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INVITED REVIEW

Extracting a needle from a haystack: reanalysis of whole genome data reveals a readily translatable finding

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There is significant unmet need for more effective treatments for bipolar disorder. The drug discovery process is becoming prohibitively expensive. Hence, biomarker clues to assist or shortcut this process are now widely sought. Using the publicly available data from the whole genome association study conducted by the Wellcome Trust Case Control Consortium, we sought to identify groups of genetic markers (single nucleotide polymorphisms) in which each marker was independently associated with bipolar disorder, with a less stringent threshold than that set by the original investigators ($p \leqslant 1 \times 10^{-4}$). We identified a group of markers occurring within the *CACNA1C* gene (encoding the alpha subunit of the calcium channel $Ca_v1.2$). We then ascertained that this locus had been previously associated with the disorder in both a smaller and a whole genome study, and that a number of drugs blocking this channel (including verapamil and diltiazem) had been trialled in the treatment of bipolar disorder. The dihydropyridine-based blockers such as nimodipine that bind specifically to $Ca_v1.2$ and are more penetrant to the central nervous system have shown some promising early results; however, further trials are indicated. In addition, migraine is commonly seen in affective disorder, and calcium channel antagonists are successfully used in the treatment of migraine. One such agent, flunarizine, is structurally related to other first-generation derivatives of antihistamines such as antipsychotics. This implies that flunarizine could be useful in the treatment of bipolar disorder, and, furthermore, that other currently licensed drugs should be investigated for antagonism of $Ca_v1.2$.

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Key words: bipolar disorder, *CACNA1C*, $Ca_v1.2$, calcium channel blockers, dihydropyridine, genetic predisposition to disease.

Introduction

Current treatment options for bipolar affective disorder (BP) are unsatisfactory for a substantial proportion of sufferers. Many are symptomatically ill for almost half of their lives (Judd *et al*. 2002) and others experience frequent relapses (Gitlin *et al*. 1995). Fifty per cent of patients fail to respond to lithium, and of those that do, 50–90% are symptomatic within 1 year (Licht, 1998). Moreover, many drugs licensed for BP have significant adverse drug reactions (ADRs), with potential negative consequences on long-term physical health that may adversely affect compliance and thereby mental state. Thus, maintaining patients on effective, well-tolerated medication regimes is a clinical priority.

The development of new drugs for the treatment of BP is dependent on the identification and subsequent validation of novel, more effective targets. Although *in vitro* testing of disease-relevant tissue and the use of animal models can be helpful in the identification stage, validation of drug targets ultimately relies on the time-consuming and expensive drug discovery pipeline that ends with clinical trials. This process leads to a high rate of attrition; that is the drug is identified as unsuitable for clinical use. Indeed, the total cost of bringing a new drug to the market is now estimated at over 800 million US dollars (DiMasi *et al*. 2003). An attractive alternative is to identify which potential drug targets are linked to the pathophysiology of the disorder before they are trialled in the clinic. Until recently, identification of the aetiological genetic variation associated with BP has represented a significant challenge because, in linkage or association studies, the results have been inconsistent and rarely replicated (Kato, 2007).

More consistent findings have been shown in three recently published large genome-wide association studies of bipolar disorder: the Wellcome Trust Case Control Consortium (WTCCC, 2007), Baum *et al*. (2008) and Sklar *et al*. (2008). In our own reanalysis of the publicly available WTCCC data (www.wtccc.org.uk), applying a less stringent significance threshold and seeking to identify independent signals within

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Table 1. *ID, position, genotypic frequency and p value of each of the SNPs identified in the publicly available WTCCC data*

			Controls				Cases				
SNP	SNP ID	Position	AA	AB	BB	Null	AA	AB	BB	Null	*p* value
1	rs10848628	2 182 750	5664	6491	2140	16	648	905	312	3	1.10×10^{-4}
2	rs12422554	2 182 881	2135	6563	5397	216	311	912	619	26	1.50×10^{-4}
3	rs4765902	2 183 226	5625	6529	2135	22	646	908	313	1	1.18×10^{-4}
4	rs10848632	2 186 254	2071	6557	5628	55	301	911	646	10	1.82×10^{-4}
5	rs10848633	2 186 280	5631	6526	2143	11	645	908	313	2	1.17×10^{-4}
6	rs10848634	2 186 388	2140	6525	5636	10	313	909	645	1	9.96×10^{-5}
7	rs10848635	2 186 456	2140	6442	5645	84	309	902	648	9	1.98×10^{-4}
8	rs10848636	2 186 754	2118	6507	5636	50	311	909	646	2	8.67×10^{-5}
9	rs11062156	2 187 784	5625	6531	2141	14	646	909	313	0	1.32×10^{-4}
10	rs2238054	2 187 905	5613	6522	2138	38	639	906	313	10	7.38×10^{-5}
11	rs1006737	2 215 556	1588	6182	6527	14	239	859	767	3	1.59×10^{-4}
12	rs4765905	2 219 845	6490	6173	1576	72	760	860	234	14	2.54×10^{-4}
13	rs10744560	2 257 360	6456	6209	1615	31	763	858	247	0	1.48×10^{-4}
14	rs4765914	2 290 638	591	4701	9006	13	92	678	1097	1	3.61×10^{-4}
15	rs10774037	2 290 787	8982	4735	587	7	1097	677	94	0	3.88×10^{-4}

SNP, Single nucleotide polymorphisms; WTCCC, Wellcome Trust Case Control Consortium.

the same locus, we identified a moderate association between 15 single nucleotide polymorphisms (SNPs) and BP, in intron 2 of the calcium channel, voltage-dependent, L-type, alpha 1C subunit gene *CACNA1C*. Each SNP had a p value of between 7.38×10^{-5} and 3.88×10^{-4} (Table 1). According to HapMap, these association signals were located in three blocks of largely distinct regions of linkage disequilibrium (LD), and may therefore be considered as three relatively independent associations between *CACNA1C* and BP (Fig. 1).

In a subsequent study, Sklar *et al*. (2008) reported a moderate association of one of these SNPs (rs1006737) ($p = 6.96 \times 10^{-4}$), and by combining the results from both their own study and those from the WTCCC, they reported a nearly genome-wide significant association between rs1006737 and BP ($p = 3.15 \times 10^{-6}$). This chromosomal region, 12p13.3, has been previously identified as showing moderate linkage in a study of BP (Detera-Wadleigh *et al*. 1999), and an earlier study by Sklar *et al*. (2002) had reported a significant association of BP with an exonic synonymous SNP in the *CACNA1C* gene.

CACNA1C codes for the α_1 subunit of a voltage-dependent, L-type calcium ion channel (LTCC) known as $Ca_v1.2$. The α_1 subunit is part of the pore-forming unit of $Ca_v1.2$ and is also the binding site for LTCC modulators such as the dihydropyridine (DHP) group of drugs. Replication of *CACNA1C* variation in BP is therefore important not only in understanding the aetiology of the disorder, but also potentially in the development of, and prediction of response to, new drugs for the treatment of BP and related disorders.

BP as a calcium channelopathy

There is evidence to suggest that intracellular calcium ion dysregulation is an important aetiological component of BP. Calcium ion concentrations have been consistently found to be elevated in blood platelets and lymphocytes of patients with BP (Dubovsky *et al*. 1994). In addition, several calcium channelopathies [disorders associated with genetic variation in voltage-gated calcium channel (VGCC) genes, including LTCCs], such as epilepsy and familial hemiplegic migraine (Ophoff *et al*. 1996; Mulley *et al*. 2003), show phenotypic overlap with BP. Moreover, associations between BP and migraine (Radat & Swendsen, 2005) and between BP and epilepsy (Ettinger *et al*. 2005) are well documented and indicate a shared aetiology (Sheftell & Atlas, 2002). This has led to the suggestion that calcium channel dysfunction may also contribute to the genetic aetiology of other polygenic psychiatric disorders (Gargus, 2006) such as schizophrenia, where an association with the calcium channel gene *CACNA1F* has been reported (Wei & Hemmings, 2006).

Calcium channel agonists and antagonists (CCAs) in the treatment of BP

CACNA1C is expressed in both the heart and the brain and, along with $Ca_v1.3$, $Ca_v1.2$ is considered the main

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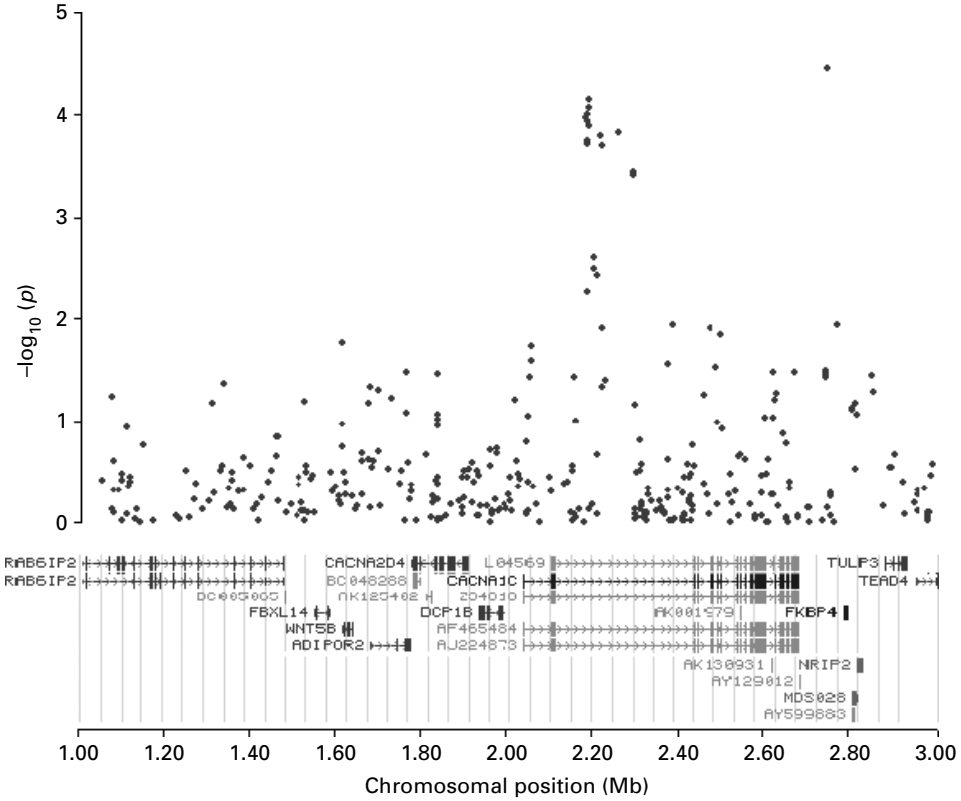

Fig. 1. $-\text{Log}_{10}$ of p values given by the Wellcome Trust Case Control Consortium (WTCCC) plotted against chromosomal position and the position of known genes identified using the University of California, Santa Cruz (UCSC) genome browser.

DHP-sensitive LTCC in the brain. Both DHP-based calcium channel antagonists (CCAs) and agonists have been developed. In mice, the administration of agonists such as BayK8644 (known as 'BayK') causes severe dystonic neurobehavioural symptoms such as self-biting and prolongation of duration of immobility in the 'behavioural despair test', whereas the administration of DHP CCAs such as nifedipine has been shown to have antidepressant effects, that is reduction of BayK-induced prolongation of immobility (Mogilnicka *et al*. 1988). Of note, the effect of BayK8644 is also antagonized by desipramine and imipramine. Sinnegger-Brauns *et al*. (2004) attempted to produce a DHP-resistant mice strain by inducing a mutation in *CACNA1C*. In these mice, BayK induced a 'behavioural despair' phenotype, and attempts at blocking $Ca_v1.2$ with nifedipine no longer had an antidepressant effect. This suggests that nifedipine and other DHPs work selectively at the $Ca_v1.2$ LTCC and that the sensitivity of $Ca_v1.2$ to these drugs is directly related to variation in the *CACNA1C* gene.

There are three main groups of CCAs: DHPs (e.g. nifedipine), phenylalkylamines (e.g. verapamil) and benzothiapines (e.g. diltiazem). Although these three groups of CCAs function in the same way, there are significant differences in the calcium channels they target. The DHP group are highly selective for cerebrovascular and neuronal voltage-gated LTCCs (Triggle, 1992).

Because of their apparent similarity to lithium in their effect on interneuronal calcium ion activity, several CCAs have already been trialled in patients with BP. Verapamil has been shown to be more successful than lithium in the treatment of some cases of mania (Garzatrevino *et al*. 1992), although evidence regarding its efficacy is not consistent (Levy & Janicak, 2000). Unlike verapamil, the efficacy of the DHP group relates directly to $Ca_v1.2$ functioning. Although most members of the DHP group have generally been prescribed for angina and hypertension, only nifedipine and nimodipine have been trialled in the treatment of BP.

Nifedipine was successfully trialled in a small case study with the CCA diltiazem (De Beaurepaire, 1992). In two patients, the withdrawal of both drugs was followed by relapse and readmission. However, the readministration of nifedipine in these patients as monotherapy had no effect on their manic symptoms. It is possible that nifedipine works predominantly on the depressive phase of BP, which would be supported by the observation that nifedipine has apparent antidepressant properties in mice.

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The second-generation DHPs show greater central nervous system (CNS) penetration and therefore may be more promising agents for BP, particularly nimodipine, which has the greatest affinity for neuronal rather than vascular LTCCs. Indeed, nimodipine has shown some success in both an open (Brunet *et al*. 1990) and a controlled trial as a monotherapy in rapid cycling and refractory recurrent BP (Pazzaglia *et al*. 1993, 1998). However, the limited number of studies with small sample sizes means that replications are required before any firm conclusions can be drawn regarding the efficacy of the drug. Nicardipine, isradipine, amlodipine and felodipine have been designed to be more selective for vascular rather than neuronal LTCCs, which may explain why they have not yet been trialled in BP, other than in the above study, in which Pazzaglia *et al*. (1998) substituted nicardipine for nimodipine in those who responded to the drug. Of interest, nicardipine was able to show maintenance of the efficacy.

Finally, most of the above LTCC antagonists have been trialled in migraine. Moreover, there is pharmacological overlap between CCAs and medications with antihistaminergic and antidopaminergic activity. One such agent, flunarizine, blocks calcium channels, including LTCCs, and is among the most effective agents for migraine prophylaxis (Amery, 1983; Silberstein & Goadsby, 2002). This drug has not yet been trialled in BP.

As there is substantial phenotypic overlap with between BP and unipolar depressive disorder (UPD) (McGuffin *et al*. 2003), and migraine is associated with both disorders, the relevance of *CACNA1C* may also extend to other affective disorders such as UPD. Although clinical trials of DHPs in UPD have so far been disappointing, these have been relatively few and limited by sample size (Levy & Janicak, 2000). Should BP and/or UPD indeed be associated with sequence variation in *CACNA1C*, it is also possible that this could influence the response of individuals with these disorders to agents (such as DHPs) that affect this channel. We suggest that *CACNA1C* should be considered as a potential candidate gene for exploration in pharmacogenetic studies in both BP and UPD, and in any further relevant emerging genome-wide association studies (GWAS) data.

Conclusion

Given the phenotypic overlap between BP and other known channelopathies, the pharmacological overlap in their treatment, the promising efficacy of some CCAs in the treatment of BP and evidence that suggests calcium is dysregulated in BP, we suggest that VGCCs (particularly LTCCs) are both relevant candidate genes for BP and promising drug targets for its treatment. Although evidence regarding the efficacy of CCAs is overall mixed, DHPs, which target $Ca_v1.2$ and may therefore relate directly to variation in *CACNA1C*, have shown the most promising results. Second-generation DHPs, especially nimodipine, are of particular interest because of their good CNS penetration and we therefore suggest that clinical trials should be conducted within this group. In addition, flunarizine and the first-generation derivatives of antihistamines should be reconsidered and investigated for their effect on ion channels, especially for individuals with both BP and migraine.

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Declaration of Interest

None.

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