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# Case-case genome wide association analysis reveals markers differentially associated with schizophrenia and bipolar disorder and implicates calcium channel genes

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## Abstract

**Objectives—**There are theoretical reasons why comparing marker allele frequencies between cases of different diseases, rather than with controls, may offer benefits. The samples may be better matched, especially for background risk factor common to both diseases. Genetic loci may also be detected which influence which of the two diseases occurs if common risk factors are present.

**Method—**We used samples of UK bipolar and schizophrenic cases which had previously been subject to genome wide association studies and compared marker allele frequencies between the two samples. When these differed for a marker, we compared the case sample allele frequencies with those of a control sample.

**Results—**Eight markers were significant at $p<10^{-5}$. Of these, the most interesting finding was for rs17645023 which was significant at $p<10^{-6}$ and which lies 36kb from CACNG5. Control allele frequencies for this marker were intermediate between those for bipolar and schizophrenic cases.

**Conclusion—**Application of this approach suggests that it does have some merits. The finding for CACNG5, taken together with the prior implication of CACNA1C and CACNA1B, strongly suggests a key role for voltage-dependent calcium channel genes in the susceptibility to bipolar disorder and/or schizophrenia.

### Keywords

Schizophrenia; bipolar disorder; association; calcium channel

## Introduction

The traditional design for an association study is of course to compare marker genotypes of a sample of cases affected with a disease against those of an unaffected control sample.

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However there are a number of reasons to propose that there could be benefits from comparing cases suffering from one disease against cases with a different disease.

One possibility to consider is that the samples might be better matched. If the two diseases were similar in terms of their epidemiology then the subjects suffering from either disease might be similar in terms of genetic and non-genetic background. Typically, unaffected controls will have been recruited from a non-clinical setting and might differ in subtle ways from a patient sample. By contrast, samples of patients suffering from different diseases might be similar to each other in a number of respects. If they are recruited from the same clinical setting they might be similar in terms of their geographical background, their social class and factors influencing presentation to services. If the two diseases share risk factors, which might be genetic or non-genetic, the two patient samples might be well matched for such risk factors and this would be expected to confer an advantage when seeking to identify genetic risk factors specific to one or other disease. Having well matched samples reduces background noise and is expected to increase both the power and specificity of association studies. In the case of bipolar disorder and schizophrenia, evidence has been presented to support the hypothesis that both share genetic risk factors (Purcell et al. 2009). Arguably, comparing samples of both would to some extent control for the presence of this shared component and would enhance the ability to detect genes of major effect.

A very extreme example of the possible advantage conferred by comparing samples of patients with different diseases is that there might be a common genetic variant which did not usually produce any increased risk of one or other disease but which did influence which of the two diseases an individual was likely to develop if a sufficient quantity of other risk factors, shared by both diseases, were present. Thus, one might postulate that there were a number of factors which combined to produce a high probability that a subject would develop some form of psychotic illness but that there might also be a common variant which would influence whether this illness took the form of schizophrenia or bipolar disorder. If one studied a sample of cases of either illness compared with control subjects there might be only a minor difference in frequency of this variant whereas if samples of cases of the two illnesses were compared this difference might become far more striking and more easily detectable.

Arguably, another benefit of the case-case approach is that it may become possible to identify markers specifically associated with one or other disease as opposed to less specific shared risk factors. For example, if it were the case that both coronary artery disease and type 2 diabetes were associated with obesity then in a case-control study of one disease one would not know whether a gene which demonstrated association influenced the specific molecular pathogenesis of the disease in question or whether it exerted its effect more indirectly, for example by influencing obesity. However if cases of both disease were compared then one could conclude that any associated marker pointed to an effect specific to one or other disease and such information might be more helpful in elucidating the related molecular pathology.

The corollary of this is that the case-case method would have an important disadvantage, namely that it would be expected to fail to identify any genetic variants which might influence susceptibility to both diseases simultaneously. Thus it could at best be seen as complementary to the case-control approach and certainly could not claim to replace it.

We have recruited samples of cases with bipolar disorder and schizophrenia and a control sample and these have been subjected to genome wide association (GWA) studies (Ferreira et al. 2008; Sklar et al. 2008; Purcell et al. 2009). In the case of bipolar disorder, these provided some evidence to implicate MYO5B, CACNA1C and ANK3 while the study of

schizophrenia implicated markers in the HLA region. Additional association studies using these samples have implicated a number of genes as being involved in both diseases including BRD1, DISC1 and DAOA (Bass et al. 2009; Hennah et al. 2009; Nyegaard et al. 2009) while separately the schizophrenia sample has been reported to show association with epsin 4 and PCM1 (Pimm et al. 2005; Datta et al. 2008) and the bipolar sample with P2RX7 (McQuillin et al. 2009). Here, we report our application of a case-case analysis of these samples in order to throw some light on the possible value of this approach.

## Method

The research has received UK NHS Multicentre Research Ethics Committee (MREC) approval from the London Metropolitan MREC. The samples used consisted of 506 with bipolar 1 disorder, 523 with schizophrenia and 505 controls. Subjects were recruited on the basis of having European, non-Jewish ancestry and at least three grand-parents who were from the UK with the fourth possibly coming from a different European country as defined before the 2004 enlargement. All subjects were interviewed using the lifetime version of the Schizophrenia and Affective Disorders Schedule and assigned a Research Diagnostic Criteria diagnosis. The bipolar subjects and controls were genotyped using the Affymetrix 500K array whereas the schizophrenia subjects were genotyped using the Affymetrix Genome Wide Human SNP Array 5.0 and both sets of cases were shown to be genetically well-matched to the control sample (ISC 2008; Sklar et al. 2008).

There were 302,482 markers for which genotypes were available for both sets of cases. Both sets of cases had previously been shown to be genetically well-matched to the control sample. For these markers, allele frequencies were compared between bipolar disorder and schizophrenia using a two-by-two chi-squared test. For markers significant at $p<10^{-5}$ the allele frequencies of each case sample were also compared with that of the control sample. Positions of nearby genes were obtained from the UCSC Human Genome Browser (UCSC).

## Results

Table 1 shows genotype counts for all markers significant at $p<10^{-5}$. It should go without saying that any or all of these results could have occurred by chance, given the number of markers tested and also the fact that this is a secondary analysis, the original case-control analyses being primary. With this proviso, arguably the most interesting finding is that obtained for rs17645023 which is significant at $p=10^{-6.1}$. It can be seen that the allele frequencies in the control sample are intermediate between those in the bipolar and schizophrenia samples, meaning that neither case-control comparison produces similar levels of statistical significance. . This marker lies between CACNG5 and CACNG4, which are genes coding for subunits of a voltage-dependent calcium channel. It is 36kb from CACNG5 and 79kb from CACNG4.

There are a number of other markers which show more marked differences in allele frequencies between cases of bipolar disorder and schizophrenia than between either sample of cases and controls, comprising rs11210359, rs1795648, rs6459804 and rs6459806. The last two of these are in complete LD with each other and are located in PTPRN2, the gene for a receptor-type protein tyrosine phosphatase. rs1795648 is in ERC2, which is thought to be involved in the organization of the cytomatrix at the nerve terminals active zone, which regulates neurotransmitter release.

Two markers, rs17075286 and rs1203847, show differences in frequencies between both case samples but show a similar effect when the schizophrenia sample is compared with the controls. Neither of these markers was reported significant at $p<10^{-5}$ in the original GWA of

schizophrenia because results were reported for the combined samples obtained from several different centres rather than individually for the sample of UK subjects collected at University College London (UCL).

Finally, rs7065696, shows some difference in allele frequencies between schizophrenia cases and controls ($p=10^{-4.6}$) but the difference is more marked when both sets of cases are compared ($p=10^{-6.4}$). Although this difference in statistical significance may seem noteworthy, in fact it is accounted for by a very small difference between the bipolar counts and the control counts. This marker is in PHF8, the gene for PHD finger protein 8.

With regard to CACNA1B and CACNA1C, which have been implicated previously and which code for subunits of the same voltage-dependent L-type calcium channel as contains the subunits coded by CACNG5 and CACNG4, no markers were significant at $p<0.01$ in the case-case analysis.

## Discussion

Comparing allele frequencies between two sets of cases has drawn attention to a number of markers which would not have been picked up by case-control comparisons using these samples. Of course, we cannot be sure that any of the results represents a genuine effect and determining this will involve genotyping additional markers in the implicated regions and carrying out tests in additional samples. Nevertheless, we feel that the findings are of some interest.

CACNA1C has previously been implicated in susceptibility to bipolar disorder (Ferreira et al. 2008) (Keers et al. 2009). It codes for an alpha subunit of a voltage-dependent L-type calcium channel. The related voltage-dependent calcium channel gene CACNA1B has also been implicated in susceptibility to schizophrenia (Moskvina et al. 2009). Here we show that there is a difference in allele frequencies between bipolar and schizophrenic cases for rs17645023, which lies 36kb from CACNG5, coding for a gamma unit of the same type of calcium channel. As we have argued previously (Curtis et al. 2007) when GWA studies are carried out it makes sense to accord more prominence to findings relating to markers which relate to candidate genes than to anonymous markers with similar p values. Hence we regard this result as possibly indicating a real effect of variation at CACNG5 in modifying the susceptibility to bipolar disorder and/or schizophrenia.

If they indicate a real effect, findings such as that obtained for rs17645023, in which the allele frequency difference is more marked for the case-case than case-control comparison, may be suggestive of variants which exert their effect in the presence of background risk factors which produce an overall increased susceptibility to psychotic illness. On the other hand, obtaining findings which are more significant for the case-case comparison may simply reflect chance variation in the allele frequencies between the control sample and one of the case samples, thus exaggerating a real but relatively small effect. It would not be possible to elucidate this further without carrying out additional studies.

This study demonstrates some of the potential benefits of carrying out comparisons between samples of cases of different but related diseases. Arguably, the finding of association with a marker close to CACNG5 when taken with the previous evidence implicating CACNA1C and CACNA1B makes a compelling case that abnormalities of voltage-dependent calcium channel genes may represent important risk factors for bipolar disorder and/or schizophrenia.

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UCSC. UCSC browser: genome.ucsc.edu. from http://genome.ucsc.edu/cgi-bin/hgGateway

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**Table 1**

Markers significant at p<0.0001 in comparison between schizophrenia and bipolar disorder cohorts.

| Marker | Chr | Position | | Genotype counts | | | Minus log(p) | | Nearest gene | Distance to nearest gene |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Schizophrenia (SZ) | Bipolar disorder (BP) | Controls (C) | SZ v. BP | SZ v. C | BP v. C | | |
| rs11210359 | 1 | 74339167 | 76 276 171 | 55 205 241 | 70 237 197 | 5.1 | 1.0 | 2.2 | LRRIQ3 | 152kb |
| rs17075286 | 3 | 43230733 | 10 33 462 | 0 13 483 | 0 14 491 | 6.1 | 5.9 | 0.05 | C3orf39 | 98kb |
| rs1795648 | 3 | 55571760 | 63 217 242 | 27 185 290 | 49 195 263 | 5.2 | 1.3 | 2.0 | ERC2 | 0kb |
| rs1203847 | 4 | 2517447 | 4 29 481 | 0 7 484 | 0 3 486 | 5.0 | 6.9 | 0.4 | RNF4 | 0kb |
| rs6459804 | 7 | 157510195 | 74 254 195 | 112 264 130 | 89 248 168 | 5.1 | 1.1 | 2.2 | PTPRN2 | 0kb |
| rs6459806 | 7 | 157510483 | 75 253 195 | 113 263 130 | 89 248 168 | 5.1 | 1.0 | 2.2 | PTPRN2 | 0kb |
| rs17645023 | 17 | 64917033 | 14 158 331 | 31 208 24 | 25 180 288 | 6.2 | 2.1 | 1.7 | CACNG5 | 36kb |
| rs7065696 | X | 53974054 | 43 32 447 | 0 34 451 | 1 39 449 | 6.4 | 4.6 | 0.4 | PHF8 | 0kb |