A Point of Rarity in Genetic Risk for Bipolar Disorder and Schizophrenia

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Context: Recent studies suggest that copy number variation in the human genome is extensive and may play an important role in susceptibility to disease, including neuropsychiatric disorders such as schizophrenia and autism. The possible involvement of copy number variants (CNVs) in bipolar disorder has received little attention to date.

Objectives: To determine whether large (>100,000 base pairs) and rare (found in <1% of the population) CNVs are associated with susceptibility to bipolar disorder and to compare with findings in schizophrenia.

Design: A genome-wide survey of large, rare CNVs in a case-control sample using a high-density microarray.

Setting: The Wellcome Trust Case Control Consortium.

Participants: There were 1697 cases of bipolar disorder and 2806 nonpsychiatric controls. All participants were white UK residents.

Main Outcome Measures: Overall load of CNVs and presence of rare CNVs.

Results: The burden of CNVs in bipolar disorder was not increased compared with controls and was significantly less than in schizophrenia cases. The CNVs previously implicated in the etiology of schizophrenia were not more common in cases with bipolar disorder.

Conclusions: Schizophrenia and bipolar disorder differ with respect to CNV burden in general and association with specific CNVs in particular. Our data are consistent with the possibility that possession of large, rare deletions may modify the phenotype in those at risk of psychosis: those possessing such events are more likely to be diagnosed as having schizophrenia, and those without them are more likely to be diagnosed as having bipolar disorder.

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Family, twin, and adoption studies have provided an impressive and consistent body of evidence supporting the existence of genes that predispose to bipolar disorder, with a gradation of risk as the genetic relatedness to a proband diminishes. Large-scale collaborative genome-wide association studies (GWASs; hundreds of thousands of single-nucleotide polymorphisms [SNPs] studied in large numbers of cases and controls) have started to deliver genome-wide significant genetic associations for bipolar disorder, thereby implicating common genetic polymorphisms in susceptibility to illness. A meta-analysis of GWAS data from approximately 10,000 individuals has shown strong evidence for association with susceptibility to bipolar disorder at variants within 2 genes involved in ion channel function: ANK3 (encoding the protein ankyrin G) and CACNA1C (encoding the a1C subunit of the L-type voltage-gated calcium channel). In addition, other strong associations have been reported in individual GWASs for several other loci including PALB2, MYO5B, and DSGK. Current evidence suggests that, consistent with findings in GWASs of nonpsychiatric disorders, a large number of susceptibility loci will be identified as larger samples are studied.

The focus of GWASs is identification of common polymorphisms that influence susceptibility to illness. Another important source of genetic variation is genomic structural variation. It has recently been recognized that structural genomic variants are a common cause of genetic variation in humans, and such variants have been reported to substantially increase the
risk of a number of neuropsychiatric phenotypes, including autism, mental retardation, and schizophrenia.10-16

The overall load of copy number variants (CNVs) has been shown to be greater in individuals with schizophrenia than controls.11,13,17 There is also convincing evidence for association between schizophrenia and a number of specific rare CNVs (<1% population frequency), particularly those at 22q11 (the velocardiofacial syndrome deletion), 1q21.1, and 15q13.3.12,13 and also to the class of rare CNVs larger than 1 megabase (Mb).14 Furthermore, some specific CNVs associated with risk of schizophrenia confer risk to multiple neuropsychiatric phenotypes, including autism, attention-deficit/hyperactivity disorder, and epilepsy (eg, 1q21.1, 15q13.3, 22q11.2, and 16p13.1).18,19 The estimated effect sizes for these CNVs are substantially greater than those conferred by the SNPs discussed earlier. However, the typical effect sizes and population frequencies of pathogenically relevant CNVs are not yet fully characterized, nor is the full extent to which CNVs contribute to the total population variance in risk of schizophrenia. The only systematic study to date for bipolar disorder did not find any increase in overall CNV load, although there was a nominally significant increase in “singleton” CNVs in cases compared with controls.20 There have been reports of specific CNVs in individuals with bipolar disorder, but none has addressed the question of whether CNVs show significant overrepresentation in cases compared with controls.21,22

The aim of the present study was to investigate CNVs in a large sample of patients with bipolar disorder (n = 1697) by means of data from a gene mapping system (GeneChip Human Mapping 500K Array Set; Affymetrix Inc, Santa Clara, California), genotyped in the Wellcome Trust Case Control Consortium (WTCCC) study.3 We compared the findings in our bipolar cases with those in a large sample of nonpsychiatric controls (n = 2806) and also with a sample of cases meeting criteria for schizophrenia (n = 440), all of whom were genotyped as part of the WTCCC pipeline with the use of identical methods.19

SAMPLING

The bipolar disorder cases and the controls are those reported by the WTCCC. A detailed description of the sample and methods has been published previously.7

The bipolar disorder cases (n = 1868) were older than 16 years, living in the United Kingdom, and of European white ancestry. Clinical assessment included semistructured interview and review of case notes. All patients received information and signed a consent form for participation in genetic studies. Of those, 1697 individuals passed the quality control (QC) filtering for the CNV analysis undertaken in the current study (described in the next section). The diagnoses according to Research Diagnostic Criteria were bipolar I disorder (n = 1209), schizoaffective disorder bipolar type (n = 243), bipolar II disorder (n = 156), and manic disorder (n = 89).

The controls were all white UK residents and were collected from 2 sources: the 1958 birth cohort and the UK Blood Service. It has previously been shown that these 2 control samples can be combined for use as controls in genetic association studies using UK disease samples, including the bipolar disorder sample.4 Of the 2938 controls, 2806 passed the QC filtering and were used for the CNV analysis (1411 individuals from the 1958 cohort and 1395 from the UK Blood Service).

GENOTYPING AND CNV ANALYSIS

The DNA from case and control samples were genotyped with the mapping array set at the laboratory of the manufacturer, as previously described.4 The array set consists of 2 arrays: NspI and StyI. On average, 250 000 SNPs were genotyped per array. It is possible to infer data for the copy number variation by using the intensity data from the SNP chip. Affymetrix Genotyping console v2.1 software was used (http://www.affymetrix.com). Because the SNP call rate is known to reflect sample quality, an initial basic QC filter was applied to the data (using the default parameters of the software) to exclude samples having an average SNP call rate less than 93%.

For reference sets we used samples from the same processing batches because using one reference set for all samples produced poor-quality data owing to systematic confounding effects. Because we sought to reduce to a minimum the false-positive rate of calling CNVs, we applied the same stringent QC filtering to the data that we used in our previous study of schizophrenia.14 A deletion or duplication was called only if it was 100 kilobase (kb) or greater in length and comprised 10 or more consecutive SNPs separately on each of the 2 arrays. The interquartile range (IQR) of the log2 ratio was used to evaluate the quality of the arrays for the copy number analyses. Samples with an IQR greater than 0.40 (default parameter in the Affymetrix genotyping console v2.1 software) were excluded from the analysis. Samples with an IQR greater than 0.40 produced more than 20 deletions and/or duplications (and > 100 CNVs when the IQR was ≥ 0.50), suggesting that observations corresponding to 20 or more CNV segments per person were likely to be false-positives. Therefore, samples with more than 20 segments were also excluded even if the observed IQR was 0.40 or less.

We accepted only deletions or duplications found independently on both arrays, with the overlap of the segments identified by the StyI and the NspI arrays of 100 kb or greater. Segments were matched by means of a stand-alone program developed in-house (available at http://s004-psycm.uwcm.ac.uk/). We also excluded any CNVs that had very low SNP density (< 3 SNPs per 100 kb) because these tend to intersect low copy repeats or “difficult-to-sequence” regions of the genome and have an increased probability of being false-positives. After these stringent QC filter criteria were applied, 1697 cases and 2806 controls were retained for analysis.

Because there are difficulties in using this method to genotype common CNVs and consistent with recent studies using this approach,13 common CNVs (found in ≥ 1% of the samples) were excluded. Any CNVs that overlapped by more than 50% of their length with common CNVs were also excluded. Using PLINK14 version 1.05, all rare deletions and duplications that remained after these criteria were listed in a custom track for visualization in UCSC Genome Browser (University of California, Santa Cruz; http://genome.ucsc.edu/), available at http://s004-psycm.uwcm.ac.uk/~detelina/.

We had previously performed a similar analysis on cases affected with schizophrenia recruited from the same population compared against the same set of WTCCC controls.15 As part of that study, we validated 22 CNVs (most of them > 1 Mb) with a second platform (Human Genome CGH Microarray Kit 44K; Agilent Technologies, Santa Clara, California). All validated correctly, reflecting our stringent filtering criteria and the fact that each CNV had already been called independently by 2 arrays (StyI and NspI). We therefore did not attempt to validate any CNVs in the current study.
STATISTICAL ANALYSIS

The CNV association analyses were performed with PLINK\textsuperscript{24} version 1.05, obtained from http://pngu.mgh.harvard.edu/~purcell/plink/.

The P values throughout this article are 2-tailed, based on comparing the rate of CNVs in 2 independent samples (usually cases vs controls), and were derived with the use of 10,000 permutations. The genomic coordinates used in the study are based on the March 2006 human genome sequence assembly (UCSC Hg18, National Center for Biotechnology Information build 36).

RESULTS

GLOBAL BURDEN OF CNVs

The total number of rare CNVs, and the corresponding P value from the comparison between cases and controls, are presented in Table 1. The rate of CNVs was not increased in bipolar disorder cases compared with controls, and there was even a nominally significant association in the opposite direction for deletions ($P = .01$) (see the supplementary Results section for more details and a breakdown of these results according to CNV size in eTable 1; available at: http://www.archgenpsychiatry.com).

Although we did not observe an overall increase in CNV burden in bipolar cases compared with controls, some individual CNVs were more common in cases than controls (although none showed a significant association after correction for multiple testing). These CNVs are shown in eTable 2. We did not observe any CNV events in cases in the few regions in which there have been reports of CNV events in individuals with bipolar disorder (for details see the supplementary Results section).

ANALYSIS OF SINGLETON CNVs

To test the recent report of an increased rate of rare singleton CNV events in bipolar disorder,\textsuperscript{20} we estimated the global burden of single-occurrence deletions and single-occurrence duplications. We found no difference between the proportion of cases and controls with singleton CNVs as a whole or when deletions and duplications were considered separately (Table 2). The previous report\textsuperscript{20} observed that singleton deletions were particularly more common in cases with mania with an onset of illness before age 18 years. In the 65 patients in our sample with such an early age at onset, there was no difference in the rate of singleton CNVs compared with the rest of the sample (data not shown).

Further details about the analysis of the singleton events, and genes affected by CNVs only in cases, can be found in the supplementary Results section (eTable 3).

ANALYSIS RESTRICTED TO CNVs LARGER THAN 1 Mb

In our study of schizophrenia using the same methods and samples from the same UK population,\textsuperscript{14} we observed a significant increase in CNV load only for CNVs larger than 1 Mb. We therefore undertook an analysis of this category in bipolar disorder (Table 3). There were no significant differences between cases and controls; indeed, the trend was toward fewer CNV events in bipolar disorder cases.

When we compared our bipolar disorder cases against our set of schizophrenia cases that had been examined for this class of CNVs by the same methods\textsuperscript{14} (n=440), we observed a significant excess in the schizophrenia cases for deletions and total CNVs (both $P < .001$). There was also a trend toward an excess of large duplications ($P = .053$) in schizophrenia compared with bipolar disorder. The rate of large CNVs in schizophrenia cases was approximately 5-fold higher for deletions and approximately 2-fold higher for duplications compared with the bipolar cases (eTable 1). It should be noted that the P values reported herein for the comparison between schizophrenia cases and controls vary slightly from those published in our schizophrenia-
nia report because of exclusion of schizoaffective cases from the current analysis. Further details are provided in the supplementary Results section.

ANALYSIS RESTRICTED TO CNVs THAT DISRUPT GENES

Following a previous study of schizophrenia,13 we examined in the bipolar cases the burden of CNVs that delete, duplicate, or disrupt genes (method described in the supplementary Results section). This analysis was performed for all CNVs and then for CNVs that occurred only once in the data. Again, no significant differences were found (data not shown). A list of all genes disrupted in CNVs in cases that were not disrupted in any controls is provided in eTable 4.

ANALYSIS OF CNVs PREVIOUSLY REPORTED TO BE ASSOCIATED WITH RISK OF SCHIZOPHRENIA

Table 4 shows the main chromosomal regions reported to be associated with schizophrenia and the respective number of the observed CNVs in the bipolar disorder cases and in the controls. We have included only loci reported in multiple studies or with strong statistical support from at least 1 large study.

The total burden of these specific rare CNVs that have been associated with risk of schizophrenia was not increased in bipolar disorder cases compared with controls (frequency per individual: cases, 0.010; controls, 0.016). Only 2 regions showed a trend for overrepresentation in cases. Duplications at 16p11.2 were found in 3 cases and 1 control, a 5-fold increase in frequency (0.2% vs 0.04%; Fisher exact test, P = .15). One of these had arisen de novo and was not present in the proband's father, who also had bipolar disorder (see the supplementary Results section). Two cases and no controls had duplications of 15q13.3, deletion of which has been confirmed to confer susceptibility to schizophrenia.12,13 More information on the loci in Table 4 is given in the supplementary Results section.

We have undertaken a comparison of the occurrence of rare CNVs in a large white UK set of bipolar cases and controls using genome-wide SNP data generated within the WTCCC study.4 In contrast to several recent reports of an increased burden of rare CNVs, particularly large deletions, in schizophrenia cases compared with controls, we observed no such increase in bipolar disorder cases, and there was even a trend toward a reduced rate for deletions. Furthermore, direct comparison of our bipolar disorder cases with our set of schizophrenia cases recruited from the same UK clinical population, assessed by similar clinical methods and analyzed by means of the same genotyping platform, QC filtering, and statistical methods, showed that deletions greater than 1 Mb were about 5 times more common in the schizophrenia cases (P < 5 × 10−5). Thus, our data demonstrate a significant difference between bipolar disorder and schizophrenia with respect to global burden of rare and large CNVs.

In addition, we did not find evidence of even a trend toward an increase in bipolar disorder in the frequency of specific CNVs that have been reported to show a robust

### Table 3. Large Copy Number Variants (CNVs) (>1 Mb) in Cases and Controls

<table>
<thead>
<tr>
<th>Large CNVs</th>
<th>Cases (n=1697)</th>
<th>Controls (n=2806)</th>
<th>CNVs per Case</th>
<th>CNVs per Control</th>
<th>Case to Control Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletion</td>
<td>7</td>
<td>20</td>
<td>0.004</td>
<td>0.007</td>
<td>0.58</td>
<td>.24</td>
</tr>
<tr>
<td>Duplication</td>
<td>33</td>
<td>65</td>
<td>0.019</td>
<td>0.023</td>
<td>0.84</td>
<td>.47</td>
</tr>
<tr>
<td>Deletion + duplication</td>
<td>40</td>
<td>85</td>
<td>0.024</td>
<td>0.030</td>
<td>0.78</td>
<td>.20</td>
</tr>
</tbody>
</table>

### Table 4. CNVs Previously Implicated in Schizophrenia and Corresponding Findings in the Current Study

<table>
<thead>
<tr>
<th>Locusa</th>
<th>Type</th>
<th>References</th>
<th>Positionb</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1q21.1c</td>
<td>del</td>
<td>12, 13</td>
<td>chr1:144.9-146.3</td>
<td>1 dup</td>
<td>2 del; 2 dup</td>
</tr>
<tr>
<td>2p16.3 (NRXN1)</td>
<td>del</td>
<td>11, 31, 32</td>
<td>chr2:50-51.3</td>
<td>0</td>
<td>4 del (various lengths)</td>
</tr>
<tr>
<td>7q34-36.1</td>
<td>del</td>
<td>33</td>
<td>chr7:145.6-148.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15q11.2c</td>
<td>del</td>
<td>12</td>
<td>chr15:20.31-20.78</td>
<td>3 del; 7 dup</td>
<td>14 del; 10 dup</td>
</tr>
<tr>
<td>15q13.3c</td>
<td>del</td>
<td>12, 13</td>
<td>chr15:28.7-30.30</td>
<td>2 dup</td>
<td>0</td>
</tr>
<tr>
<td>16p11.2</td>
<td>dup</td>
<td>11</td>
<td>chr16:29.5-30.3</td>
<td>3 dup</td>
<td>3 del; 1 dup</td>
</tr>
<tr>
<td>17p12</td>
<td>del</td>
<td>14</td>
<td>chr17:14.0-15.4</td>
<td>1 dup</td>
<td>0</td>
</tr>
<tr>
<td>22q11.2c</td>
<td>del</td>
<td>12-14, 34</td>
<td>chr22:17.2-19.8</td>
<td>0</td>
<td>8 dup</td>
</tr>
<tr>
<td>Total</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>3 del; 14 dup</td>
<td>23 del; 21 dup</td>
</tr>
</tbody>
</table>

Abbreviations: CNV, copy number variant; del, deletion; dup, duplication; NA, not applicable.
aLocus refers to the chromosome band.
bPosition refers to chromosome and physical location in megabases of DNA from the p terminus (thus, “chr1:144.9-146.3” designates the region of DNA between 144.9 and 146.3 megabases from the p terminus of chromosome 1).
cNote that the current evidence for association of these loci with schizophrenia is strongest for 1q21.1, 15q11.2, 15q13.3, and 22q11.2 (where, for each, significance approaches or exceeds P < 5 × 10−5).
association with schizophrenia. It is important to recognize that power to detect a significant association at any specific rare CNV is low, although our power to detect the joint association of the 4 robustly schizophrenia-associated deletions (1q21.1, 15q11.2, 15q13.3, and 22q11.2) at P < .05 exceeds 96%. It is of particular interest that we observed no instances of a 22q11 deletion in our bipolar sample. In contrast, in our much smaller schizophrenia sample we observed 2 such deletions. Thus, our data do not support the hypothesis that 22q11 deletion syndrome (associated with velocardiofacial syndrome) is associated with bipolar disorder, as others have suggested.32 The only locus implicated in schizophrenia that showed a trend for overrepresentation in bipolar cases was duplication at 16p11.2, with a 5-fold increased rate over controls, but this did not reach statistical significance.

Genetic epidemiology suggests the existence of some genetic factors that confer risk to both schizophrenia and bipolar disorder and others that confer relatively specific risk to one or other of the dichotomous categories.33,34 A recent study of approximately 10,000 individuals has provided strong evidence of association between bipolar disorder and SNPs within 2 genes involved in ion channel function: ANK3 and CACNA1C.3 The SNP in CACNA1C that was most strongly associated with bipolar disorder shows a similar magnitude of association in UK schizophrenia and unipolar depression samples.35 Thus, variation at this locus influences susceptibility across the mood-psychosis spectrum. A study of approximately 20,000 individuals has provided strong evidence of association between schizophrenia and an SNP within 2NF804A (encoding a protein of unknown function, but that, on the basis of sequence similarity, may act as a transcription factor).36 The same SNP in 2NF804A also showed evidence of association with bipolar disorder,37 again demonstrating that variation at this locus also has an effect on illness susceptibility across the traditional diagnostic boundaries. Thus, for 2 of the recent strongly supported SNP associations that have been reported with the use of large samples and GWAS methods, there is evidence of an overlap in susceptibility across the traditional Kraepelienian dichotomy. Further recent support for the existence of shared genetic risk comes from the observation of overlapping sets of genes showing gene-wide significance in a gene-based analysis of genome-wide SNP data for bipolar disorder and schizophrenia.38 In contrast, the CNV findings to date suggest that copy number variation may have a relatively specific influence on susceptibility to illness that meets diagnostic criteria for schizophrenia.

This possibility has substantial face validity in that CNVs are known to be associated with some persistent neuropsychiatric phenotypes that are known to occur in, or be risk factors for, illnesses that meet diagnostic criteria for schizophrenia, including mental retardation, epilepsy, and autism.39,40 Thus, CNVs may predispose to persistent brain dysfunctions that affect intellectual functioning and personality development that may modify expression of the phenotype in those who have a propensity to develop psychoses.39,40

In our analyses, we found that the CNV burden was lower in our bipolar disorder cases than controls, and this difference was statistically significant when deletions were considered separately (Table 1; P = .01). Because this finding is not highly significant in the context of multiple testing, it may well be a chance finding, and therefore we consider that our data should be interpreted conservatively as showing no increase in burden of CNVs in bipolar cases compared with controls.

To our knowledge, there has been only 1 previous systematic genome-wide CNV analysis in bipolar disorder,41 and, like the present study, it showed no increase in the total burden of CNVs in cases compared with controls. However, our data do not replicate the finding of a significant increase in singleton CNV events in bipolar cases compared with controls. Our study is larger than that of Zhang et al,42 and power exceeded 95% to detect the reported effect size at P < .05. Power is obviously lower if there is a true, but smaller, increase in singleton CNV burden or if our stringent QC method resulted in our detecting a smaller proportion of the relevant loci. With respect to very weak effects, no single study can provide definitive results, and therefore in this context a type II error in the present bipolar vs control analysis study cannot be fully discounted. However, the findings of Zhang and colleagues are much weaker than those reported in much smaller studies of schizophrenia and autism, and it is possible the results of that study represent a type I error because they would not withstand correction for testing multiple plausible hypotheses concerning CNV size, type, and frequency. Although additional studies will be required to finally resolve this discrepancy, our study does provide strong evidence that any contribution to bipolar disorder from CNVs in the size range we tested for is significantly smaller than it is to schizophrenia.

Although we have no evidence to support any specific locus, even single observations of occurrence of a CNV in a disorder can ultimately make an important contribution to understanding of disease pathogenesis, e.g., the observation of a rare deletion in the NRXN1 gene in a proband with schizophrenia.43 To facilitate this, and downstream meta-analyses, we presented the full list of rare CNVs found in cases and controls in our supplementary material.

The strengths of our study include the large sample size, the rigorous QC filtering used, and our ability to make direct comparisons between bipolar disorder and schizophrenia cases that have been recruited from similar clinical populations and analyzed by means of the same genotyping pipeline and statistical methods. Some important limitations are inherent in all studies of CNVs. The first, as mentioned earlier, concerns power to detect very small increases in CNV burden. Second, CNV analyses using SNP data are not straightforward and require several analytic steps that are difficult to standardize precisely across studies by different investigators. This does not, however, affect comparisons across our own bipolar disorder and schizophrenia samples. The genotyping platform used in the WTCCC study (Affymetrix 500K chip) was one of the earliest GWAS genotyping platforms and, hence, has less resolution for CNVs than later platforms. This has much less influence on large CNVs (the focus of our study) but, because of this limitation, we have not tested the contribution of smaller CNVs (<100 kb) to disease susceptibility. In the future, it will
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be important to undertake further studies that use much higher-resolution platforms and that allow CNVs to be called at similar levels of accuracy as SNPs to provide further information about a wider spectrum of CNV sizes. The number of CNVs increases exponentially with smaller CNV size, so most CNVs remain to be identified and examined for association with disease.

Finally, CNV burden analysis alone cannot identify specific risk factors; nevertheless, the approach we present herein has proved pivotal in pointing to the involvement of CNVs in schizophrenia and other disorders. In conclusion, we have used genome-wide association data to undertake a comparison of CNV burden in a large sample of bipolar disorder cases, nonpsychiatric controls, and schizophrenia cases. We found that CNV load was not elevated in bipolar disorder compared with controls and that deletions larger than 1 Mb were less common in probands with bipolar disorder than in those with schizophrenia. Our findings suggest that schizophrenia and bipolar disorder differ with respect to CNV burden in general and association with specific schizophrenia-associated CNVs in particular.

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