCRIT checklist was completed.[42] Key clinical variables relating to psychosis were rated using the Bipolar Affective Disorder Dimension Scale (BADDS).[43] In this scale, a score in the range 1 to 100 on the psychosis dimension shows the best estimate of the proportion of total episodes of illness in which psychotic features (defined as delusions or hallucinations) occurred. A score in the range 1 to 100 on the incongruence dimension shows the best estimate of the overall balance between mood-congruent and mood-incongruent psychotic features. Mood incongruence was defined according to the guidelines in the *DSM-IV*[4] and BADDS.[43] In our sample, bipolar cases had a lifetime occurrence of 1 or more psychotic features in 62% and a lifetime occurrence of predominantly mood-incongruent psychotic features (ie, mood-incongruent features constitute 50% or more of the psychotic features that occur) in 36%. Interrater reliability was high for the measures used in this study. This was formally assessed using 20 cases and resulted in mean κ statistics of 0.85, 0.83, and 0.80 for *DSM-IV*, *ICD-10*, and RDC diagnoses, respectively. Mean intraclass correlation coefficients were 0.89 for the BADDS incongruence dimension and 0.86 for the psychosis dimension. Formative team reliability meetings took place weekly throughout recruitment.

CONTROLS

Controls (n=1011; 57% men; mean age, 42 years [SD, 12 years]), all white and of UK origin, were collected from 2 sources. One source was the British Blood Transfusion Service. This sample (n=902) did not undergo specific screening for psychiatric illness, but individuals were not taking regularly prescribed psychiatric medications. In England and Wales, blood donors are not remunerated, even for expenses, and are therefore not overrepresented by the indigent or the socially disadvantaged in whom the rate of psychosis might possibly rise above a threshold that would influence power.[44] The second source was individuals who were recruited from among those attending a family practitioner clinic for nonpsychiatric reasons. This sample (n=109) was screened to exclude a personal history of mood disorder.

GENOTYPING

The core haplotype of Stefansson et al[25] can be defined by 1 single nucleotide polymorphism (SNP) and 2 microsatellites that were typed as follows. The SNP8NRG221533 was genotyped using a fluorescence polarization–based primer extension assay[45] with the AcycloPrime Kit (Perkin Elmer Life Sciences, Boston, Mass) according to manufacturer's instructions and an Analyst genotyping platform (LJL Biosystems, Sunnyvale, Calif). The microsatellites 478B14848 and 420M91395 were genotyped after analysis on an ABI3100 capillary sequencer (Applied Biosystems, Foster City, Calif).

STATISTICAL ANALYSIS

Departure from Hardy-Weinberg equilibrium was tested using a χ^2 goodness-of-fit test. Tests for differences between allele and haplotype frequencies were performed using UNPHASED software, version 2.40.[46] The effect of haplotypes was assumed to be additive. The primary analysis was a comparison of the frequency of the core haplotype between patients and controls, allowing a 1-*df* test that maximizes power to test our hypothesis. We also examined a global haplotype test using the restriction of excluding rare haplotypes (frequencies of <1%). Uncertain haplotypes were estimated using the expectation/maximization algorithm within UNPHASED. Two-tailed *P* values are reported. Nominally significant asymptotic *P* values were confirmed by permuting the case-control status for 50 000 replicates and observing the maximum test statistic in each case. We undertook testing for population stratification as a potential cause of finding genetic differences between our case and control samples using the program STRUCTURE,[47] with 18 SNPs and 20 microsatellites distributed across the autosomes and unlinked to the *NRG1* locus.

RESULTS

The controls underwent genotyping for the *NRG1* polymorphisms in 2 waves. The first underwent genotyping with our schizophrenia sample (618 controls), with the results reported in Williams et al[31]; the second underwent genotyping with the bipolar sample (393 controls). Duplicate samples were typed and allele coding was verified. There were no significant deviations from Hardy-Weinberg equilibrium in the separate or in combined control sets. The allele frequencies for each of the 3 markers showed no significant difference between the 2 control sets. Further, the 2 control samples had similar levels of linkage disequilibrium between the loci (data not shown). Pooling the controls yielded a total sample of 1011 control individuals who underwent genotyping for all loci.

There were no significant differences in the allele distributions between bipolar cases and controls for any of the markers. We found a significant difference between

Table. Frequency of the Core Haplotype at *NRG1* in Controls, Bipolar Cases, and Schizophrenia Cases

Test	Frequency of Core Haploytype, %	*P* Value for Comparison vs Controls	OR (95% CI)
Controls (n = 1011)	7.8*		
Cases			
Bipolar (n = 529)	10.2	.04	1.37 (1.03-1.80)
Bipolar-P (n = 329)	10.1	.11	1.34 (0.94-1.90)
Bipolar-I (n = 193)†	12.5	.009	1.71 (1.29-2.59)
Bipolar-I (n = 95)‡	14.1	.01	1.95 (1.11-3.41)
Bipolar-NI (n = 336)§	9.1	.33	1.19 (0.82-1.72)
Schizophrenia (n = 573)	9.5	.18	1.22 (0.92-1.61)
Schizophrenia-M (n = 27)	12.2	.29	1.64 (0.54-5.01)
Schizophrenia-NM (n = 545)	9.2	.24	1.20 (0.90-1.60)
Schizophrenia-M + bipolar-I (n = 220)	12.2	.007	1.70 (1.15-2.51)
Schizophrenia-NM + bipolar-NI (n = 881)	9.2	.17	1.20 (0.96-1.51)
RDC SABP (n = 75)	12.7	.05	1.72 (1.06-2.10)

Abbreviations: BADDS, Bipolar Affective Disorder Dimension Scale; bipolar, bipolar I disorder; CI, confidence interval; core haplotype, "core at-risk haplotype"; I, predominantly mood-incongruent psychotic features; M, manic episodes; NI, no predominantly mood-incongruent psychotic features; NM, no manic episodes; *NRG1*, neuregulin 1 gene; OR, odds ratio; P, with lifetime occurrence of 1 or more psychotic features; RDC, Research Diagnostic Criteria; SABP, schizoaffective disorder of bipolar type.

*It is a property of the estimation algorithms that when estimating control haplotype frequencies, the estimates vary slightly according to the composition of the case sample. Thus, estimates range from 7.7% to 7.9%, according to the analysis.

†Indicates a score greater than or equal to 20 on the incongruence dimension of the BADDS.

‡Indicates a score greater than or equal to 30 on the incongruence dimension of the BADDS.

§Excludes those with a score greater than or equal to 20 on the incongruence dimension of the BADDS.

the overall distribution of 3-locus haplotypes between bipolar cases and controls ($P=.003$). Inspection of these haplotypes showed that the largest absolute difference in haplotype frequencies was found for the core haplotype, with the global signal also coming from a series of small differences at several rarer haplotypes (but all having a frequency >1%). Results of our primary analyses of the core haplotype frequency comparisons with controls are presented in the **Table**. Consistent with our hypothesis, the core haplotype was significantly more common in our bipolar cases than in the controls (odds ratio [OR], 1.37; 95% confidence interval [CI], 1.03-1.80; $P=.04$). The magnitude of this overrepresentation was very similar in the subset of 329 bipolar cases who had lifetime experience of psychotic features (OR, 1.34; 95% CI, 0.94-1.90; $P=.11$), but consistent with our hypothesis, it was increased within the subset of bipolar individuals who had experienced psychotic features that were predominantly mood incongruent (defined by a BADDS incongruence dimension score ≥20; OR, 1.71; 95% CI, 1.29-2.59; $P=.009$). This analysis had been decided a priori, and clinically this corresponds to at least half of the psychotic features experienced during a subject's lifetime being rated as mood incongruent. Comparing this subset of bipolar cases (ie, with an incongruence dimension score ≥20) against all remaining bipolar cases demonstrated a significant difference in the core haplotype frequency ($P=.04$). All effect sizes were very similar when cases were separately compared against the controls undergoing genotyping in the first or second waves or when cases were separately compared against the subset of blood donor controls or the subset of family practice controls. We explored the effect on the estimate of effect size (measured by the OR) of variation in the preponderance of mood incongruence in psychotic symptoms experienced and found that the estimated OR increased with increasing mood incongruence, as measured by the incongruence dimension of BADDS (**Figure 2**). For example, when comparing the group of bipolar cases with BADDS incongruence dimension scores greater than or equal to 30, we found an OR of 1.95 (95% CI, 1.11-

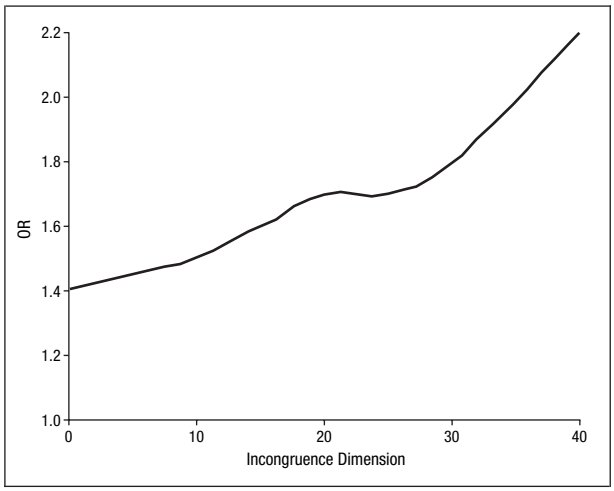

Figure 2. Variation in estimated odds ratios (ORs) with score on the incongruence dimension of the Bipolar Affective Disorder Dimension Scale (BADDS) for the "core at-risk haplotype" (core haplotype) for bipolar I disorder cases compared with controls. The ORs were estimated for the set of cases with a score equal to or exceeding the BADDS incongruence dimension value shown on the x-axis, compared with the set of controls. Thus, as the incongruence dimension score increases on the x-axis, a successively smaller subset of cases (with greater mood incongruence) is compared against controls. Only cases with lifetime psychotic symptoms and sufficient information to make a consensus judgment about the balance of mood congruence had a rating on the incongruence dimension (n=320) and therefore contributed to this analysis. The significance of the upward trend was tested using a sign test of the direction of changes in the OR with the increase of the incongruence dimension score (compared with the null expectation of a 50% chance of an increase of the OR with each increase of the incongruence dimension score). This demonstrated significance at P=.002.

3.41; $P=.01$) (Table). Clinically this corresponds to 75% or more of the psychotic features experienced being rated as mood incongruent. This trend for increase of effect size with incongruence was tested using a sign test for change in effect size at each increment of the incongruence dimension score and was statistically significant ($P=.002$) (Figure 2). When we reanalyzed our schizophrenia cases against our enlarged set of controls, there was a modest reduction in the estimated effect size (from 1.25 to 1.22) with loss of conventional levels of statistical significance for the comparison of the schizophrenia cases against controls. It is of interest that the small subset of schizophrenia cases in whom at least 1 definite manic episode had occurred (n=27) showed higher estimates for the frequency of the core haplotype, at a level similar to that for the bipolar individuals with predominantly mood-incongruent psychotic features (Table).

Analysis of the set of autosomal microsatellites (n=20) and SNPs (n=18) unlinked to the *NRG1* locus using the STRUCTURE program showed no evidence of population stratification (tested under a presumed subpopulation number, k=1, 2, and 3) in our case-control sample (data not shown).

COMMENT

Consistent with our hypotheses, we found significant evidence for the association of the schizophrenia core haplotype at *NRG1* with susceptibility to bipolar I disorder, and the pattern of results showed a stronger signal within the bipolar cases in whom psychotic features were predominantly mood incongruent. Further, there was a clear and statistically significant trend for the estimated effect size to increase with the degree of mood incongruence, as measured by the incongruence dimension of the BADDS. These findings are of interest for several reasons.

First, in our large samples recruited using similar methods and ascertainment approaches in the same geographical and clinical locations, we found that the estimated effect size of the core haplotype was very similar in our bipolar and schizophrenia cases, suggesting that *NRG1* is not a specific schizophrenia susceptibility gene but instead confers risk across the traditional kraepelinian dichotomy. An interesting observation in our data was that the effect size (measured by the OR) in our bipolar cases with psychotic features that are completely or almost completely mood incongruent (BADDS incongruent dimension score, >39) is similar in magnitude to the largest effect size reported in any of the studies of schizophrenia to date (OR, 2.2) (Figure 2).[25]

Second, although the 8p12 region has not been implicated in meta-analyses of bipolar disorder genome scans, a recent linkage analysis of psychosis in bipolar pedigrees reported genome-wide significant evidence of linkage in this region.[48] The findings reported herein suggest that *NRG1* may be responsible for the linkage signal in that scan.

Third, our phenotype analyses are consistent with the core haplotype at *NRG1* exerting some specificity on susceptibility to mania and mood-incongruent psychotic features. It might, therefore, more appropriately be regarded as a schizoaffective susceptibility haplotype. However, it is important to recognize that the effect was not restricted to the 48 bipolar cases meeting formal RDC for schizoaffective disorder but was also present in the additional 145 bipolar cases with predominantly mood-incongruent features not conventionally regarded as schizoaffective under current classifications. Identification of 1 or more variants that have specificity for an intermediate form between the traditional major categories of schizophrenia and bipolar disorder would have major importance for nosology and understanding of pathophysiology of the major psychoses. Indeed, our findings support the view that alternative approaches to classification of the functional psychoses will more closely reflect biology. For example, our data are consistent with the suggestion by Crow[7] of a dimensional or continuum approach to the functional psychosis phenotype, although they are incompatible with his hypothesis that a single gene underlies all psychosis.

Fourth, our findings suggest that in seeking bipolar cases most likely to have an overlap in pathogenesis with schizophrenia, it may not be sufficient simply to examine the subset with lifetime occurrence of psychotic features. Instead, the qualitative similarity of the psychotic features to those seen in schizophrenia is relevant. This argues for the importance of detailed phenotypic characterization of samples.

Fifth, our previously reported schizophrenia sample shows reduced support for the involvement of *NRG1* in the pathogenesis of schizophrenia. However, it should be noted that the core haplotype frequency in our schizophrenia cases is very close to that observed in the Scottish sample.[30] The difference in statistical significance comes from the different estimates in control frequency for the core haplotype. The finding of an increased effect in our manic subset of schizophrenia cases should be interpreted cautiously because of the small number of cases in this subset (n=27).

Sixth, it is of interest that the mouse model used by Stefansson et al[25] to provide support for the involvement of *NRG1* in schizophrenia has hyperactivity as 1 of the key features, a behavior, perhaps, more characteristic of mania than schizophrenia. Thus, the animal data are at least as supportive of the involvement of *NRG1* in bipolar spectrum illness as they are supportive of its involvement in schizophrenia.

Seventh, the presence of a global linkage disequilibrium signal between bipolar cases and controls cannot be entirely explained by the core haplotype, with additional evidence coming from several relatively rare haploptypes, each showing modest differences. This could be the result of multiple, independent, relatively uncommon variants that influence susceptibility to bipolar illness or the result of 1 or a small number of variants occurring on multiple haplotypes. These possibilities are entirely consistent with the current state of understanding of the role of *NRG1* in schizophrenia where no causative variants have yet been identified and multiple haplotypes have been reported that are associated with schizophrenia susceptibility within and between samples.

In any case-control study, spurious differences between cases and controls that are unrelated to disease status can be caused by the presence of so-called population structure, which may result in differential sampling of cases and controls from genetically distinct subpopulations. However, we can be confident that this is not the cause of our findings for the following reasons: (1) Results of formal tests for stratification demonstrated no evidence of stratification in our sample. (2) The direction and magnitude of effect is similar to that observed in several other case-control studies of samples from European populations, including Iceland and the United Kingdom. (3) The estimated effect sizes in our sample are closely similar in our blood donor and our family practice controls. (4) The clear and significant relationship between the OR and mood incongruence (Figure 2) is very unlikely to arise from population stratification. Nonetheless it is generally desirable that a finding from a case-control design is explored in family-based association designs that are robust to population stratification. However, there is a practical difficulty in that samples exceeding 800 parent-proband trios would be required to provide 80% power to detect the effect observed in our study (Genetic Power Calculator[49]; available at: http://statgen.iop.kcl.ac.uk/gpc/). This required sample size substantially exceeds any that are available at present.

No previous reports of studies of *NRG1* haplotypes in bipolar disorder have been published to date, and it is clearly important that other groups examine relevant independent samples. However, it will be essential that samples are adequately powered and sufficiently well characterized phenotypically, particularly in relation to the frequency and nature of psychotic symptoms. It will be appropriate to explore the haplotype-phenotype correlations within schizophrenia samples in addition to bipolar disorder samples. Analysis of sets of individuals having schizophrenic features and clear manic episodes will be of particular relevance. Given the findings with *NRG1* in schizophrenia, it would be expected that an effect with the core haplotype in bipolar disorder will be most easily replicated in samples of North European origin.

Our findings add to the increasing molecular genetic evidence consistent with the involvement of *NRG1* in the pathogenesis of psychotic illness and suggest that this may be the first gene to be identified that confers particular susceptibility to mania and mood-incongruent psychotic features. However, pathogenic variants remain to be identified, and it is important that detailed analyses are undertaken of multiple large samples using phenotypically informed approaches.

Submitted for Publication: August 18, 2004; final revision received November 8, 2004; accepted November 16, 2004.

Correspondence: Nick Craddock, MB, PhD, Department of Psychological Medicine, Henry Wellcome Building for Biomedical Research, University of Wales College of Medicine, Heath Park, Cardiff CF14 4XN, Wales (craddockn@cardiff.ac.uk).

Funding/Support: This study was supported by the Wellcome Trust, London, England; the UK Medical Research Council, London; and the Higher Education Funding Council for Wales, Cardiff.

Acknowledgment: We thank all of the families and patients who have participated in this study.

REFERENCES

1. Craddock N, Jones I. Genetics of bipolar disorder. *J Med Genet*. 1999;36:585-594.
2. Craddock N, Khodel V, Van Eerdewegh P, Reich T. Mathematical limits of multilocus models: the genetic transmission of bipolar disorder. *Am J Hum Genet*. 1995;57:690-702.
3. Kraepelin E. *Manic-Depressive Insanity and Paranoia*. Barclay RM, trans. Edinburgh, Scotland: E&S Livingstone; 1919.
4. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*. Washington, DC: American Psychiatric Press; 1994.
5. World Health Organization. *The ICD-10 Classification of Mental and Behavioural Disorders*. Geneva, Switzerland: World Health Organization; 1993.
6. Spitzer RL, Endicott J, Robins E. Research Diagnostic Criteria: rationale and reliability. *Arch Gen Psychiatry*. 1978;35:773-782.
7. Crow TJ. The continuum of psychosis and its genetic origins: the sixty-fifth Maudsley lecture. *Br J Psychiatry*. 1990;156:788-797.
8. Gershon ES, Hamovit J, Guroff JJ, Dibble E, Leckman JF, Sceery W, Targum SD, Nurnberger JI Jr, Goldin LR, Bunney WE Jr. A family study of schizoaffective, bipolar I, bipolar II, unipolar, and normal control probands. *Arch Gen Psychiatry*. 1982;39:1157-1167.
9. Frangos E, Athanassenas G, Tsitourides S, Katsanou N, Alexandrakou P. Prevalence of *DSM-III* schizophrenia among the first-degree relatives of schizophrenic probands. *Acta Psychiatr Scand*. 1985;72:382-386.
10. Baron M, Gruen R, Asnis L, Kane J. Schizoaffective illness, schizophrenia and affective disorders: morbidity risk and genetic transmission. *Acta Psychiatr Scand*. 1982;65:253-262.
11. Tsuang MT, Winokur G, Crowe RR. Morbidity risks of schizophrenia and affective disorders among first degree relatives of patients with schizophrenia, mania, depression and surgical conditions. *Br J Psychiatry*. 1980;137:497-504.
12. Valles V, Van Os J, Guillamat R, Gutierrez B, Campillo M, Gento P, Fananas L. Increased morbid risk for schizophrenia in families of in-patients with bipolar illness. *Schizophr Res*. 2000;42:83-90.
13. Kendler KS, Karkowski LM, Walsh D. The structure of psychosis: latent class analysis of probands from the Roscommon Family Study. *Arch Gen Psychiatry*. 1998;55:492-499.
14. Rice J, Reich T, Andreasen NC, Endicott J, Van Eerdewegh M, Fishman R, Hirschfeld RM, Klerman GL. The familial transmission of bipolar illness. *Arch Gen Psychiatry*. 1987;44:441-447.
15. Cardno AG, Rijsdijk FV, Sham PC, Murray RM, McGuffin P. A twin study of genetic relationships between psychotic symptoms. *Am J Psychiatry*. 2002;159:539-545.
16. McGuffin P, Reveley A, Holland A. Identical triplets: non-identical psychosis? *Br J Psychiatry*. 1982;140:1-6.
17. Badner JA, Gershon ES. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry*. 2002;7:405-411.
18. Berrettini W. Evidence for shared susceptibility in bipolar disorder and schizophrenia. *Am J Med Genet C Semin Med Genet*. 2003;123:59-64.
19. Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H, Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H, Bougueleret L, Barry C, Tanaka H, La Rosa P, Puech A, Tahri N, Cohen-Akenine A, Delabrosse S, Lissarrague S, Picard FP, Maurice K, Essioux L, Millasseau P, Grel P, Debailleul V, Simon AM, Caterina D, Dufaure I, Malekzadeh K, Belova M, Luan JJ, Bouillot M, Sambucy JL, Primas G, Saumier M, Boubkiri N, Martin-Saumier S, Nasroune M, Peixoto H, Delaye A, Pinchot V, Bastucci M, Guillou S, Chevillon M, Sainz-Fuertes R, Meguenni S, Aurich-Costa J, Cherif D, Gimalac A, Van Duijn C, Gauvreau D, Ouellette G, Fortier I, Raelson J, Sherbatich T, Riazanskaia N, Rogaev E, Raeymaekers P, Aerssens J, Konings F, Luyten W, Macciardi F, Sham PC, Straub RE, Weinberger DR, Cohen N, Cohen D, Ouelette G, Realson J. Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. *Proc Natl Acad Sci U S A*. 2002;99:13675-13680.
20. Schumacher J, Jamra RA, Freudenberg J, Becker T, Ohlraun S, Otte AC, Tullius M, Kovalenko S, Bogaert AV, Maier W, Rietschel M, Propping P, Nothen MM, Cichon S. Examination of G72 and D-amino-acid oxidase as genetic risk factors for schizophrenia and bipolar affective disorder. *Mol Psychiatry*. 2004;9:203-207.
21. Hattori E, Liu C, Badner JA, Bonner TI, Christian SL, Maheshwari M, Detera-Wadleigh SD, Gibbs RA, Gershon ES. Polymorphisms at the G72/G30 gene locus, on 13q33, are associated with bipolar disorder in two independent pedigree series. *Am J Hum Genet*. 2003;72:1131-1140.
22. Chen YS, Akula N, Detera-Wadleigh SD, Schulze TG, Thomas J, Potash JB, De-

Paulo JR, McInnis MG, Cox NJ, McMahon FJ. Findings in an independent sample support an association between bipolar affective disorder and the G72/G30 locus on chromosome 13q33. *Mol Psychiatry*. 2004;9:87-92.
23. Moises HW, Zoega T, Gottesman II. The glial growth factors deficiency and synaptic destabilization hypothesis of schizophrenia. *BMC Psychiatry*. 2002;2:8.
24. Corfas G, Roy K, Buxbaum JD. Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. *Nat Neurosci*. 2004;7:575-580.
25. Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, Brynjolfsson J, Gunnarsdottir S, Ivarsson O, Chou TT, Hjaltason O, Birgisdottir B, Jonsson H, Gudnadottir VG, Gudmundsdottir E, Bjornsson A, Ingvarsson B, Ingason A, Sigfusson S, Hardardottir H, Harvey RP, Lai D, Zhou M, Brunner D, Mutel V, Gonzalo A, Lemke G, Sainz J, Johannesson G, Andresson T, Gudbjartsson D, Manolescu A, Frigge ML, Gurney ME, Kong A, Gulcher JR, Petursson H, Stefansson K. Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet*. 2002;71:877-892.
26. Blouin J-L, Dombroski BA, Nath SK, Lasseter VK, Wolyniec PS, Nestadt G, Thornquist M, Ullrich G, McGrath J, Kasch L, Lamacz M, Thomas MG, Gehrig C, Radhakrishna U, Snyder SE, Balk KG, Neufeld K, Swartz KL, DeMarchi N, Papadimitriou GN, Dikeos DG, Stefanis CN, Chakravarti A, Childs B, Housman DE, Kazazian HH, Antonarakis SE, Pulver AE. Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. *Nat Genet*. 1998;20:70-73.
27. Kaufmann CA, Suarez B, Malaspina D, Pepple J, Svrakic D, Markel PD, Meyer J, Zambuto CT, Schmitt K, Matise TC, Harkavy Friedman JM, Hampe C, Lee H, Shore D, Wynne D, Faraone SV, Tsuang MT, Cloninger CR; NIMH Genetics Initiative Millennium Schizophrenia Consortium. Linkage analysis of African-American pedigrees. *Am J Med Genet*. 1998;81:282-289.
28. Kendler KS, MacLean CJ, O'Neill FA, Burke J, Murphy B, Duke F, Shinkwin R, Easter SM, Webb BT, Zhang J, Walsh D, Straub RE. Evidence for a schizophrenia vulnerability locus on chromosome 8p in the Irish study of high-density schizophrenia families. *Am J Psychiatry*. 1996;153:1534-1540.
29. Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I, Williams NM, Schwab SG, Pulver AE, Faraone SV, Brzustowicz LM, Kaufmann CA, Garver DL, Gurling HM, Lindholm E, Coon H, Moises HW, Byerley W, Shaw SH, Mesen A, Sherrington R, O'Neill FA, Walsh D, Kendler KS, Ekelund J, Paunio T, Lonnqvist J, Peltonen L, O'Donovan MC, Owen MJ, Wildenauer DB, Maier W, Nestadt G, Blouin JL, Antonarakis SE, Mowry BJ, Silverman JM, Crowe RR, Cloninger CR, Tsuang MT, Malaspina D, Harkavy-Friedman JM, Svrakic DM, Bassett AS, Holcomb J, Kalsi G, McQuillin A, Brynjolfson J, Sigmundsson T, Petursson H, Jazin E, Zoega T, Helgason T. Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia. *Am J Hum Genet*. 2003;73:34-48.
30. Stefansson H, Sarginson J, Kong A, Yates P, Steinthorsdottir V, Gudfinnsson E, Gunnarsdottir S, Walker N, Petursson H, Crombie C, Ingason A, Gulcher JR, Stefansson K, St Clair D. Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. *Am J Hum Genet*. 2003;72:83-87.
31. Williams NM, Preece A, Spurlock G, Norton N, Williams HJ, Zammit S, O'Donovan MC, Owen MJ. Support for genetic variation in neuregulin 1 and susceptibility to schizophrenia. *Mol Psychiatry*. 2003;8:485-487.
32. Iwata N, Suzuki T, Ikeda M, Kitajima T, Yamanouchi Y, Inada T, Ozaki N. No association with the neuregulin 1 haplotype to Japanese schizophrenia. *Mol Psychiatry*. 2004;9:126-127.
33. Corvin AP, Morris DW, McGhee K, Schwaiger S, Scully P, Quinn J, Meagher D, Clair DS, Waddington JL, Gill M. Confirmation and refinement of an "at-risk" haplotype for schizophrenia suggests the EST cluster, Hs.97362, as a potential susceptibility gene at the neuregulin-1 locus. *Mol Psychiatry*. 2004;9:208-213.
34. Yang JZ, Si TM, Ruan Y, Ling YS, Han YH, Wang XL, Zhou M, Zhang HY, Kong QM, Liu C, Zhang DR, Yu YQ, Liu SZ, Ju GZ, Shu L, Ma DL, Zhang D. Association study of neuregulin 1 gene with schizophrenia. *Mol Psychiatry*. 2003;8:706-709.
35. Li T, Stefansson H, Gudfinnsson E, Cai G, Liu X, Murray RM, Steinthorsdottir V, Januel D, Gudnadottir VG, Petursson H, Ingason A, Gulcher JR, Stefansson K, Collier DA. Identification of a novel neuregulin 1 at-risk haplotype in Han schizophrenia Chinese patients, but no association with the Icelandic/Scottish risk haplotype. *Mol Psychiatry*. 2004;9:698-704.
36. Zhao X, Shi Y, Tang J, Tang R, Yu L, Gu N, Feng G, Zhu S, Liu H, Xing Y, Zhao S, Sang H, Guan Y, St Clair D, He L. A case control and family based association study of the neuregulin1 gene and schizophrenia. *J Med Genet*. 2004;41:31-34.
37. Hall D, Gogos JA, Karayiorgou M. The contribution of three strong candidate schizophrenia susceptibility genes in demographically distinct populations. *Genes Brain Behav*. 2004;3:240-248.
38. Thiselton DL, Webb BT, Neale BM, Ribble RC, O'Neill FA, Walsh D, Riley BP, Kendler KS. No evidence for linkage or association of neuregulin-1 (NRG1) with disease in the Irish study of high-density schizophrenia families (ISHDSF). *Mol Psychiatry*. 2004;9:777-783.
39. Falls DL. Neuregulins: functions, forms, and signalling strategies. *Exp Cell Res*. 2003;284:14-30.
40. Leckman JF, Sholomskas D, Thompson WD, Belanger A, Weissman MM. Best estimate of lifetime psychiatric diagnosis: a methodological study. *Arch Gen Psychiatry*. 1982;39:879-883.
41. Wing JK, Babor T, Brugha T, Burke J, Cooper JE, Giel R. SCAN: Schedules for Clinical Assessment in Neuropsychiatry. *Arch Gen Psychiatry*. 1990;47:589-593.
42. McGuffin P, Farmer A, Harvey I. A polydiagnostic application of operational criteria in studies of psychotic illness: development and reliability of the OPCRIT system. *Arch Gen Psychiatry*. 1991;48:764-770.
43. Craddock N, Jones I, Kirov G, Jones L. The Bipolar Affective Disorder Dimension Scale (BADDS): a dimensional scale for rating lifetime psychopathology in bipolar spectrum disorders. *BMC Psychiatry*. 2004;4:19.
44. Owen MJ, Holmans P, McGuffin P. Association studies in psychiatric genetics. *Mol Psychiatry*. 1997;2:270-273.
45. Chen X, Levine L, Kwok P-Y. Fluorescence polarization in homogenous nucleic acid analysis. *Genome Res*. 1999;9:492-498.
46. Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol*. 2003;25:115-121.
47. Pritchard JK, Stephens M, Donnelly P. Inference of population structure from multilocus genotype data. *Genetics*. 2000;155:945-959.
48. Park N, Juo SH, Cheng R, Liu J, Loth JE, Lilliston B, Nee J, Grunn A, Kanyas K, Lerer B, Endicott J, Gilliam TC, Baron M. Linkage analysis of psychosis in bipolar pedigrees suggests novel putative loci for bipolar disorder and shared susceptibility with schizophrenia. *Mol Psychiatry*. 2004;9:1091-1099.
49. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics*. 2003;19:149-150.