Investigation of rare non-synonymous variants at \textit{ABCA13} in schizophrenia and bipolar disorder

\textit{Molecular Psychiatry} (2011) \textbf{16}, 790–791; doi:10.1038/mp.2011.2; published online 1 February 2011

It has recently been reported that rare genetic variants in \textit{adenosine triphosphate (ATP)-binding cassette} \textit{A13} (\textit{ABCA13}) increase susceptibility to schizophrenia, bipolar disorder and major depression.\(^1\) The gene was targeted following the observation of a complex chromosomal rearrangement, inv(7) (p12.3;q21.11), t(7;8)(p12.3;p23) in an individual with severe chronic schizophrenia.\(^1\) Only one breakpoint disrupted a gene, that at 7p12.3 to which maps \textit{ABCA13}. This led the authors to postulate the involvement of rare non-synonymous variants at \textit{ABCA13} in major psychiatric disorders. They resequenced the exons of \textit{ABCA13} that encode key functional domains in 100 individuals with schizophrenia and 100 controls, and observed a total of 32 novel variants. In all, 10 of these were observed in not more than one control and were predicted to cause a non-conservative amino-acid change and these were tested for involvement in psychiatric disorders using case samples comprising individuals with schizophrenia, bipolar disorder, major depression, as well as controls.

In these follow-up samples, the minor allele was observed in at least one additional case for only six of the markers. Of these, three variants were nominally significantly associated with bipolar disorder and one was nominally significantly associated with schizophrenia (indicated by * and † in Table 1, respectively). Although no variant was significantly associated after correcting for multiple testing, an aggregate test of minor alleles at all 10 loci under the assumption of marker independence revealed that the finding arose simply by chance. These two statements are difficult to reconcile. Examination of the two data sets (Supplementary table 1) shows that cases in the original study are significantly more likely to carry a risk allele than our cases (\(P=0.008\), whereas the controls in the original study are significantly less likely to do so than our controls (\(P=0.01\)). Both studies used controls that had not been screened for an absence of psychiatric illness, including Birth Cohort and Blood Donor controls (the latter in the United Kingdom are unpaid and not enriched for groups at high risk of drug abuse or other psychiatric illness) making control ascertainment an unlikely source of the difference between studies. We cannot exclude the possibility that the difference was confounded by other factors.

Seeking support for the original finding, which as yet is unreplicated, we genotyped the six informative variants in a United Kingdom schizophrenia (\(n=831\)), bipolar disorder (\(n=1734\)) and control sample (\(n=4536\)). The control sample consisted of 1456 samples from the 1958 British Birth Cohort and 3080 United Kingdom blood donors. Both control groups were ascertained for the Wellcome Trust Case Control Consortium, and, along with the bipolar sample, have been described elsewhere.\(^2\) Of the schizophrenia sample, 593 have been described elsewhere.\(^3\) The remainder (\(n=238\)) were ascertained using the same diagnostic and ascertainment practices. With the exception of variant R4278X, the genotyping call rate for all variants was >98.5%. The apparently lower call rate for R4278X in the bipolar cases resulted from the removal of an entire 384-well plate, which had a high PCR failure rate. The power of our study to detect association at \(z=0.05\) for the effect described across samples is \(\sim 100\%\) (1-tailed; Genetic Power calculator\(^4\)) or, for the lower CI estimate, \(80\%\) at this threshold, and \(90\%\) at a trend level (\(P<0.1\)).

Of the six variants examined, only R4843C was significantly more common in a case sample than control (bipolars vs controls; \(P=0.03\); bipolar plus schizophrenia \(P=0.04\); 1-tailed), although these would not remain significant after correction for multiple testing (Table 1). In the previous study, this variant was associated with schizophrenia rather than bipolar disorder, and was not significant in all combined phenotypes.

A global analysis of the occurrence of minor alleles at all variant sites was performed exactly as in the earlier study. No significant excess of minor alleles was observed in any group of cases (Table 1) or in schizophrenia and bipolar disorder combined.

Knight \textit{et al.}\(^1\) also reported homozygosity or compound heterozygosity in six cases but no controls. We found four cases (three bipolar and one schizophrenia) and four controls were compound heterozygotes for minor alleles at the variants. One bipolar and one control were compound heterozygotes for H3609P and T4550A. One schizophrenia case carried H3609P and R4590W and one control carried both R4590W and T4550A. Two bipolar cases and two controls were heterozygous for T4031A and T4550A.

Given the high power our study had to replicate the previous findings, even at the lower end of the 95% confidence interval reported in that study, our failure to confirm the earlier finding is not likely to be a type II error. However, it should also be noted that the reported type I error rate in the earlier study was \(\sim 0.0002\) (all diagnoses combined), making it unlikely that the finding arose simply by chance. These two statements are difficult to reconcile. Examination of the two data sets (Supplementary table 1) shows that cases in the original study are significantly more likely to carry a risk allele than our cases (\(P=0.008\), whereas the controls in the original study are significantly less likely to do so than our controls (\(P=0.01\)). Both studies used controls that had not been screened for an absence of psychiatric illness, including Birth Cohort and Blood Donor controls (the latter in the United Kingdom are unpaid and not enriched for groups at high risk of drug abuse or other psychiatric illness) making control ascertainment an unlikely source of the difference between studies. We cannot exclude the possibility that the difference...
in the rate of rare variant occurrence in cases might reflect some as yet unknown phenotypic differences between the samples, although again, we do not think that this a particularly likely explanation as ascertainment of the samples in both studies and the diagnostic methodologies used are similar. Moreover, the signal in the earlier study was observed in both schizophrenia and bipolar disorder suggesting broad rather than narrow phenotypic effects.

Additional potential sources of discrepancy between the studies include stratification and genotyping artefacts. Stratification (in one or both studies) is unlikely as most of the samples used in each study have also been included in single-nucleotide polymorphism-based genome-wide association studies, in which there was no evidence that this made an appreciable impact.\textsuperscript{1,2,3} Regarding genotyping artefacts, we have no specific reason to propose that this explains the discrepant results. Addressing our own study, the genotyping clusters for all markers were excellent, automated and manual calls by two independent experienced researchers blind to the results of the other were 100% congruent, as were duplicate genotypes for each of the markers in 5% of the sample. Genotyping call rates were high for our primary analysis, and, moreover, when we implemented an additional high stringency quality control step, in which we restricted the analysis of each marker to samples for which the call rate was 100% for the other markers, which might be particularly prone to missing or unsatisfactory calls, we obtained virtually identical results as in the primary analysis (data not shown), this despite call rates ranging from 0.998–1.000. Thus, using conventional measures of genotyping quality control, we conclude our results are unlikely to differ from those of the previous study as a result of genotyping artefacts in this study.

Although the source of discrepancy between the two studies is unknown, our overall conclusion is that despite high power to do so if it is correct, our study does not support the hypothesis of Knight et al. that the rare variants they detected at \textit{ABCA13} are enriched for association with schizophrenia and bipolar disorder.

Conflict of interest

The authors declare no conflict of interest.

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References

2 WTCCC. \textit{Nature} 2007; 447: 661–678.
4 Purcell S, Cherny SS, Sham PC. \textit{Bioinformatics} 2003; 19: 149–150.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)