The impact of CACNA1C gene, and its epistasis with ZNF804A, on white matter microstructure in health, schizophrenia and bipolar disorder

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Genome-wide studies have identified allele A (adenine) of single nucleotide polymorphism (SNP) rs1006737 of the calcium-channel CACNA1C gene as a risk factor for both schizophrenia (SZ) and bipolar disorder (BD) as well as allele A for rs1344706 in the ZNF804A gene. These illnesses have also been associated with white matter abnormalities, reflected by reductions in fractional anisotropy (FA), measured using diffusion tensor imaging (DTI). We assessed the impact of the CACNA1C psychosis risk variant on FA in SZ, BD and health. 230 individuals (with existing ZNF804A rs1344706 genotype data) were genotyped for CACNA1C rs1006737 and underwent DTI. FA data was analysed with tract-based spatial statistics and threshold-free cluster enhancement significance correction ($P < 0.05$) to detect effects of CACNA1C genotype on FA, and its potential interaction with ZNF804A genotype and with diagnosis, on FA. There was no significant main effect of the CACNA1C genotype on FA, nor diagnosis by genotype(s) interactions. Nevertheless, when inspecting SZ in particular, risk allele carriers had significantly lower FA than the protective genotype individuals, in portions of the left middle occipital and parahippocampal gyri, right cerebellum, left optic radiation and left inferior and superior temporal gyri. Our data suggests a minor involvement of CACNA1C rs1006737 in psychosis via conferring susceptibility to white matter microstructural abnormalities in SZ. Put in perspective, ZNF804A rs1344706, not only had a significant main effect, but its SZ-specific effects were two orders of magnitude more widespread than that of CACNA1C rs1006737.

Keywords: Bipolar disorder, CACNA1C, diffusion tensor imaging, fractional anisotropy, genome-wide association, rs1006737, schizophrenia, white matter, ZNF804A epistasis

Received 19 October 2016, revised 24 October 2016, accepted for publication 25 October 2016

Schizophrenia (SZ) and bipolar disorder (BD) are major psychiatric illnesses that have profound effects on a person’s mood, cognition and behaviour. Psychotic and negative symptomatology, and cognitive impairments are common to both, albeit these are more severe for SZ (Green et al. 2010). Lifetime prevalence of SZ and BD is about 4% (Bhugra 2005) and 0.5%, respectively (Merikangas et al. 2007). Both illnesses are highly heritable: up to 80% (SZ) and 93% (BD) (Gurung & Prata 2015) but their common and specific aetiological and pathophysiological causes are poorly understood.

Genome-wide association studies (GWAS) have identified two genetic variants conferring, so far the most reproducible, risk for both SZ and BD: allele A of the single nucleotide polymorphism (SNP) labelled rs1006737 of the CACNA1C
gene (Ferreira et al. 2008; Green et al. 2010; Guan et al. 2014; He et al. 2014; Jiang et al. 2015; Nyegaard et al. 2010; Sklar et al. 2008; Zheng et al. 2014) and allele A of the rs1344706 SNP of the ZNF804A gene (Donohoe et al. 2010; Sun et al. 2015; Zhang et al. 2015; Zhu et al. 2014). The latter variant has been associated with white matter (WM) microstructural abnormalities as we report elsewhere (Mallas et al. 2016).

The CACNA1C gene, located on the short arm of chromosome 12p13.3 encodes an alpha-1 subunit of a voltage dependent L-type calcium-channel (LTCC) referred to as CaV1.2. Some 12p13.3 encodes an alpha-1 subunit of a voltage dependent L-type calcium-channel (LTCC) referred to as CaV1.2. LTCCs regulate the influx of calcium ions into the cell upon polarisation of the membrane, and, being widely expressed in the central nervous system (CNS), are involved in various processes including regulation of signalling pathways, neurotransmitter release and neuronal excitability. CaV1.2 is thought to be important in synaptic plasticity and neuronal gene expression (Uemura et al. 2015).

SZ and BD have also been robustly associated with WM abnormalities. Fractional anisotropy (FA) measured using diffusion tensor imaging (DTI) is a putative proxy of WM microstructural integrity (Jones et al. 2013) and decreases in FA can thus be regarded as being indicative of reduced WM integrity. Additionally, FA is highly heritable: 75–90% of the variance in FA across almost all WM regions could be explained by genetic factors (Chiang et al. 2009). Several studies have reported FA reductions in psychotic probands, with similar abnormalities to an attenuated degree, observed in healthy first-degree relatives (Chaddock et al. 2009; Skudlarski et al. 2013; Sprooten et al. 2012), and FA has been found to decrease with increasing genetic liability to psychosis (Emserl et al. 2014; Phillips et al. 2011). Reduced FA is a replicated finding in SZ, and to a lesser extent, in BD, in a diverse range of brain regions (Ellison-Wright & Bullmore 2009; Vederine et al. 2011). Furthermore, reduced FA can be detected in both the prodromal and early stages of illness (Carletti et al. 2012; Cheung et al. 2008; Karlsgodt et al. 2008; Szeszko et al. 2005), suggesting that microstructural abnormalities in WM are involved in the underlying neuropathophysiology of the disease.

Imaging genetics evidence of the impact of CACNA1C rs1006737 on WM microstructure is sparse. The risk allele has recently been associated with reduced FA in the right hippocampal formation in Caucasian healthy subjects in a study researching this location only (Dietsche et al. 2014). In another study whole-brain but non WM-tract-based, SZ Chinese patients carrying the risk allele have also shown lower mean FA in the left frontal lobe, left parietal lobe and left temporal lobe, compared to G homozygotes while healthy G homozygotes show higher mean FA compared to A-allele carriers in the right cingulate gyrus and left temporal lobe (Woon et al. 2014). However, none of these studies have provided whole-brain WM tract-based evidence of an impact on FA, thus providing limited resolution of this SNP’s effects. Moreover, no study has researched the impact of this SNP in a clinical population.

In this study, we aimed to assess for the first time the effect of the genome-wide risk variant allele A for rs1006737 of CACNA1C on tract-based regional FA throughout the whole brain in a large (n = 230) and predominantly Caucasian (83%) sample of non-clinical controls and SZ and BD patients. Unprecedentedly, we also enquire if CACNA1C rs1006737 is modulated by diagnosis and by the other most GWAS-implicated SNP: the rs1344706 of the ZNF804A gene (for our review of GWAS-implicated genes on brain phenotypes, see (Gurung & Prata 2015); the impact of the ZNF804A gene on psychiatric disorders is discussed in more detail in our previously reported work (Mallas et al. 2016). We predicted that the risk allele (A) of CACNA1C rs1006737 would be associated with reduced FA across all subjects, irrespective of diagnosis, but might have a greater effect in the patient (SZ and BD) groups. We also anticipated that the effect of this risk allele might be augmented by the presence of the risk allele A of ZNF804A rs1344706.

Materials and methods

Participants

The total sample of 230 subjects consisted of patients with SZ (n = 63), patients with BD type 1 or type 2; 77% of which with psychosis; (n = 43) and non-clinical controls (n = 124), who had participated in seven previous research imaging studies at the Institute of Psychiatry, Psychology & Neuroscience, King’s College London (Allin et al. 2011; Chaddock 2009; Chaddock et al. 2009; Kyriakopoulos et al. 2009; Picchioni et al. 2006; Shergill et al. 2007). Individuals were collated from those sub-samples, with any relatives excluded, as detailed elsewhere (Mallas et al. 2016).

Each participant was assigned to three groups: a diagnosis group (SZ, BD or control), and after genotyping (see below), a genotype group for CACNA1C in terms of risk allele load (CAC+: A homozygotes and heterozygotes; and CAC−: allele G (guanine) homozygotes), and for ZNF804A (ZNF+: A homozygotes; and ZNF−: heterozygotes and C (cytosine) homozygotes). ZNF grouping is discussed in Mallas et al. (2016). Cell sizes (averaging 19.2 subjects) are shown in Table S1. The grouping of genotype groups had the purpose of maximising counterbalance for the rs1006737 CACNA1C SNP, as commonly practiced in the literature (Backes et al. 2014; Dietsche et al. 2014; Nieratschker et al. 2015; Ou et al. 2015; Woon et al. 2014) given the much lower frequency counts of allele A, compared to C, in the Caucasian population. The SNPs reported in this manuscript are the only SNPs analysed in this imaging data set.

The study was approved by the National Health Service (NHS) South East London Research Ethics Committee, UK (Project ‘Genetics and Psychosis (GAP)’ reference number 047/04). All subjects provided written informed consent at the time of participation. Participants were recruited from the South London and Maudsley (SLaM) NHS Trust. Diagnosis, according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, & DSM-IV, 1994) was ascertained by an experienced psychiatrist using a structured diagnostic interview with instruments detailed elsewhere (Prata et al. 2009). All SZ and BD patients were in a stable clinical state and all SZ and some BD were treated with antipsychotic medication (from which Chlorpromazine-equivalence was calculated, see Table 1).

Exclusion criteria applied to all participants were a history of significant head injury and current (last 12 months) substance dependency according to DSM-IV diagnostic criteria. Non-clinical controls were excluded if they had any personal or family history of a psychotic spectrum disorder. In order to follow the gold standard of experimental design that a control group must be matched to the experimental group on all variables except the one isolated for study, and avoid a biased ‘super-normal’ control group (Kendler 2003), participants with a previous, but not present, diagnosis of any other Axis I disorder (or family history) were not excluded given these are frequently present in SZ and BD (as such, two controls had once in the past been diagnosed with mild depression). None were psychiatrically unwell or on any psychiatric medication at the time of participation.
<table>
<thead>
<tr>
<th>Participant demographics (n = 230)</th>
<th>Diagnosis</th>
<th>Genotype</th>
<th>Test Statistic, df, P-value</th>
<th>Genotype</th>
<th>Test statistic, df, P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SZ (n = 63)</td>
<td>BD (n = 43)</td>
<td>Control (n = 124)</td>
<td>CAC+ (AA/AG; n = 138)</td>
<td>CAC− (GG; n = 92)</td>
</tr>
<tr>
<td>Age (SD)</td>
<td>33.78 (10.70)</td>
<td>41.07 (12.33)</td>
<td>35.79 (13.40)</td>
<td>34.86 (12.56)</td>
<td>38.28 (12.56)</td>
</tr>
<tr>
<td>IQ: Z-scores (SD)‡</td>
<td>−0.75 (2.89)</td>
<td>−0.87 (0.97)</td>
<td>−0.68 (3.51)</td>
<td>−0.60 (2.78)</td>
<td>−0.56 (3.27)</td>
</tr>
<tr>
<td>Antipsychotics dose (in CPZ equivalent; SD)</td>
<td>696.94 (613.02)</td>
<td>341.60 (434.56)</td>
<td>n/a</td>
<td>291.15 (530.70)</td>
<td>200.18 (376.18)</td>
</tr>
<tr>
<td>Years of Education (SD)</td>
<td>13.74 (2.61)</td>
<td>14.81 (3.10)</td>
<td>14.90 (2.79)</td>
<td>14.57 (2.98)</td>
<td>14.57 (2.68)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>50/13</td>
<td>18/25</td>
<td>67/57</td>
<td>83/55</td>
<td>52/40</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>White</td>
<td>46</td>
<td>40</td>
<td>104</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>Black Caribbean</td>
<td>6</td>
<td>1</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Black African</td>
<td>5</td>
<td>2</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Central Asian</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Mixed African and White</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Eastern Asian</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Handedness</td>
<td>Right</td>
<td>62</td>
<td>38</td>
<td>112</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Genotype (%)</td>
<td>AA</td>
<td>10 (16%)</td>
<td>15 (35%)</td>
<td>17 (14%)</td>
<td>17 (14%)</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>30 (48%)</td>
<td>15 (35%)</td>
<td>51 (41%)</td>
<td>51 (41%)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>23 (37%)</td>
<td>13 (30%)</td>
<td>56 (45%)</td>
<td>56 (45%)</td>
</tr>
</tbody>
</table>

BD, bipolar disorder; CAC+, high risk (AA/AG genotypes); CAC−, low risk (GG genotypes); df, degrees of freedom; n/a, not applicable; SZ, schizophrenia; SD, standard deviation.

*Statistically significant at p < 0.05.

†Statistically significant at 95% level.

‡Scores of full scale IQ from the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler 1999), the Wechsler Adult Intelligence Scale – Revised (WAIS-R) (Wechsler 1981) or the National Adult Reading Test (NART) (Nelson & Willison 1991), were standardised to Z-scores to permit between-group demographic comparison. The type of test used was balanced between diagnostic or genotype groups.
Genotyping
DNA was extracted from blood samples or buccal swabs following a standard protocol (Freeman et al. 1997, 2003) and a TaqMan SNP Genotyping Assay (Applied Biosystems 2010) for CACNA1C SNP rs1006737 (A/G) and ZNF804A rs1344706 (A/C) was performed blind to any phenotype data, at the Social Genetic and Developmental Psychiatry Centre (SGDP) lab, King’s College London.

Image acquisition
Magnetic resonance imaging (MRI) data were acquired using a 1.5T GE Signal LX system (General Electric, Milwaukee, WI, USA) in the Mapother House MR unit at the Maudsley Hospital, South London and Maudsley (SLAM) NHS Foundation Trust, London, UK. The scanner had actively shielded magnetic field gradients (maximum amplitude 40 mT/m), and a quadrature birdcage head coil was used for both radiofrequency (RF) transmission and signal reception. Each volume of DTI data was acquired using a multi-slice peripherally-gated echo planar imaging (EPI) sequence, optimised for eddy-current induced distortions, and then brain-extracted using the brain-extracted image) by fitting a tensor model to the raw diffusion data. The WM labelling based on the JHU ICBM-DTI-81 WM Atlas (Mori et al. 2000) provided by the Atlasquery tool FSL, was used to define clusters within the statistical images. We will also report simple effects for completeness. The ‘Cluster’ tool in FSL was used to define clusters within the statistical images. The mean FA within the largest cluster of each effect was graphically plotted for visual display of effects.

Results
Demographics
Participant demographic data are shown in Table 1. The BD patients (mean age = 41.1, SD = 12.3) were significantly younger than SZ patients (mean age = 33.8, SD = 10.7) and controls (mean age = 35.8, SD = 13.40), while there was no significant difference in age between the control and the SZ group. There were significantly ($\chi^2 = 17.2, P < 0.001$) more males (50 M:25 F) in SZ than BD (18 M:25 F) or control (67 M:57 F) groups. There was a significant difference ($\chi^2 = 11.17, df = 4, P = 0.03$) in genotype distribution between diagnostic groups. The SZ patients (mean CPZ score = 56.9, SD = 613.0) had a significantly higher (t(104) = 8.3, P < 0.001) CPZ-equivalent score than BD (mean CPZ score = 341.6, SD = 434.6). There were no other significant differences between diagnostic groups in IQ, years of education, ethnicity or handedness. There was a significant difference in the distribution of ethnicities between CAC+ and CAC− ($\chi^2 = 14.37, df = 6, P = 0.03$) but no other significant differences between the genotype groups in IQ, age, chlorpromazine (CPZ) equivalents in medication, years of education or handedness. ZNF804A genotype-wise demographics differed only for ethnicity ($\chi^2 = 20.86, df = 6, P < 0.001$) given the a priori different allele frequencies between African and Caucasian populations. Full details are reported elsewhere (Mallas et al. 2016) and displayed in Table S2.

Effect of CACNA1C genotype on FA
Whole-brain, voxel-based TBSS retrieved no significant main effect of CACNA1C rs1006737 genotype on FA in the total variance (ANOVA) in Statistical Package for Social Sciences (SPSS) v21 (Corp. 2012).

The FSL Randomise tool (Anderson & Robinson 2001) was used to perform permutation-based non-parametric inferences on the skeletonised FA data at a threshold of 0.2 (TBSS default) with 10,000 permutations. The significance level was set at $P < 0.05$ after multiple comparisons correction using threshold-free cluster enhancement (TFCE, Smith & Nichols 2009), an approach that allows the significance of a target voxel to take into account not only the amplitude of the signal (in this case FA) but also the contribution of both the spatial extent and the magnitude of supporting voxels. To assess the main effect of CACNA1C genotype, of ZNF804A genotype, and of diagnostic group as well as their interactions on FA, an ANOVA design matrix was built with CACNA1C genotype (CAC+ vs. CAC−), ZNF804A genotype (ZNF+ vs. ZNF−) and diagnosis (SZ, BD and controls) as the three independent variables, and age as a covariate in effects where age differed significantly across groups. The main effect of diagnosis and of ZNF has been reported in detail elsewhere (Mallas et al. 2016). We will also report simple effects for completeness. The ‘Cluster’ tool in FSL was used to define clusters within the statistical images. The mean FA within the largest cluster of each effect was graphically plotted for visual display of effects.

The WM labelling based on the JHU ICBM-DTI-81 WM Atlas (Mori et al. 2008) provided by the Atlasquery tool FSL, was used to determine the anatomical location of significant FA clusters; only with greater than 1% probability of being part of the WM in question were included in the cluster table. Where cluster results were retrieved as ‘Unclassified’, labelling was carried out manually by FC using the MRI Atlas of Human WM (Mori et al. 2005). Results were overlaid on MNI152 (1 mm) standard template and displayed in radiological convention.
CACNA1C impacts on white matter microstructure

Figure 1: Effect of CACNA1C rs1006737 genotype on fractional anisotropy. (a) FA was significantly reduced in SZ CAC+ group (i.e. risk A allele carriers for rs1006737) compared to the SZ CAC− group (i.e. protective G allele homozygotes (p < 0.05, TFCE-corrected) in locations labelled in red in this figure’s section. (b) The difference in FA between CAC+ and CAC− was not statistically significant anywhere in the BD or control groups alone, nor irrespective of diagnosis. (b) Figure depicting locations where in SZ, the CAC+ group showed significantly reduced FA compared to the CAC− group. Significant clusters displayed in red overlaid on MNI template in radiological view.

Table 2: White matter labels corresponding to clusters showing significant effects on fractional anisotropy (P < 0.05 after TFCE correction)

<table>
<thead>
<tr>
<th>Cluster size (Voxels)</th>
<th>Cluster maximum, Z-statistic</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>White matter labels*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SZ-specific effect CACNA1C (CAC+ &lt; CAC−)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>133</td>
<td>0.982</td>
<td>44</td>
<td>−69</td>
<td>8</td>
<td>WM of the L middle occipital gyrus</td>
</tr>
<tr>
<td>60</td>
<td>0.975</td>
<td>−31</td>
<td>−31</td>
<td>−15</td>
<td>WM of the L parahippocampal gyrus</td>
</tr>
<tr>
<td>47</td>
<td>0.967</td>
<td>8</td>
<td>−58</td>
<td>−46</td>
<td>R cerebellar WM</td>
</tr>
<tr>
<td>36</td>
<td>0.983</td>
<td>−20</td>
<td>−92</td>
<td>10</td>
<td>L optic radiation</td>
</tr>
<tr>
<td>35</td>
<td>0.967</td>
<td>−39</td>
<td>−14</td>
<td>−24</td>
<td>Subcortical WM of the L inferior temporal gyrus</td>
</tr>
<tr>
<td>24</td>
<td>0.958</td>
<td>−58</td>
<td>−40</td>
<td>−3</td>
<td>Subcortical WM of the L superior temporal gyrus</td>
</tr>
</tbody>
</table>

CAC+, high risk (AA/AG genotypes); CAC−, low risk (GG genotypes); SZ, Schizophrenia.

*Only tracts with clusters at >1% probability are included. White matter labels are provided in accordance with JHU ICBM-DTI-81 White Matter Atlas (Mori et al. 2008) using AtlasQuery in FSL unless marked with ‘*’, in which case they were labelled by FC based on MRI Atlas of Human White Matter, 1st Edition by Mori et al. (2005) due to retrieval from AtlasQuery returning as ‘Unclassified’.

sample. However, as a secondary result, there was a significant (P < 0.05, TFCE-corrected) simple effect of genotype within the SZ group alone (Fig. 1a), in which FA was significantly lower in the SZ CAC+ than in the SZ CAC− group. This effect encompasses WM portions of the left middle occipital and parahippocampal gyri, right cerebellum, left optic radiation and left inferior and superior temporal gyri (Fig. 1c; Table 2). No effect in the opposite direction was significant in any location, either as a main effect or within SZ, BD or controls separately.

**Interactions involving CACNA1C genotype, ZNF804A genotype and/or diagnosis on FA**

Before correcting for age, there was a significant (P < 0.05, TFCE-corrected) 3-way interaction between CACNA1C rs1006737 genotype, ZNF804A rs1344706 genotype and diagnosis affecting the body of the corpus callosum, the right superior corona radiata and the left anterior corona radiata, the largest cluster spanning 125 voxels. This reflected that the presence of the risk genotype of ZNF804A (ZNF+; vs. ZNF−) increased the magnitude of the effect of the
CACNA1C risk genotype (CAC+ vs. CAC−) on FA, in BD patients more than in controls. However, this effect was no longer significant when age (which was significantly higher in BD than controls or SZ groups) was added into the model as a covariate. No other locations showed alternative interaction patterns of this 3-way ANOVA.

**Effect of diagnosis on FA**

Both SZ and BD had significantly reduced FA compared to the control group. There was no significant difference in FA between patient groups, nor were there regions where FA was significantly decreased in the control group compared to patient groups. We have previously reported this in detail (Mallas et al. 2016).

**Discussion**

We assessed the effect across the human brain of the CACNA1C rs1006737 genotype on FA, unprecedentedly in a Caucasian clinical sample as well as in health, and whether this genotype effect was different between diagnostic groups and whether it interacted with the ZNF804A rs1344706 genotype. Although using a relatively large sample for imaging analysis, we detected no significant (P < 0.05, TFCE-corrected) main effects or interaction effects from an ANOVA.

As a result of a complementary analysis to our main hypotheses of main and interaction effects, we detected statistically significant, albeit small, effect (P < 0.05, TFCE-corrected), in the predicted direction, in our SZ sample alone. This is the first evidence of an association between CACNA1C rs1006737 risk allele A and reduced FA in a predominantly Caucasian clinical sample. This result may suggest the involvement of the GWA-implicated linkage disequilibrium region tagged by allele A at CACNA1C rs1006737 locus in influencing susceptibility to psychosis by demonstrating its effect in reducing FA in the SZ population. This putatively reflects changes in WM microstructure and reduction of WM integrity in SZ. Comparatively, both the effect in SZ and the overall main effect of genotype that we found for another top GWA-implicated ZNF804A rs1344706 was much larger and more spatially expansive (Mallas et al. 2016): the main effect of ZNF804A genotype spanned more than 44,054 voxels in the largest cluster and its effect in SZ only, spanned 51,260 voxels. Thus, one should put these polymorphisms’ effect in perspective: both the effects of ZNF804A genotype spanned an location of about two orders of magnitude larger than the one under the CACNA1C genotype effects we report herein.

Given that we found an effect of CACNA1C in SZ but not in controls, but a CACNA1C genotype by diagnosis interaction was not significant, it is possible that power was not sufficient to detect the latter. We encourage further studies to use a larger sample study to detect whether SZ does indeed increase susceptibility to a CACNA1C rs1006737 effects. Our finding that FA in SZ patients carrying the risk allele was significantly lower than in SZ non-risk allele carriers is consistent with those reported by Woon et al. (2014) in a Chinese SZ population of similar size (96 SZ and 64 controls). However, in our study we found an effect in the left middle occipital gyrus, left parahippocampal gyrus, left optic radiation and left temporal gyrus WM, while Woon et al. (2014) reported the left frontal lobe, left parietal lobe and left temporal lobe WM. We found significantly reduced FA in the right cerebellar WM, while Woon et al. (2014) found it on the left. Unfortunately, precise comparisons with their study are limited by the different approaches: while we report comparatively more precise (TBSS) tract-based or gyri, they report very wide lobes.

In our non-clinical volunteers, the high risk A allele carriers did not differ significantly in any brain region’s FA compared to the low risk G homozygotes, for CACNA1C rs1006737. This is inconsistent with recent positive findings reported by Dietsche et al. (2014) of decreased FA in the right hippocampal formation in non-clinical A allele carriers. A possible explanation is that Dietsche et al. (2014) focused on one region of interest (ROI) in contrast to our whole-brain analysis, thus allowing a more relaxed multiple comparisons correction in their selected location.

We also report the first test of a CACNA1C and ZNF804A interaction. We predicted their respective GWA-implicated risk SNPs would interact in an additive manner, the risk increasing when both risk allele groups were present (e.g. CAC+ and ZNF+) – in consistency with the hypothesis that both these polymorphisms increase the risk for psychosis, and that psychosis is associated with a (widespread) decrease in FA. Prior to covarying for age, we found, albeit only in BD compared to controls (and surprisingly not in SZ vs. controls), that the concomitant presence of both risk alleles was represented more in subjects with relatively decreased FA in the body of the corpus callosum, the right superior corona radiata and the left anterior corona radiata. However, when we added age as a covariate to the model this effect did not survive significance, suggesting that the difference in BD patients’ age (vs. controls or SZ) across genotype groups had driven this interaction effect. Further research therefore needs to explore this effect in a larger, age matched sample.

Structural volumetric imaging studies have also implicated the CACNA1C rs1006737 risk-allele in increased regional (Frazier et al. 2014) and total (Kempton et al. 2009; Wang et al. 2011) grey matter volumes in non-clinical controls, inclusive in a dose-dependent manner (Kempton et al. 2009). Others have found the same trend in the amygdala and hypothalamus (Perrier et al. 2011) irrespective of diagnosis, and, in SZ patients specifically, the amygdala (Wolf et al. 2014). However, a larger sample of almost 600 healthy subjects failed to replicate this association (Franke et al. 2010), as did another study in amygdala and hippocampal volumes, in BD patients and healthy controls (Soeiro-de-Souza et al. 2012). Regarding WM, the risk allele has also been associated with increased total and fronto-limbic volumes (Frazier et al. 2014).

Functional MRI studies have also implicated the CACNA1C rs1006737 risk allele (A) in candidate behavioural intermediate phenotypes for BD and SZ, it being associated with increased activation (and thus putatively ‘inefficient’) when performance differences are controlled for) in the left inferior frontal gyrus and left precuneus during a verbal fluency task (Krug et al. 2010), in which performance is often impaired in
SZ and BD (Krabbendam et al. 2005). The same allele has also been associated with increased activation in the left amygdala in BD patients in a face-matching task (Tesli et al. 2013), increased hippocampal and prefrontal activation during emotional processing and executive cognition tasks, respectively (Bigos et al. 2010), and enhanced right amygdala response both during a monetary reward task (Wessa et al. 2010) and a face recognition task (Jogia et al. 2011). In contrast, response reductions in bilateral hippocampal (Erk et al. 2010), medial frontal gyrus (Thimm et al. 2011) and right dorso-lateral prefrontal cortex (dPFC; Paulus et al. 2013) have also been associated with this allele during episodic memory, executive control of attention and working memory tasks, respectively. While the findings of reduced activation are harder to reconcile, those of (putatively inefficiently) increased activation during cognitive executive tasks provide converging support to our finding that the same risk allele is influencing WM microstructural abnormalities. Nevertheless, further research combining fMRI-based and DTI-based connectivity would be paramount to adequately test to what extent localized functional cognitive deficits are caused by FA deficits affecting the same regions.

Regarding the influence of the illnesses on the CACNA1C rs1006737 genotype effect, Jogia et al. (2011) observed a significant genotype × diagnosis interaction in the right ventro-lateral prefrontal cortex, where risk-allele carriers in the BD group, but not unaffected relatives’ nor unrelated controls, showed reduced activation. Furthermore, in a partial superset of the current sample, including 20 BD patients, 20 unaffected first-degree relatives and 20 unrelated healthy controls, Radua et al. (2013) found a similar genotype × diagnosis interaction, this time on effective brain connectivity. The risk allele A was associated with decreased outflow of information from the medial frontal gyrus during perception of fearful faces, significantly more so in BD than relatives or controls. This disruption in connectivity is a plausible consequence of WM microstructural abnormalities we found herein and thus supports the idea that this genotypic variation exerts its functional effect, to a greater extent in an illness context, via a disruption in WM microstructure.

Supporting the plausibility that genetic factors, such as CACNA1C rs1006737 A allele, could increase risk of psychosis through predisposing individuals to abnormalities in WM microstructure, are heritability studies of WM. These have suggested that genetic factors explain a large proportion of the variance in WM inter-individual differences (originally estimated at around 88%, Baaré et al. 2001) with a meta-analysis of over 50 studies further supporting the genetic basis of total and regional WM volumes (Blokland et al. 2012). Mechanism-wise, it is possible that CACNA1C rs1006737 exerts its effect through altering levels of CACNA1C mRNA expression. As rs1006737 is located in a non-coding region of the gene, it is not thought that this SNP directly interferes with CaV1.2 properties. The risk allele (A) was associated with increased mRNA expression of CACNA1C in induced human neurons, and increased density of Cav1.2-mediated currents (Yoshimizu et al. 2014), albeit it was also found to be associated with decreased expression in the human cerebellum (Gershon et al. 2014). As the latter authors state, this may suggest that either an increase or decrease of calcium influx in excitable cells might be associated with BD, as both could lead to changes in monoamine neurotransmitter synthesis and release (Gershon et al. 2014) which has been associated with psychiatric disorders (Booj et al. 2003). Nevertheless, a more comprehensive characterization of this polymorphism’s impact on mRNA levels across different brain regions is still warranted.

In conclusion, and in combination with our previous work (Mallas et al. 2016), we provide evidence that the genomic regions tagged by the two most significantly genome-wide associated psychosis risk variants for SZ and BD (in the genes for CACNA1C and ZNF804A) may increase susceptibility to abnormal WM microstructure and, as such, to psychosis. Effects of the respective causative ZNF804A polymorphism were demonstrated to be two orders of magnitude more widespread (and present as a main effect as well as a SZ-specific effect) compared to those of the CACNA1C one (where only a SZ-specific effect was found).

References


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Acknowledgments

D.P. was supported by UK National Institute for Health Research fellowship (NIHR, PDF-2010-03-047), a Marie Curie Career Integration grant (FP7-People-2013-CIG-631952) and a Fundação para Ciência e Tecnologia (FCT) Investigator grant (IF/00787/2014). E.B. was supported by a Medical Research Council (MRC) New Investigator Award (G0901310) and the Wellcome Trust (085475/B/08/Z and 085475/Z/08/Z). S.S. is supported by a European Research Council (ERC) Consolidator award and the NIHR BRC for Mental Health at the South London and Maudsley NHS Foundation Trust. G.J.B. received honoraria for teaching from General Electric Healthcare during the course of this research, and acts as a consultant for IXICO. No other author reports biomedical financial interested or declared potential conflicts of interest.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher’s web-site:

**Table S1**: Cell size counts (*n* = 230).

**Table S2**: Demographics for ZNF804A genotype.