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Author for correspondence:
Professor G. Donohoe, E-mail: gary.donohoe@nuigalway.ie

Genetically predicted complement component 4A expression: effects on memory function and middle temporal lobe activation

G. Donohoe[1], J. Holland[1], D. Mothersill[1], S. McCarthy-Jones[2], D. Cosgrove[1], D. Harold[3], A. Richards[4], K. Mantripragada[4], M. J. Owen[4], M. C. O'Donovan[4], WTCCC2, M. Gill[2], A. Corvin[2] and D. W. Morris[1]

[1]The Cognitive Genetics & Cognitive Therapy Group, The School of Psychology and Discipline of Biochemistry, The Centre for Neuroimaging & Cognitive Genomics, National University of Ireland Galway, University Road, Galway, Ireland; [2]Neuropsychiatric Genetics Research Group, Department of Psychiatry & Institute of Molecular Medicine, Trinity College Dublin, Dublin, Ireland; [3]School of Biotechnology, Dublin City University, Dublin, Ireland and [4]MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University School of Medicine, Cardiff, UK

Abstract

Background. The longstanding association between the major histocompatibility complex (MHC) locus and schizophrenia (SZ) risk has recently been accounted for, partially, by structural variation at the complement component 4 (*C4*) gene. This structural variation generates varying levels of *C4* RNA expression, and genetic information from the MHC region can now be used to predict *C4* RNA expression in the brain. Increased predicted *C4A* RNA expression is associated with the risk of SZ, and *C4* is reported to influence synaptic pruning in animal models.

Methods. Based on our previous studies associating MHC SZ risk variants with poorer memory performance, we tested whether increased predicted *C4A* RNA expression was associated with reduced memory function in a large ($n = 1238$) dataset of psychosis cases and healthy participants, and with altered task-dependent cortical activation in a subset of these samples.

Results. We observed that increased predicted *C4A* RNA expression predicted poorer performance on measures of memory recall ($p = 0.016$, corrected). Furthermore, in healthy participants, we found that increased predicted *C4A* RNA expression was associated with a pattern of reduced cortical activity in middle temporal cortex during a measure of visual processing ($p < 0.05$, corrected).

Conclusions. These data suggest that the effects of *C4* on cognition were observable at both a cortical and behavioural level, and may represent one mechanism by which illness risk is mediated. As such, deficits in learning and memory may represent a therapeutic target for new molecular developments aimed at altering *C4*'s developmental role.

Introduction

Schizophrenia (SZ) is a highly heritable disorder associated with disturbances in perception, cognition and affect, the biological basis of which is only partly understood. Successful identification of over 100 genetic risk loci to date has provided an important basis from which to begin to identify relevant biological mechanisms and their functional significance. Recently, a study of the major histocompatibility complex (MHC) region by Sekar *et al.* (2016) identified one potential such mechanism involving a locus containing the complement component 4 (*C4*) gene isotypes *C4A* and *C4B*. In that study, *C4* structural variation was associated with significantly altered *C4* RNA expression (as measured in post-mortem brain tissue) such that copy number and structure of these genes could be used to predict *C4A* and *C4B* brain expression levels. Predicted *C4A* RNA expression was highly significantly associated with SZ risk ($p = 3.6 \times 10^{-24}$) in the Psychiatric Genomics Consortium (PGC) SZ GWAS data (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), driven by an allelic series of SZ risk levels that corresponded to each allele's relationship to *C4A* expression levels. The GWAS signal at the MHC region appeared to arise from at least three distinct genome-wide significant signals, one of which involves this collection of allelic influences on *C4A* expression. Finally, in a region of the mouse thalamus responsible for visual processing (an established model for experience-dependent synaptic refinement) *C4* RNA was expressed in neurons during a period of peak synaptic pruning, and mediated synaptic refinement in this system (Sekar *et al.* 2016). Whether or how predicted *C4* expression is associated with perceptual and cognitive function in humans is unknown.

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The MHC region contains scores of genes with roles in the adaptive and innate immune systems and is the location of SZ's most significant genetic association (for common genetic variation) at a population level. Our group has previously reported a series of studies highlighting the cognitive and cortical effects of SZ-associated genetic risk loci in the MHC region and in non-MHC genes potentially related to complement regulation. We have shown that the SZ risk allele at rs10503253 within *CSMD1*, which encodes a regulator of *C4*, was associated with poorer general cognitive ability and episodic memory function in large independent samples of patients and healthy participants (Donohoe *et al.* 2013). We further showed that the same risk allele was associated with reduced cortical activation within the occipital cortex and cuneus during a spatial working memory task (Rose *et al.* 2013). We have also shown that the SZ risk allele at rs6904071, a perfect proxy for the top MHC SZ risk SNP rs13194053 identified by both the International Schizophrenia Consortium (Purcell *et al.* 2009) and Molecular Genetics of Schizophrenia (Shi *et al.* 2009) studies, was associated with episodic memory performance in the same large datasets, and – in a third independent sample – with decreased hippocampal volume (Walters *et al.* 2013). Given the demonstrated role for *C4* in a model of experience-dependent synaptic pruning, we speculated that *C4*'s effects on synaptic pruning may also be apparent behaviourally and cortically during performance of perceptual and cognitive tasks. The findings from our previous *CSMD1* and MHC studies, which have been supported by studies of other complement genetic variants (Athanasiu *et al.* 2017; Zhang *et al.* 2017), caused us to specifically hypothesize a role for *C4* variation in memory function.

The purpose of the present study was to examine the relationship between predicted *C4A* RNA expression (based on structural variation in the C4 gene) and cognition in a large Irish sample of cases and healthy participants. In terms of the evidence and justification for the use of predicted C4A expression based on C4 structural variation, the following is noteworthy. In the Sekar *et al.* (2016), based on eight panels of post-mortem human adult brain samples (674 samples from 245 distinct donors in three cohorts), RNA expression of C4A and C4B increased proportionally with copy number of C4A and C4B, respectively; the results of these expression analyses were consistent across all five brain regions analysed. Similarly, in serum, a previous study also reported that C4 gene dosage was positively correlated with serum C4 protein concentrations *in vivo*, mirroring the observations in the Sekar *et al.* post-mortem samples paper (Yang *et al.* 2003). Sekar *et al.* (2016) further measured *C4A* RNA expression levels in brain tissue samples from 35 SZ patients and 70 individuals without SZ. The median expression of *C4A* in brain tissues from SZ patients was 1.4-fold greater and was elevated in each of the five brain regions assayed. This was consistent with earlier reports that elevated the levels of complement proteins that were present in the serum of SZ patients (Rudduck *et al.* 1985; Hakobyan *et al.* 2005).

Based on this evidence above, and our previous studies, we hypothesised that increased predicted *C4A* RNA expression (which is associated with increased SZ risk) would be associated with *poorer* memory function in patients with SZ and in healthy participants. Given Sekar *et al.*'s report that *C4* expression may influence visual development in an animal model, we also investigated, using functional MRI, whether predicted *C4A* expression would explain variation in cortical activity during a visual processing task in a healthy participant sample.

Methods

Participants

In total, 908 cases and 330 healthy participants completed a full neuropsychological assessment battery and had full genome-wide SNP data available on the basis of which predicted *C4* expression levels could be calculated (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Cases consisted of $n = 676$ clinically stable patients with a diagnosis of SZ and schizoaffective disorder (SZA), and an additional $n = 232$ patients with 'broad sense' psychosis – diagnosed with either bipolar disorder with psychotic features, major depressive disorder with psychotic features, delusional disorder, or psychosis not otherwise specified. Patients were diagnosed by trained psychiatrists using the Structured Clinical Interview for DSM-IV Axis I Diagnosis (First, 2005). These patients were recruited from five sites across Ireland. Inclusion criteria required participants to be clinically stable at the time of cognitive assessment, aged between 18–65 years, no history of co-morbid psychiatric disorder, no substance abuse in the preceding 6 months, no prior head injury with loss of consciousness, no history of seizures and with Irish ancestry (all four grandparents born in Ireland). Symptom severity was measured using the SAPS and SANS scores as previously described by us (Donohoe *et al.* 2009; Walters *et al.* 2010).

Healthy participants were recruited from the general population through local media advertisements. All were aged between 18 and 65 years and had Irish-born paternal and maternal grandparents, and satisfied, on the basis of clinical interview, the criteria of having no history of major mental health problems, intellectual disability or acquired brain injury, and no substance abuse in the preceding 6 months. Exclusion criteria also included having a first-degree relative with a history of psychosis. All assessments were conducted in accordance with the relevant ethics committees' approval from each participating site, and all participants provided written informed consent. In this study, healthy participants did not represent a control group as no direct phenotypic comparison are made with patients; instead healthy participants are included both to establish whether comparable effects of predicted *C4* expression levels were observed in both groups and, in a subset of these samples, to test for cortical effects using MRI.

Cognitive assessment

Memory recall was assessed using the Logical Memory subtest (immediate and delayed conditions) from the Wechsler Memory Scale, Third Edition (WMS III) (Wechsler, 1997) and the Paired Associated Learning task (PAL; stages completed and total errors) from the Cambridge Automated Neuropsychological Test Battery (Robbins *et al.* 1994). Working memory was assessed using the Spatial Working Memory (SWM) subtest from the Cambridge Automated Neuropsychological Test Battery (Robbins *et al.* 1994) and Letter-Number Sequencing (LNS task) from the WMS III. Finally, measures of general cognitive ability (derived from the Wechsler Adult Intelligence Scale, Third Edition) (Wechsler, 1997) and attentional control [Continuous Performance Task, Identical Pairs version; CPT-IP (Cornblatt *et al.* 1988)] were also included as patients with SZ frequently show deficits in these areas of function. The published norms from the Wechsler test battery, the CANTAB test batteries, and the CPT-IP indicate a high level of test–retest validity, and, having been widely used in SZ

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research, have consistently showed a high sensitivity to cognitive deficits.

Functional MRI assessment

A subgroup of the healthy participants ($n = 87$) underwent functional imaging during a visual processing task as described by us previously (Grosbras & Paus, 2006; Donohoe *et al.* 2007; Rose *et al.* 2012; Mothersill *et al.* 2014*a*, *b*). In this task, a face processing task developed by Grosbras & Paus (2006), participants watched a series of 2–5-s black-and-white videos of either contrasting circular images (expanding/contracting black-and-white concentric circles; 'baseline' condition), or faces which started from a neutral expression, and then turned into an angry expression or neutral expression. Overall, there were 28 blocks of 18-s duration each consisting of 4–7 video clips: nine blocks of concentric circles, five blocks of neutral face videos, five blocks of angry face videos. Attention to task was confirmed on the basis of a face recognition task following completion of the fMRI task and outside the scanner. Six of the 87 participants scored <4/5 on this task and were excluded from further analysis.

Imputation of C4 structural variation and genetically predicted C4A expression

Genotyping was conducted on DNA extracted from blood or saliva from patient and healthy participant participants. SNP data were obtained from two different sites; a GWAS using the Affymetrix SNP Array 6.0 platform, conducted as part of the Wellcome Trust Case Control Consortium 2 (Irish Schizophrenia Consortium & The Wellcome trust Case Control Consortium 2, 2012) and a collaborative GWAS with Cardiff University using an Illumina HumanCoreExome (+custom) SNP array. Direct genotypes for SNPs in the region of 23–35 Mb on chromosome 6 from the Affymetrix ($n = 3657$ SNPs) and Illumina ($n = 3712$) data were used to impute *C4* structural alleles and predicted expression. This analysis of our data was undertaken by a member of the McCarroll group using the same methods described previously by them (Sekar *et al.* 2016). In brief, this involved imputation of *C4* structural alleles in the study populations using a 222 haplotype integrated SNP and *C4* reference panel. Imputed structural alleles were used to determine copy number of *C4* structural elements (*C4A*, *C4B*, *C4L* and *C4S* and their co-occurrence) in each individual, and expected expression of *C4A* and *C4B* in the brain was inferred based on the previously determined relationship of copy number of *C4* structural elements to gene expression in human brain samples. This resulted in a normally distributed range of predicted C4 expression scores of between 0 and 1.87 (mean 1.23, S.D. 0.45).

Statistical analysis – neuropsychological tests

To estimate the correlation between predicted *C4A* expression levels and performance of memory and other cognitive tasks, a series of correlational analysis was performed using Pearson's *r*, followed by multiple regression analysis for significant variables using IBM SPSS Statistics (IBM Corp, 2012). As this regression analysis focused on memory tasks known to be correlated with each other, and observed here to be correlated with predicted *C4* expression levels, an unrotated principal components analysis was undertaken based on the four episodic memory test available to reduce the multiple testing burden. This resulted in one component which explained 72% of the variance in memory scores being extracted (with factor loadings of 0.881 for logical memory 1, 0.889 for logical memory 2, 0.766 for PAL stages and −0.813 for PAL total errors); participants scores on this factor were used as the dependent variable in the regression analysis. Age and gender were entered into the regression analysis as covariates of no interest. As cognitive profiles of patients with SZ and SZA are typically reported to differ from other kinds of psychosis (e.g. bipolar disorder), the analysis was undertaken both in the full group, and with psychosis patients with disorders other than SZ and SZA removed. Power calculations for these regression analyses indicated that sample sizes of $n = 385$ or greater would be required to observe small effects. This suggests that in the present study of 908 cases and 330 health participants (total sample $N = 1238$), we were adequately powered to detect small effects based on the full sample and the patient-only sample, but were somewhat underpowered to detect small effects in the healthy participant-only sample.

Imaging pre-processing and statistical analysis

Spatial pre-processing and statistical analysis of MRI data was performed using Statistical Parametric Mapping (SPM8, revision 4290, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) and MATLAB R2011b (v7.13; http://www.mathworks.co.uk/). Functional images were realigned to the mean functional image, normalised to Montreal Neurological Institute (MNI) space with a voxel size of 3 mm × 3 mm × 3 mm and smoothed using a 10 mm full width at half maximum (FWHM) isotropic Gaussian filter. After spatial pre-processing, graphical plots of the estimated time series of translations and rotations were inspected for excessive motion, which we defined as more than 3 mm translation and/or 3° rotation. One participant was excluded from further analysis due to movement, and six participants were excluded due to low-quality MRI data and/or significant artefacts, resulting in a final sample of 74 participants. For the face processing task, three task conditions (angry faces, neutral faces and baseline) and four contrasts consistent with our examination of neural activity associated with this task in SZ patients (Grosbras & Paus, 2006; Mothersill *et al.* 2014*a*): neutral faces *v.* baseline, angry faces *v.* baseline, all faces (angry and neutral) *v.* baseline and angry faces *v.* neutral faces. Participants' contrast maps were entered into a second-level analysis to investigate effects of predicted *C4* expression on neural activity. Results were examined at a $p < 0.001$ (uncorrected) level and clusters were considered statistically significant at a $p < 0.05$ level after family-wise error corrected for multiple comparisons across the whole brain at the cluster level. For each of these clusters, MNI coordinates of significant maxima were entered into the Anatomy toolbox in SPM 8 (Eickhoff *et al.* 2005, 2006, 2007) and probable anatomical regions were identified using the AllAreas_v18_MPM atlas.

Results

C4 neuropsychological results

Demographic and clinical characteristics for patients and healthy participants appear in Table 1. Predicted *C4A* expression levels were not associated with age, gender or years of education. In terms of clinical symptom severity, no association was observed between predicted *C4A* RNA expression levels and either positive, negative or disorganized symptom factor scores [based on a

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Table 1. Mean demographic, clinical and predicted C4A expression levels for participants included in the neuropsychological analysis

	Schizophrenia/ Schizoaffective disorder ($n = 676$) *Mean* (*s.d.*)	Other psychosis ($n = 232$) *Mean* (*s.d.*)	Healthy participants ($n = 330$) *Mean* (*s.d.*)	*C4A* expression (all participants) r (p *value*)
Age	42.33 (12.48)	44.94 (12.08)	35.87 (12.64)	$r = 0.016$ ($p = 0.62$)
Gender (M:F)	476:200	123:109	147:183	t (961) = 0.414, ($p = 0.697$)
Year in education	12.60 (2.46)	12.89 (2.82)	15.9 (2.3)	$r = -0.13$ (0.79)
SAPS	21.74 (19.72)	12.86 (15.92)	–	$r = -0.06$ (0.17)
SANS	26.23 (20.01)	14.06 (16.39)	–	$r = 0.00$ (0.99)
Chlorpromazine equivalents	520.42 (482.29)	275.45 (307.31)	–	$r = 0.014$ (0.72)
Predicted *C4A* expression levels (*n*)	1.26 (0.44) ($n = 578$)	1.20 (0.46) ($n = 190$)	1.19 (0.45) ($n = 195$)	$r = 1$

principal components analysis of SAPS and SANS scores previously described by us (Donohoe *et al.* 2009)]. Similarly, no association between predicted *C4A* expression and medication dosage, measured in terms of chlorpromazine equivalents was observed.

Based on a correlational analysis, increased predicted *C4A* RNA expression levels were associated with poorer performance on all indexes of both verbal and non-verbal episodic memory performance (see Table 2). Given the correlation between these measures, to estimate the amount of variance in memory function explained by predicted *C4A* expression levels, these four memory scores were combined using an unrotated principal components analysis, the first extracted component of which explained 72% of variance on these measures. Participant's scores on this memory factor were then used as the dependent variable in the regression analysis. After the effects of age and gender were accounted for (as covariates of no interest), predicted *C4A* expression continued to significantly predict variation in memory performance (F change = 8.07; df = 1653; $p = 0.005$), explaining 1.2% of variation in memory factor scores (see Table 3). On the basis of a Bonferroni correction for the four cognitive constructs included in this study, this finding survives correction for multiple testing [corrected p value (0.005×4) = 0.02]. Re-running the analysis to account for diagnosis (entered as a covariate on the step prior to entering predicted *C4 expression level*), the results were unchanged (F change = 9.3; df1639; $p = 0.002$; r^2 change = 1.1%). Similarly, results remained significant when only patients and not healthy participants were included in the analysis (F change = 4.71; df1499; $p = 0.030$; r^2 change = 0.8%), or when only narrow psychosis and healthy participants were included and not non-SZ psychotic cases (F change = 8.2; $d = 1513$; $p = 0.004$; r^2 change = 1.3%). Finally, in an analysis of the healthy participant group only (which was less than half the size of the patient sample),

Table 2. Correlation coefficients for predicted C4A expression and cognitive function in psychosis cases and healthy controls

Cognitive function	Broad psychosis cases and controls r (p value)	SZ/SA cases only and controls r (p value)
Episodic memory		
Logical memory immediate	−0.064 (0.049)	−0.064 (0.079)
Logical memory delayed	−0.069 (0.036)	−0.068 (0.065)
Paired associate learning – stages completed	−0.105 (0.007)	−0.120 (0.014)
Paired associate learning – total errors	0.017 (0.616)	0.107 (0.012)
Working memory		
Spatial working memory – total errors	0.052 (0.169)	0.050 (0.232)
Letter number sequencing	0.021 (0.526)	0.028 (0.455)
General cognitive ability		
Premorbid IQ	0.032 (0.363)	0.036 (0.364)
Verbal IQ	−0.013 (0.690)	−0.007 (0.839)
Performance IQ	−0.042 (0.240)	−0.047 (0.238)
Full scale IQ	−0.029 (0.417)	−0.038 (0.343)
Attentional control		
CPT D-prime – two numbers	0.008 (0.870)	0.021 (0.702)
CPT D-prime – 3 numbers	0.009 (0.860)	0.039 (0.478)
CPT D-prime – 4 numbers	0.018 (0.718)	0.060 (0.297)

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Table 3. Regression analysis of C4 expression levels and episodic memory scores in patients and controls

Groups	*N*	*F* (model)	*F* change	R^2 (model)	R^2 change	df	p
Whole group							
–	655	8.07	8.066	0.012	0.012	1653	0.005
+	655	38.96	7.214	0.152	0.009	1651	0.007
++	644	60.42	9.355	0.274	0.011	1639	0.002
Broad psychosis							
–	503	5.42	5.416	0.011	0.011	1501	0.020
+	503	20.03	4.710	0.107	0.008	1499	0.030
HCs and SZ/SA							
–	517	7.467	7.467	0.014	0.014	1515	0.007
+	517	34.31	8.175	0.167	0.013	1513	0.004
++	517	123.43	3.52	0.491	0.003	1512	0.061
HCs only							
+	141	0.154	0.154	0.001	0.001	1139	0.696
–	141	5.268	0.582	0.103	0.004	1137	0.447

– Analysis without adding covariates.
+ Analysis including covariates of age and gender in regression block 1.
++ Analysis including covariates of age, gender and diagnosis as other covariate.

predicted *C4* expression showed the same direction of association as in patients but was not statistically significant.

This relationship between predicted *C4A* expression and episodic memory was observed in the absence of any correlation with working memory. Similarly, predicted *C4A* expression was not observed to correlate with either general cognitive ability or attentional control (see Table 2).

Two other variants within the MHC region were each associated with the risk in the Sekar *et al.* study, independently of *C4* and of each other. For one of these, rs210133, we did not find any association with memory (r^2 change = .001, N.S.). The other SNP, rs13194504, was not available in our dataset. Instead we use a linkage disequilibrium (LD) proxy SNP rs148082388 ($r^2 = 0.87$) 82.5 kb away to investigate whether the same memory effects were associated with this SNP; a comparable association with poorer memory function was observed (r^2 change 0.6%; *F* change = 4.46; $p = 0.035$). This SNP is also in moderately high LD ($r^2 = 0.67$) with the MHC risk variant rs115329265 reported on by the PGC (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), for which we observed a similar association with poorer memory function (r^2 change 0.5%; *F* change = 5.23; $p = 0.022$). Finally, to relate our *C4* predicted expression findings to our earlier cognitive findings with MHC SNP rs6904071 (Walters *et al.* 2013), a Pearson's *r* correlation was carried out, based on which a statistically significant positive correlation was observed ($r = 0.32$, df = 610, $p = 7.56 \times 10–16$).

Table 4. MRI participant demographics

Age	27.87 ± 7.59[a]
Gender	40 M/34 F
Years of education	17.58 ± 3.32
C4A expression	1.194 ± 0.520

[a]Mean ± standard deviation reported.

C4 *fMRI analysis in healthy participants*

In the subset of participants for whom fMRI data were available, differences in predicted *C4A* expression were not observed to associate with either age or gender ($p > 0.05$; see Table 4). A nominally significant (positive) correlation with years of education was observed ($p = 0.04$). We therefore examined the effects of education on neural activity across our sample for all experimental conditions examined but no significant effects of education were observed, so education was not considered further.

Neural activity during face processing task

Based on a whole brain analysis, increasing levels of genetically predicted *C4A* expression significantly correlated with decreased activity in a cluster incorporating the middle temporal gyrus during neutral face processing compared to baseline [$t_{(74)} = 5.49$; corrected $p < 0.05$; see Table 5 and Fig. 1]. This relationship was also observed during angry face processing *v.* baseline and all faces *v.* baseline, but only at trend levels (uncorrected $p < 0.001$). To check for outlier effects, each participant's mean parameter estimates for all voxels were calculated for the temporal cluster showing a significant correlation with predicted *C4A* expression. These parameter estimates were then inputted into SPSS to check for outlier values, which were defined as any value more than 1.5 times the interquartile range of the values. No outliers were detected.

Discussion

This study examined the effects of genetically predicted *C4A* RNA expression on neuropsychological function in a large dataset of psychosis cases and healthy participants, and on task-dependent

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Table 5. Cluster showing significantly decreased activity with increasing predicted C4A expression during neutral face processing relative to baseline, corrected for multiple comparisons at the cluster level

Extent (voxels)	*p* value	Cluster number	Cluster peak	*t*-value	*Z*-value	Peak coordinates (MNI)
202	0.004	1	Right middle temporal gyrus	5.49	5.00	51–67.7
			Not found on any probability map	3.56	3.40	36–67.19
			Not assigned	3.53	3.38	33–58.28

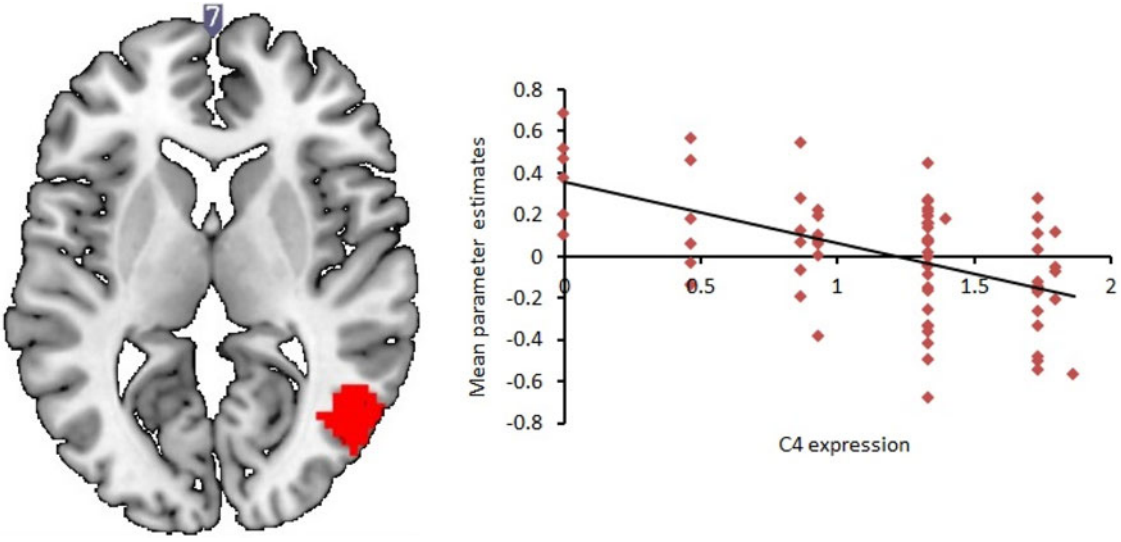


Fig. 1. Increased *C4A* expression is associated with decreased right middle temporal response during neutral face processing. Red: cluster showing decreased neural response with increasing *C4A* predicted expression levels [$N = 74$; multiple regression with *C4A* expression as covariate of interest; significance set at $p < 0.05$, family-wise error (FWE) corrected for multiple comparisons across the whole brain at the cluster level; df = 71]. The two-dimensional axial slice is labelled with an MNI coordinate. Cluster is rendered on the 'ch256' brain template using MRIcroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). Parameter estimates displayed in arbitrary units.

cortical activation during a visual task in a subset of healthy samples. Based on recent evidence of an association between predicted *C4A* RNA expression and increased SZ risk in humans, and between *C4* deficiency and altered synaptic pruning in mice (Sekar *et al.* 2016), and our previous neurocognitive studies of variants at this locus, we hypothesised that variation in predicted *C4A* RNA expression would be associated with reduced memory function and altered neural activity. In testing this hypothesis, we observed that increased predicted *C4A* RNA expression was significantly correlated with, and predictive of, poorer performance on measures of episodic memory in both patients and healthy participants. Furthermore, based on an analysis carried out in a subset of our healthy participants, we found that increased predicted *C4A* RNA expression was associated with a pattern of reduced cortical activity in the middle temporal gyrus during a measure of visual processing.

Among the cognitive deficits associated with SZ, deficits in memory function are among the largest observed (Heinrichs & Zakzanis, 1998). The association between predicted *C4A* RNA expression and poorer episodic memory observed in this study are highly consistent with our previous studies of other genetic risk variants either at this locus or known to directly interact with *C4*. *C4* was selected for a study by Sekar *et al.* (2016) on the basis of the MHC signal previously reported both in the PGC GWAS and by previous GWAS (Ripke *et al.* 2013). On the basis of our analysis of the MHC risk allele at rs6904071, we previously reported an association with poorer episodic memory and, in an independent cohort, with decreased hippocampal volume. Even though the correlation between rs6904071 and predicted C4 expression moderate (r^2 estimate of shared variance ~10.2%), the patterns of cognitive results here are highly consistent with both the specific phenotype and direction of those previous findings. At present, other cognitive datasets in which predicted C4 expression levels have been calculated are not available; although supportive of our earlier MHC findings, independent replication of these results will be required to confirm *C4*'s effects on cognition. Finally, the association with memory performance observed here is unlikely to be solely attributable to inattentiveness, as these associations were observed in the absence of an association with attentional performance as measured by the CPT-IP.

Sekar *et al.* reported two other variants within the MHC region which were each associated with risk, independently of *C4* and of each other. Based on an analysis of an LD proxy for one of these – rs148082388, a comparable association with poorer memory function was observed. As noted, this SNP is in moderately high LD with the MHC risk variant rs115329265 reported on by the PGC (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), and for which we observed a similar association with poorer memory function. While it is highly unlikely that all SZ-associated variants within the MHC locus would show the same phenotypic effects, the consistency of

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these genetic effects on memory function is interesting. Returning to *C4* in particular, the basis for this study reported here, it is interesting to note that Sekar *et al.* found that of the five brain regions assessed, cells expressing *C4* were most abundant in the hippocampus, the subcortical region most strongly associated with memory recall.

A key observation of the Sekar *et al.* (2016) *C4* study was the observation of reduced levels of synaptic refinement in mice that lacked *C4*. In an experimental model of synaptic pruning in the visual system, Sekar *et al.* reported that *C4*-deficient mice showed decreased *C4* expression in the lateral geniculate nucleus (LGN) of the visual thalamus, and that this was associated with defects in experience-dependent synaptic remodelling. In linking these findings to our cortical activation findings, in which we observed predicted C4 expression-related difference in the middle temporal gyrus and not the thalamic regions, the following points are noteworthy: (1) the functional specialization of *C4* into *C4A* and *C4B* in humans does not have an analogy in mice, and (2) the mice findings related to developmental (rather than cross-sectional) differences in synaptic pruning) in the thalamic dLGN region; furthermore, (3) our study employed a visual processing task designed to index face processing – an aspect of visual information processing involving the ventral stream that is consistently shown to be impaired in patients with SZ (Mothersill *et al.* 2014*a*, *b*). Given that this task is unlikely to specifically highlight regions serving basic visual processing, it is therefore unsurprising that the between-group differences in thalamic activation are not observed; (4) in genetic terms, using the same task, Dickie *et al.* (Dickie *et al.* 2014) found that task-related BOLD response within a cluster incorporating the middle temporal cortex was strongly genetically influenced. Consistent with these findings, our study highlights the role of *C4* in the activity of the right middle temporal gyrus during task performance. Given that this effect was significant for the neutral faces *v.* baseline contrast but not others (e.g. association between predicted C4 expression and activation during angry faces *v.* baseline, all faces *v.* baseline, did not survive correction), confirmation of these results in further samples will be important.

The right middle temporal gyrus plays an important role in facial recognition (Carvajal *et al.* 2013), and is activated by both neutral and angry facial expressions (Fusar-Poli *et al.* 2009; Dickie *et al.* 2014), consistent with the view that healthy participants respond similarly to both neutral and angry faces at both a behavioural and neural level (Lee *et al.* 2008; Ille *et al.* 2011). Nevertheless, participants may interpret neutral faces differently, not only due to the fact that no overt anger is being displayed, but also due to the presentation context – for example, neutral faces are sometimes interpreted more positively if immediately following negative faces and more negatively if following happy faces (Lee *et al.* 2008). In this study, we found that C4A expression affected right middle temporal activity during both neutral and angry face processing, but this effect was only significant at a corrected level during neutral face processing. Future imaging genetics studies based on face processing will be needed to examine why neural response to neutral faces might be more sensitive to *C4A* genetic variation compared to angry faces.

Finally, in the absence of a memory component to this visual fMRI task, whether these cortical abnormalities are related to, and account for, the behavioural memory impairments observed on neuropsychological testing is unknown. Similarly, as there was not a behavioural component to this task, it was not possible to correlate task performance with memory task performance. Whether these findings implicate the pleiotropic effects of predicted C4 expression differences, or the behavioural and cortical effects of a common pathway, therefore, remains to be elucidated. From a translational perspective, this will be important for determining the extent to which any pharmacological attempt to target the deleterious cortical effects of C4 variation should be specific to, or broader than, memory function alone.

The finding of comparable cognitive effects of predicted C4 expression in patients and healthy participants is consistent with our general expectation that while risk-associated biological processes will, by definition, occur at higher frequency in cases than controls, the phenotypic effects will be comparable in healthy participants who carry that risk factor. Comparable phenotypic effects in cases and healthy participants have previously been reported for other SZ risk variants (e.g. *MIR137*; Mothersill *et al.* 2014*b*), although for some cases this expectation has not been met (e.g. Walters *et al.* 2010). The cortical effects of predicted *C4* expression reported here are based on the analysis of healthy participants only, an approach previously used in psychiatric genetics studies given the challenges of imaging sufficiently large samples of cases. Whether the same cortical effects of *C4*, based on one contrast (neutral faces *v.* baseline) but not others (angry faces *v.* either neutral faces or baseline), will be observed in patients is currently unknown, and further imaging studies of patients will be required to establish how C4 expression effects visual processing in this group.

Conclusion

The recent association of SZ risk with increased predicted *C4* expression is a major step towards understanding the aetiology of SZ. Based on the hypothesis that *C4*'s effect would be most pronounced in cortical regions whose development is highly experience-dependent, we hypothesised and then observed that increased predicted *C4A* RNA expression was predictive of poorer memory performance and reduced cortical activity in middle temporal cortex during a measure of visual processing. Doing so further elucidates the pathway between genetically mediated altered development and illness-related disability.

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