Schizophrenia Research 204 (2019) 30–37

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Investigation of peripheral complement factors across stages of psychosis

Liliana Laskaris [a,b,m], Andrew Zalesky [a,b,c], Cynthia Shannon Weickert [a,e,f,g,j], Maria A. Di Biase [a,b], Gursharan Chana [c,m], Bernhard T. Baune [d], Chad Bousman [a,b,n,o], Barnaby Nelson [h,i], Patrick McGorry [h,i], Ian Everall [b,n,q], Christos Pantelis [a,b,k,l,m,n,1], Vanessa Cropley [a,b,p,*,1]

[a] Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne and Melbourne Health, Carlton South, VIC, Australia
[b] Department of Psychiatry, The University of Melbourne, Parkville, VIC, Australia
[c] Melbourne School of Engineering, The University of Melbourne, Parkville, VIC, Australia
[d] Discipline of Psychiatry, The University of Adelaide, SA, Australia
[e] Neuroscience Research Australia, Randwick, NSW, Australia
[f] Schizophrenia Research Institute, Randwick, NSW, Australia
[g] School of Psychiatry, University of New South Wales, Sydney, NSW, Australia
[h] Orygen, The National Centre of Excellence in Youth Mental Health, VIC, Australia
[i] Centre for Youth Mental Health, The University of Melbourne, Parkville, VIC, Australia
[j] Brain & Mind Centre, The University of Sydney, NSW, Australia
[k] North Western Mental Health, Melbourne Health, Parkville, VIC, Australia
[l] Florey Institute for Neurosciences and Mental Health, Parkville, VIC, Australia
[m] Centre for Neural Engineering, Department of Electrical and Electronic Engineering, University of Melbourne, Carlton South, VIC, Australia
[n] Cooperative Research Centre for Mental Health, Carlton, VIC, Australia
[o] Departments of Medical Genetics, Psychiatry, Physiology & Pharmacology, University of Calgary, Calgary, AB, Canada
[p] Centre for Mental Health, Faculty of Health, Arts and Design, School of Health Sciences, Swinburne University, VIC, Australia
[q] Institute of Psychiatry, Psychology & Neuroscience, King's College London, UK

ARTICLE INFO

Article history:
Received 8 March 2018
Received in revised form 2 October 2018
Accepted 30 November 2018
Available online 6 December 2018

Keywords:
Psychosis
Schizophrenia
Complement protein
Biomarkers

ABSTRACT

The complement cascade has been proposed to contribute to the pathogenesis of schizophrenia. However, it remains unclear whether peripheral complement levels differ in cases compared to controls, change over the course of illness and whether they are associated with current symptomatology. This study aimed to: i) investigate whether peripheral complement protein levels are altered at different stages of illness, and ii) identify patterns among complement protein levels that predict clinical symptoms.

Complement factors C1q, C3 and C4 were quantified in 183 participants [n = 83 Healthy Controls (HC), n = 10 Ultra-High Risk (UHR) for psychosis, n = 40 First Episode Psychosis (FEP), n = 50 Chronic schizophrenia] using Multiplex ELISA. Permutation-based *t*-tests were used to assess between-group differences in complement protein levels at each of the three illness stages, relative to age- and gender-matched healthy controls. Canonical correlation analysis was used to identify patterns of complement protein levels that correlated with clinical symptoms.

C4 was significantly increased in chronic schizophrenia patients, while C3 and C4 were significantly increased in UHR patients. There were no differences in C1q, C3 and C4 in FEP patients when adjusting for BMI. A molecular pattern of increased C4 and decreased C3 was associated with positive and negative symptom severity in the pooled patient sample.

Our findings indicate that peripheral complement concentration is increased across specific stages of psychosis and its imbalance may be associated with symptom severity. Given the small sample size of the UHR group, these findings should be regarded as exploratory, requiring replication.

1. Introduction

Epidemiological, molecular and genetic research converge on inflammatory pathways as one of the primary lines of investigation in schizophrenia (Dickerson et al., 2015). Prenatal inflammation has been linked to increased risk of developing schizophrenia (Brown and Derkits, 2010; Ellman et al., 2010), while molecular architects of

* Corresponding author at: Melbourne Neuropsychiatry Centre, Level 3, Alan Gilbert Building, The University of Melbourne, Carlton South, VIC 3053, Australia.
E-mail address: vcropley@unimelb.edu.au (V. Cropley).
[1] Joint senior authors.

https://doi.org/10.1016/j.schres.2018.11.035
0920-9964/

inflammation such as peripheral cytokines, chemokines and acute phase molecules are increased in patients and unaffected first degree relatives (Miller et al., 2011; Upthegrove et al., 2014) and increased pro-inflammatory cytokines are found in the brain of people with schizophrenia (Fillman et al., 2015; Volk et al., 2015). In addition, some of the strongest genetic markers of schizophrenia are located in the immune system related major histocompatibility complex (MHC) region (Ripke et al., 2014). Recently, attention has focused on the complement cascade, a key component of the immune system, in schizophrenia. In a series of studies, Sekar et al. (2016) demonstrated that copy number variants of the C4 gene were associated with both increased C4A mRNA levels in brain and increased risk of schizophrenia. In this same report, the authors demonstrated a role for C4 in synaptic pruning in developing mice (Sekar et al., 2016), supporting previous studies showing that developmentally timed synaptic pruning in the brain required both C1q and C3 (Bilimoria and Stevens, 2014; Stevens et al., 2007). Complement proteins are therefore understood to not only support the immune system but also play a critical role in neurodevelopment (e.g. synaptic elimination) (Stephan et al., 2012; Stevens et al., 2007). This suggests that abnormal activation of the complement cascade might contribute to the pathogenesis of schizophrenia, and in particular the loss of cortical grey matter observed in the illness (Olabi et al., 2011; Sekar et al., 2016).

Despite the interpretation that an over-active complement system may predispose individuals to develop schizophrenia, findings concerning complement levels in the disease are mixed. While a number of studies have found increased levels of C1q (Arakelyan et al., 2011; Hakobyan et al., 2005; Severance et al., 2012), C3 (Boyajyan et al., 2010, 2008; Hakobyan et al., 2005; Maes et al., 1997; Santos Soria et al., 2012) and C4 in peripheral blood in schizophrenia (Hakobyan et al., 2005; Mayilyan et al., 2006); decreased C1q (Idonije et al., 2012), C3 (Idonije et al., 2012; Li et al., 2016) and C4 (Li et al., 2012; Mayilyan et al., 2008) levels have also been observed. Several studies have also found no difference in C3 (Arakelyan et al., 2011; Kopczynska et al., 2017) or C4 (Idonije et al., 2012; Kopczynska et al., 2017; Santos Soria et al., 2012) levels between psychosis patients and healthy controls. Furthermore, recent studies suggest decreased complement proteins in individuals with first episode psychosis (FEP) (Idonije et al., 2012; Li et al., 2012), but increased levels at later stages of the illness (Boyajyan et al., 2010; Kopczynska et al., 2017; Santos Soria et al., 2012). To date, only one study has examined complement proteins across different stages of schizophrenia in the same study (Idonije et al., 2012). Contrary to evidence for increased activation of the complement cascade in the development of schizophrenia (Sekar et al., 2016), this study found evidence for decreased levels in the illness, with deficient C1q present at both early and established stages of illness, whereas C3c levels were deficient in only recently diagnosed patients (Idonije et al., 2012). Nevertheless, another study found no difference in complement protein levels, including C1q and C3, between FEP patients and healthy controls (Kopczynska et al., 2017). Therefore, it remains unclear whether peripheral complement levels differ in cases compared to controls, and change over the course of illness.

Despite reports of altered complement proteins in schizophrenia, the direction of change and the clinical relevance of such abnormalities remain unclear. To date, only two studies have explicitly examined the association between complement proteins and current symptomatology; these studies reported associations between increases in C3 and C4 (Morera et al., 2007) and decreases in C3 (Li et al., 2016) and symptom severity. However, examination of relationships between single proteins and symptom domains are typically inconsistent. Rather, a multivariate approach assessing the relationship between the *profile* or *pattern* of complement proteins and symptoms might be more informative, as demonstrated with other sets of variables (Moser et al., 2017). Patterns involving multiple proteins are likely to yield more potent predictors than any single protein considered in isolation. Molecular patterns among complement proteins have not been evaluated to date in schizophrenia.

Here, we aimed to characterise peripheral complement protein levels in individuals at different stages of a psychotic illness; specifically, chronic schizophrenia, individuals experiencing their FEP and individuals at ultra-high risk of developing psychosis (UHR). We aimed to i) compare C1q, C3 and C4 protein levels in schizophrenia across various stages of illness relative to age and gender matched healthy controls (HCs); and ii) examine the relationship between complement protein levels and clinical symptom profiles using an integrated multivariate analysis. The significance of this study is the identification of increased complement protein levels in patients with psychosis and schizophrenia at different stages of illness and identification of a molecular pattern of complement proteins that predict symptom severity in one of the largest samples to date.

2. Materials and methods

2.1. Subjects and methods

A total of 183 subjects (all genetically unrelated) participated in this study: 10 individuals at UHR for psychosis, 40 FEP patients, 50 chronic schizophrenia patients (illness duration > 7 years), and 83 healthy controls. Due to the small sample size, findings relating to the UHR group should be regarded as exploratory. Healthy controls were divided into mutually exclusive younger (n = 29; age 18.1–24.8 years) and older (n = 54; 25.2–61.6 years) groups that were matched on age and gender to their respective patient group. That is, the older control group was matched in mean age to chronic patients, whereas the younger control group was matched to both UHR and FEP participants. Demographic data for these subjects are shown in Table 1.

Healthy controls were assessed by trained researchers to confirm that they had no diagnosable psychopathology using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID;48). The status of UHR subjects was confirmed using the Comprehensive Assessment of At Risk Mental State (CAARMS), a clinical instrument for assessing sub-threshold psychotic symptoms (Yung et al., 2005). FEP and chronic schizophrenia subjects were diagnosed using the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and diagnoses confirmed using the Mini-International Neuropsychiatric Interview (MINI; Sheehan et al., 1998) or the Structured Clinical Interview for DSM-IV (SCID) (Spitzer et al., 1992).

Exclusion criteria for the study included pregnancy, history of an auto-immune disorder (Hashimoto's thyroiditis or Graves disease, lupus, arthritis), neurological or endocrine disorder (diabetes), as well as a history of head injury and seizures. Current inflammatory conditions (e.g. influenza) requiring treatment with immunosuppressive, corticoid/glucocorticoid, steroidal or non-steroidal anti-inflammatory medication within two weeks of blood draw also constituted exclusionary criteria.

The study was approved by the Melbourne Health and Austin Health Human Research Ethics Committees and all participants provided written consent prior to participation.

2.2. Clinical measures

Clinical symptoms were assessed on the day of the blood draw and included the following symptom measures: the SCID, the Expanded Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) or the Positive and Negative Syndrome Scale (PANSS) to provide assessments of positive and general psychopathology (Kay et al., 1987) and the Scale for the Assessment of Negative Symptoms (SANS) (Blanchard and Cohen, 2006) for the assessment of negative symptoms. PANSS scores were converted to BPRS scores as previously described (Leucht et al., 2013) to allow for the assessment of total and domain psychopathology scores.

Table 1
Clinical cohort characteristics.

Characteristic	Young HC	Older HC	UHR	FEP	Chronic
	n = 29	n = 54	n = 10	n = 40	n = 50
Age, mean (sd) years	22.2 (1.9)	41.1 (9.5)	20.9 (2.2)	20.9 (2.2)	40.6 (8.7)
Sex, % (n) female	31 (9)	33 (18)	40 (4)	35 (14)	37 (19)
Ethnicity, % (n) Caucasian	72 (21)	87 (47)	70 (7)	55 (22)	78 (39)
BMI, median (IQR)	22.9 (5.6)	24.3 (5.1)	22.7 (5.2)	26.2 (6.8)	27.8 (8.4)
Current smoker, % (n)	35 (10)	19 (10)	50 (5)	53 (21)	52 (26)
Current cannabis user, % (n)	28 (8)	11 (6)	30 (3)	45 (18)	20 (10)
Age at diagnosis, mean (sd) years	–	–	19.3 (2.2)	19.5 (1.7)	22.2 (6.0)
Duration of illness, mean (sd) years	–	–	–	1.3 (0.5)	18.7 (7.7)
SANS Total, mean (sd)	–	–	21.4 (9.5)	19.4 (12.0)	40.1 (17.0)
Total psychopathology (BPRS Total), mean (sd)	–	–	44.7 (9.6)	38.5 (9.6)	39.1 (12.6)
Positive symptom subscore (BPRS), mean (sd)	–	–	14 (3.9)	14.1 (5.5)	17.3 (6.7)
Negative symptom subscore (BPRS), mean (sd)	–	–	4.3 (2.0)	5.2 (2.4)	7.5 (3.1)
General symptom subscore (BPRS), mean (sd)	–	–	15.4 (3.9)	14.1 (4.4)	13.0 (3.9)
CPZ equivalents, median (IQR) dose	–	–	–	229.5 (265.6)	900.0 (506.8)

HC, healthy controls; UHR, ultra-high risk of psychosis; FEP, first episode psychosis; BMI, body mass index; IQR, interquartile range; SANS, scale for the assessment of negative symptoms; BPRS, Brief Psychiatric Rating Scale; CPZ, chlorpromazine.

2.3. Laboratory procedures

All subjects were fasting and blood samples were obtained between 8:00 and 11:00 am. Three complement proteins (C1q, C3 and C4) from the MILLIPLEX MAP Human Complement Panel 2 - Immunology Multiplex Assay (HCMP2MAG-19K, Merck Millipore, Billerica, MA, USA) were quantified in duplicate using the Luminex Magpix assay. Inter-assay coefficients were below 15% (CV < 15%) and intra-assay CVs were below 5% (CV < 5%).

Serum samples were thawed at 4 °C and centrifuged at 1400*g* for 5 min in order to remove any aggregate protein. All reagents including samples were assayed according to manufacturer instructions. A 7-point standard curve was generated using the standards supplied in the assay. The data was generated by applying a 5 parameter logistic standard curve fit corrected for sample dilutions in the Millipore Analyst Software (Merck Millipore, USA).

2.4. Statistical analyses

2.4.1. Demographic variables

Statistical analyses of demographic variables were performed using the Statistical Package for the Social Sciences (SPSS) version 22 (IBM). Differences in these variables between groups were analysed with independent *t*-tests or Pearson's χ^2 statistics for continuous and categorical variables, respectively. The Mann-Whitney *U* test was performed for non-parametric variables. Differences in sex, ethnicity, tobacco and cannabis consumption between groups was analysed with Pearson's χ^2 statistics. Correlations between age, body mass index (BMI) and current chlorpromazine (CPZ) equivalents with serum complement levels were analysed with Spearman's rank correlation coefficient. The association of each complement protein with ethnicity, and tobacco/cannabis consumption were analysed with the Kruskal Wallis H and the Mann Whitney *U* test respectively.

2.4.2. Between-group differences

Permutation-based two-sample *t*-tests (two tailed, 4000 iterations) were used to test the null hypothesis of equality in group-averaged complement proteins between the following pairs of groups: i) UHR and the younger control group ii) FEP and the younger control group and iii) Chronic patients and the older control group. A general linear model (GLM) comprising two regressors was formulated: main effect of group and the confound of BMI. Permutation testing was used to estimate a p-value for the main effect of group. Specifically, for each permutation, the data were fit to the BMI confound and the confound-only residuals were permuted. The estimated confound signal was then added back to the permuted residuals, yielding a sample of the data from the null distribution. The full GLM was fitted to this sample and the t-statistic for the main effect stored. A p-value was estimated as the proportion of permutations with a t-statistic that exceeded or equalled the observed t-statistic. The Benjamini-Hochberg procedure was used to control the false discovery rate (FDR) across the nine comparisons. A false discovery rate threshold of 5% was deemed significant. Statistical inference was performed in Matlab (Mathworks 2014). Reported p-values are FDR-corrected.

2.4.3. Correlations with symptoms

Canonical correlation analysis (CCA) was performed to identify any potential latent relations between the set of complement proteins (C1q, C3, C4) and the set of clinical symptom scores (BPRS positive symptom subscore, BPRS negative symptom subscore and BPRS general symptom subscore). CCA was performed using data from the pooled patient group (UHR, FEP and chronic). CCA sought to identify a set of complement protein weights, denoted u_1, u_2 and u_3, as well as a set of symptom weights, denoted v_1, v_2 and v_3, such that the weighted combination of complement proteins $U_i = u_1\mathrm{C1q}_i + u_2\mathrm{C3}_i + u_3\mathrm{C4}_i$ was maximally correlated with the weighted combination of symptoms $V_i = v_1\mathrm{BPRSpos}_i + v_2\mathrm{BPRSneg}_i + v_3\mathrm{BRPSgen}_i$, where the index *i* is used to denote a particular patient (41). In other words, CCA identified a set of weights to maximize the correlation between (U_i, V_i), $i = 1, 2, \ldots, N$, where *N* is the total number of patients. Permutation was used to compute a p-value for this correlation. This involved randomly permuting the complement proteins among individuals on 5000 separate occasions, each time performing CCA on the permuted data to generate a null distribution for the sample canonical correlation coefficient; p-values < 0.05 were deemed significant. While permutation does not mandate data normality, ensuring normality can potentially improve the sensitivity of CCA. Normality assumptions were rejected for the following variables: BPRS positive and negative subscore, C1q and C3. These variables were transformed with a logarithm function (BPRS positive and negative, C1q) or a square-root function (C3). Transformation resulted in acceptable normality based on skewness and visual inspection. Canonical correlations were performed in Matlab (Mathworks) 2014 based on the *canoncor* function. Associations between the C4/C3 ratio and clinical symptoms were assessed using Pearson's correlations.

3. Results

3.1. Demographics

Age and gender between patients and their respective control group did not significantly differ. In the entire sample, age was not

significantly associated with C1q or C4, but was negatively correlated with C3 ($r_s = -0.276$, $p < 0.001$). Similarly, in the pooled patient sample, age showed no correlation with C1q or C4, but was negatively correlated with C3 (rs = −0.293, $p < 0.001$). Complement proteins did not significantly differ between current smokers and non-smokers (C1q: U = 3616, p = 0.797; C3: U = 3607, p = 0.851; C4: U = 3660, p = 0.776) or between current cannabis users and non-users (C1: U = 3041, p = 0.191; C3: U = 2184, p = 0.417; C4: U = 2847, p = 0.370) and were not associated with current CPZ equivalents in patients (C1 $r_s = 0.069$, p = 0.507; C3 $r_s = -0.184$, p = 0.075; C4 $r_s = 0.137$, p = 0.185). There were no associations between ethnicity and diagnosis ($\chi^2 = 20.767$, p = 0.188) or ethnicity and any of the complement proteins (C1: H(4) = 2.894, p = 0.576; C3(4): H(4) = 2.613, p = 0.625; C4: H(4) = 2.564, p = 0.633). Body mass index was positively correlated with C1q ($r_s = 0.178$, p = 0.020), C3 ($r_s = 0.266$, $p < 0.0005$) and C4 ($r_s = 0.326$, $p < 0.0005$) in the entire sample and C4 only in the pooled patient sample (C4: rs = 0.237, p = 0.023; C1q: rs = 0.153, p = 0.148; C3: rs = 0.130, p = 0.218). BMI was therefore treated as a nuisance variable and included as a covariate in the between-group comparisons of complement proteins.

3.2. Between-group comparisons of complement protein levels

3.2.1. C1q

C1q did not significantly differ between UHR and younger HCs (p = 0.82), FEP and younger HCs (p = 0.15), and chronic and older HCs (p = 0.26; see Table 2 and Fig. 1A).

3.2.2. C3

C3 was significantly higher in UHR (t (38) = 2.59, p = 0.041), Cohen's d = 0.770) but not in FEP (t (68) = 1.71, p = 0.19) or chronic schizophrenia patients (t (103) = 1.12, p = 0.262), with FDR adjustment for the nine comparisons (see Table 2 and Fig. 1B).

3.2.3. C4

C4 protein was significantly greater in UHR (t (38) = 2.58, p = 0.041, Cohen's d = 0.849) and chronic schizophrenia (t (103) = 2.79, p = 0.041, d = 0.635) but not in FEP (t (68) = 1.32, p = 0.29, d = 0.577) with FDR adjustment for the nine comparisons.

3.3. Relationship between complement protein levels and symptoms

CCA was undertaken to identify any latent relations between clinical symptoms (BRPS positive, BPRS negative, BPRS general) and complement proteins (C1q, C3 and C4). A single significant CCA mode was identified (r = 0.37, p = 0.0358). CCA weights (canonical coefficients) with 95% confidence intervals residing outside zero were considered significant contributors to the correlation between symptoms and complement proteins. Of the three symptoms, the CCA weights corresponding to positive and negative subscores (positive association) were significant contributors, while of the three complement proteins, the CCA weights corresponding to C4 (positive association) and C3 (negative association) were significant. The general symptom subscore and C1q did not significantly contribute. This CCA mode therefore demonstrated that increased C4 together with decreased C3 levels were associated with increased positive and negative symptoms but not general psychopathology (see Fig. 2). This relationship remained after controlling for age (C3) (r = 0.385, p = 0.0462) and BMI (all proteins; p = 0.0358).

The CCA analysis led us to formulate the hypothesis that an imbalance between C4 and C3 may be related to symptom severity. We therefore calculated the ratio of C4 to C3 (C4/C3) and assessed whether this ratio correlated with the symptom measures examined in the pooled patient sample. A partial correlation controlling for age detected a significant association between the C4/C3 ratio and the BPRS positive subscore (r = 0.211, p = 0.038) (see Fig. 3). This relationship remained after outlier removal (r = 0.206, p = 0.045).

Table 2
Peripheral complement proteins examined by cohort.

Cohort	C1q	C3	C4
HC[a] young, mean (sd)	44.3 (12.1)	17.6 (4.1)	244.5 (49.5)
HC older, mean (sd)	45.8 (12.5)	17.1 (6.5)	252.0 (52.9)
UHR, mean (sd)	42.8 (7.8)	21.2 (5.4)	287.3 (51.3)
FEP, mean (sd)	48.2 (9.4)	21.4 (7.7)	279.3 (69.5)
Chronic, mean (sd)	48.9 (12.1)	16.9 (5.5)	292.2 (72.3)
Group differences			
Cohen's d effect size (sig)	C1q	C3	C4
UHR vs. young HC	d = 0.147 (p = 0.82)	d = 0.770 (p = 0.04)*	d = 0.849 (p = 0.04)*
FEP vs. young HC	d = 0.369 (p = 0.15)	d = 0.616 (p = 0.19)	d = 0.577 (p = 0.29)
Chronic vs. older HC	d = 0.249 (p = 0.26)	d = 0.035 (p = 0.26)	d = 0.635 (p = 0.04)*

HC, healthy controls; UHR, ultra-high risk for psychosis; FEP, first episode psychosis; sd, standard deviation.
[a] All values adjusted for BMI.
* $p < 0.05$ adjusted for BMI and multiple comparisons.

4. Discussion

We found a specific increase in some complement protein levels, particularly C4, in individuals with psychosis and psychosis risk compared to age and gender matched controls. This was particularly prominent in the putative prodromal period, with significant elevations of both C3 and C4 found in those at ultra-high risk for psychosis. Individuals with chronic schizophrenia also showed increased C4 compared to healthy controls. Finally, we identified a molecular pattern of decreased C3 and increased C4 that was associated with increases in positive and negative symptom severity in the pooled patient group.

A number of previous studies have examined peripheral complement proteins in schizophrenia, producing mixed results. Our finding of increased C4 level in established schizophrenia is in agreement with some previous findings (Hakobyan et al., 2005; Maes et al., 1997; Mayilyan et al., 2006), but is in contrast to other studies reporting increased C3 (Boyajyan et al., 2008, 2010; Hakobyan et al., 2005; Maes et al., 1997; Santos Soria et al., 2012) and C1q (Arakelyan et al., 2011; Hakobyan et al., 2005) proteins in these patients. Likewise, our finding of no difference in complement protein concentration in first-episode psychosis concurs with some (Kopczynska et al., 2017) but not all (Idonije et al., 2012) studies. The reason for these discrepancies is unclear but may be related to a number of factors including variation in molecular technique, medication status, methodological considerations, and clinical heterogeneity of patients including stage of illness. For example, some studies in established schizophrenia measured the activity of C3 (Hakobyan et al., 2005) and C1q (Arakelyan et al., 2011; Hakobyan et al., 2005), whereas our study assayed for total C3 and C1q levels, which may account for some of the differences between our, and previous studies. Likewise, studies varied in the extent that they considered potential confounds, such as BMI, smoking and other demographic variables, on complement levels (see discussion below). Given the majority of studies have not considered such confounds, it remains difficult to reconcile across studies. These discrepancies highlight the need for consistency in molecular techniques and methodology across studies in order to elucidate the nature of the complement cascade in schizophrenia.

In addition to the above factors, differences in stage of psychotic illness may also contribute to the inconsistent results across studies. To our knowledge, we are the first to examine peripheral complement protein levels in individuals at high risk for developing psychosis. Our finding of increased C3 and C4 factors in these individuals suggests that the complement cascade may be elevated prior to illness onset or in

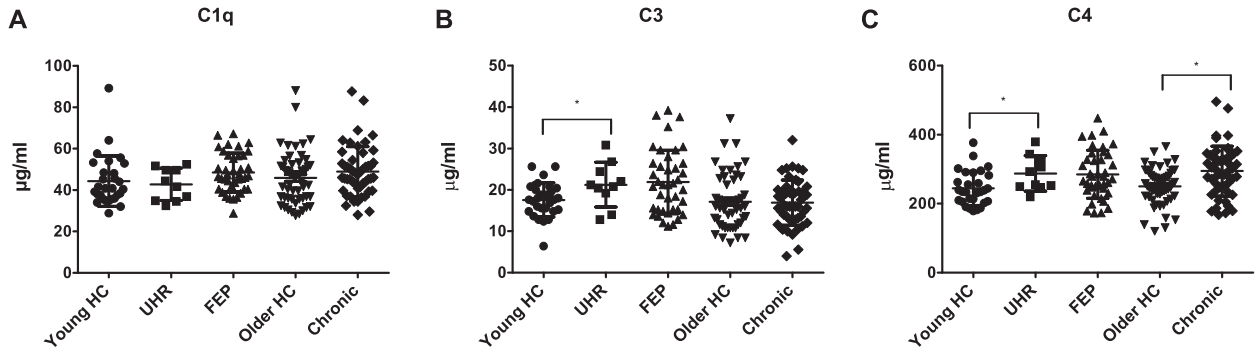

Fig. 1. Comparison of C1q (panel A), C3 (panel B) and C4 (panel C) complement protein levels in individuals at ultra-high risk for psychosis, first-episode psychosis and chronic schizophrenia compared to age and gender matched comparison subjects. Data points denote individuals and bars the mean value of each complement protein. HC – healthy controls. UHR – ultra-high risk for psychosis. FEP – first-episode psychosis. Chronic – chronic schizophrenia. *p ≤ 0.05; corrected for BMI and nine comparisons with the Benjamini-Hochberg false discovery rate.

individuals vulnerable to the development of a psychotic disorder, irrespective of later transition. This is in line with theories of immune dysregulation in the prodrome of psychosis (Cannon, 2015) and is supported by recent studies reporting an association between certain complement components (serum C1q and C4A gene copy number) and increased risk of developing schizophrenia (Severance et al., 2014; Sekar et al., 2016). In addition, our study is only the second to examine the same proteins across stages in the course of schizophrenia. Indeed, a motivation of this study was to elucidate whether alterations (if any) in peripheral complement are seen in very early illness and are maintained in later disease stages, or whether they fluctuate across stages of illness. Our findings may suggest the latter, whereby elevations in complement proteins are evidenced in a putative prodromal, or attenuated psychotic period, followed by decreases at the onset of frank psychosis, and elevations with illness chronicity. However, given the cross-sectional design and comparatively small sample of the UHR cohort, most who will not develop a psychotic illness, this interpretation should be regarded preliminary, requiring large, prospectively planned longitudinal studies to confirm and extend these findings. Alternatively, interactions between stage of illness and unrelated demographic or illness-related factors (e.g. BMI) may also explain our differential findings with illness stage.

Over recent years, a growing body of evidence has demonstrated important roles for the complement system in normal brain development (Stephan et al., 2012). In mice, activation of the classical complement cascade, including C1q-C3 (Stephan et al., 2012; Stevens et al., 2007) and, most recently, C4 (Sekar et al., 2016), has been found to promote synapse elimination or 'pruning' at critical neurodevelopmental time points by tagging weaker synapses for elimination (Sekar et al., 2016; Stephan et al., 2012). In humans, elimination of synapses is proposed to take place in young adulthood, thus forming part of the normal maturational process of the brain (Andreasen et al., 2011; Faludi and Mirnics, 2011). Given the typical onset of schizophrenia occurs in adolescence and early adulthood, which corresponds to this putative 'late stage' synaptic maturation, it has been proposed that an exaggeration of normal synaptic pruning may underlie the increased rate of cortical thinning observed prior to, and at the earliest stages of psychotic illness (Cannon et al., 2015), contributing to the manifestation of the disorder (Feinberg, 1982). Our finding (although preliminary), of increased complement proteins (especially C3) in the younger UHR cohort, whose brains may be undergoing late stage synaptic pruning and refinement (though see Catts et al., 2013), provides preliminary support to this hypothesis. Nevertheless, given our measure of complement in peripheral blood rather than the brain, this proposition remains speculative.

There are a variety of mechanisms through which elevated peripheral complement proteins may be exerting their effects, including on brain development and function. For instance, complement components are acute phase proteins, increasing vascular permeability, vasodilation, and leukocyte extravasation (Markiewski and Lambris, 2007), and thus may represent an acute response to inflammation or infection. Nevertheless, this remains unlikely given that all participants in the current study reported to be well at the time of assessment and a subsample of the current cohort showed no evidence of classical inflammation

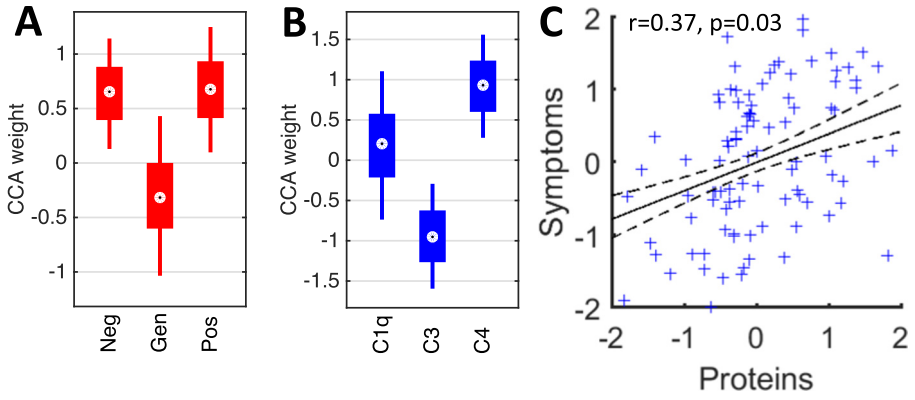

Fig. 2. Multivariate relation between complement protein levels and clinical symptoms identified with canonical correlation analysis (CCA). Canonical coefficients (CCA weights) are shown for each clinical symptom (panel A) and complement protein (panel B). Bars denote 95% confidence intervals, computed with bootstrapping (5000 bootstraps). Weights with confidence intervals excluding zero were deemed significant contributors. Canonical correlation ($r = 0.37$, $p = 0.03$) between protein and symptoms scores (panel C). Protein score computed for each individual by summing the product of complement protein level and corresponding CCA weight across all three proteins. Data points denote individuals (crosses). Solid line denotes line of best fit and dashed lines denote 95% confidence intervals. Neg. – BPRS negative symptoms. Pos. – BPRS positive symptoms. Gen. – BPRS general symptoms.

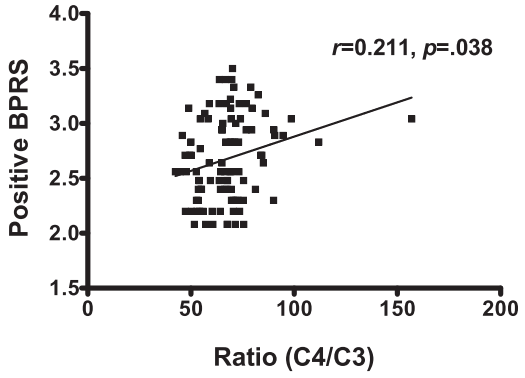

Fig. 3. Partial correlation controlling for age of the ratio of complement C4 to C3 (C4/C3) with positive and negative symptoms in the pooled patient sample. The ratio was significantly positively correlated with BPRS positive scores. BPRS – Brief Psychiatric Rating Scale. SANS – Scale for the Assessment of Negative Symptom.

such as increased pro-inflammatory cytokines or microglial activation (Di Biase et al., 2017). Secondly, given the increasingly recognised functions of the complement system in the central nervous system (Presumey et al., 2017), our findings also raise the possibility that increased peripheral complement activity may influence the brain. Despite the immune privileged notion of the brain there are a number of mechanisms through which this may occur. For example, complement protein circulating in peripheral blood can enter the brain via receptors on the vagus nerve or through increases in the permeability of the blood brain barrier (BBB), given that the epithelial cells of the BBB express complement protein receptors (Jacob and Alexander, 2014; Veerhuis et al., 2011) and schizophrenia patients are proposed to have a particularly permeable BBB (Stolp and Dziegielewska, 2009; Vasic et al., 2012). Furthermore, increases in some complement proteins could lead to decreases in other components of the complement pathway, which may impair the ability of this pathway to clear apoptotic cells and debris, leading to further accentuation of inflammation and the development of autoimmunity(Markiewski and Lambris, 2007), which has also been linked to psychosis (Benros et al., 2014). Although speculative, peripheral increases in complement protein may reflect a primed immune system (possibly due to intrauterine inflammation), which has been shown to lead to changes in neuronal migration in the developing brain (Mayilyan et al., 2008). Alternatively, complement increases peripherally may reflect a higher C4 genetic load (e.g. Sekar et al., 2016; Yang et al., 2012) that may affect brain development and function, though the association between C4 gene copy number and peripheral levels of complement is yet to be established in psychosis and in relation to specific C4 isoforms. Finally, the effects of complement in the brain may occur primarily through endogenous mechanisms (Veerhuis et al., 2011); however to date it remains unclear whether endogenous and peripheral proteins levels are related. Our study provides impetus for future work to directly examine complement levels in the brain (and periphery) as well as their relationship, with longitudinal imaging and biological measures of neuronal-glia function in psychosis.

In order to explore the clinical relevance of altered complement proteins in schizophrenia, we examined the interplay between complement proteins and symptom severity. We found that increases in positive and negative symptom subscores were associated with increases in C4 and decreases in C3 protein levels. The association between complement protein was confined to positive and negative but not general symptoms, indicating that this relationship may be specific to schizophrenia-like symptoms. Interestingly, there was no association when symptom subscores were examined in relation to each complement protein individually. This indicates that the *imbalance* between C4 and C3, rather than C4 and C3 levels per se, is associated with symptom severity. Indeed, when we examined the ratio of C4 to C3 across patients (adjusted for age), we found a significant association with positive symptoms. Thus far, only two studies have explored the relationship between complement proteins and symptom severity and have produced contradictory results. While one study found that increases in C3 and C4 were associated with reductions in total PANSS psychopathology scores (Li et al., 2016), the second study reported an association between increases in C3 and C4 and the severity of certain negative and general (though not positive) PANSS symptom items (Morera et al., 2007). Nevertheless, this latter study was hindered by the small sample size and the inherent difficulty in inferring global patterns from isolated correlations between single symptom items and complement proteins (Mayilyan et al., 2008). To this end, our finding that a specific molecular *pattern* was associated with increases in positive and negative symptom severity extends the research beyond single molecular associations and provides a potential avenue of exploration. Should future studies confirm the validity of this molecular pattern, the levels of C4 and C3 could be examined in relation to each other and clinical indicators.

Our finding of increased C3 or C4 protein level in UHR and chronic schizophrenia appears independent of age, gender, medication, smoking and cannabis use, and BMI. It is noteworthy, however, that BMI, which was positively associated with each of the complement proteins in the entire sample, showed a differential effect on complement level in each of the three groups. Whereas complement was elevated in the UHR in exploratory findings in addition to chronic schizophrenia groups when controlling for BMI, this adjustment completely negated the elevations seen in FEP when BMI was not considered. This effect is illustrated in Fig. 1, which shows higher levels of C3 and C4 in FEP compared to younger controls (as the data points reflect raw values), despite the absence of statistically significant group differences. Indeed, whereas complement proteins were positively associated with BMI in FEP, there was no evidence for this relationship in UHR or chronic schizophrenia (data not shown). It is unclear why BMI was strongly associated with complement levels in only the FEP group. It is possible that the effect of BMI in FEP may be a by-product of medication commencement (leading to rapidly increasing BMI), which has yet to take effect in UHR but has stabilised in chronic patients. Co-varying for BMI may therefore mask increases in complement levels in FEP that may have been present prior to medication commencement and become apparent once weight gain has stabilised. Nevertheless, this proposition remains speculative and requires longitudinal studies that measure complement protein levels and BMI both prior to, and after, the initiation of antipsychotic treatment. In addition, given that the majority of studies examining peripheral complement in psychosis have not controlled for BMI (Arakelyan et al., 2011; Boyajyan et al., 2010; Idonije et al., 2012; Kopczynska et al., 2017; Mayilyan et al., 2006) our study highlights the need to consider this confound in future studies.

There are several limitations of our study that should be noted. First, our UHR sample size was limited. Therefore, these findings should be regarded as preliminary, with longitudinal analyses in larger sample sizes required to draw more definitive conclusions. Second, we examined only three proteins within the complement cascade (C1q, C3, C4) and assayed for total complement protein, rather than complement isotypes (e.g. C4A and C4B and their long and short forms). It is possible that other proteins, or specific isoforms, within this cascade may be altered at various stages of a psychotic illness. Finally, complement proteins were measured in peripheral blood and it is as yet unclear how peripheral complement is related to complement activity in the brain.

In conclusion, this is the first study to examine chronic, FEP and UHR cohorts under the same conditions. We found elevated C4 protein in chronic and those at ultra high risk of psychosis and elevated C3 protein in the UHR or putative prodromal period. Finally, we identified a molecular pattern of increased C4 and decreased C3 associated with positive and negative symptom severity, thus suggesting that the imbalance in these protein levels might contribute to the symptomatic manifestation of the illness.

Author disclosure

Funding body agreements and policies

The authors have not received funding from the funding bodies listed in relation to Elsevier's existing agreements and policies.

Conflict of interest

This study was supported by an Australian National Health and Medical Research Council (NHMRC) Project Grant (1065742) and University of Melbourne Early Career Researcher grant (601253) to Vanessa Cropley; and a Royal Melbourne Hospital Grant in Aid (GIA-030-2016) and NARSAD Distinguished Investigator Grant to Christos Pantelis (18722). Liliana Laskaris was supported by the Australian Postgraduate Award (APA), Vanessa Cropley and Barnaby Nelson by a University of Melbourne Faculty Fellowship, Maria Di Biase by the Australian Rotary Health, Ian Scott PhD Scholarship in Mental Health, Christos Pantelis by NHMRC Senior Principal Research Fellowships (628386, 1105825), Cynthia Shannon Weickert by the Schizophrenia Research Institute (utilizing infrastructure funding from the NSW Ministry of Health and the Macquarie Group Foundation), the University of New South Wales, and Neuroscience Research Australia. Cynthia Shannon Weickert is a recipient of an NHMRC Australian Principal Research Fellowship (PRF) (1117079).

Over the last 5 years, Christos Pantelis has participated on Advisory Boards for Janssen-Cilag, Astra-Zeneca, Lundbeck, and Servier. He has received honoraria for talks presented at educational meetings organised by Astra-Zeneca, Janssen-Cilag, Eli-Lilly, Pfizer, Lundbeck and Shire. His research work has received major support from NHMRC and Australian Research Council (ARC), as well as Melbourne Health and The University of Melbourne. Cynthia Shannon Weickert is on an advisory board for Lundbeck, Australia Pty Ltd. and in collaboration with Astellas Pharma Inc., Japan. BT Baune is member of advisory boards, received funding and/or gave presentations for the following companies: AstraZeneca, Lundbeck, Pfizer, Servier, and Wyeth. He receives funding from the National Health and Medical Research Council, Australia. Ian Paul Everall has received grant support from the Australian Commonwealth Government, Australian National Health Medical Research Council and from Lundbeck.

All other authors declare no conflict of interest.

CRediT authorship contribution statement

Liliana Laskaris: Conceptualization, Data curation, Formal analysis, Investigation, Writing - original draft. **Andrew Zalesky:** Formal analysis, Writing - review & editing, Methodology. **Cynthia Shannon Weickert:** Conceptualization, Resources, Supervision, Methodology. **Maria A. Di Biase:** Writing - review & editing. **Gursharan Chana:** Supervision. **Bernhard T. Baune:** Writing - review & editing. **Chad Bousman:** Writing - review & editing, Project administration. **Barnaby Nelson:** Writing - review & editing. **Patrick McGorry:** Resources. **Ian Everall:** Resources. **Christos Pantelis:** Supervision, Writing - review & editing. **Vanessa Cropley:** Supervision, Writing - review & editing, Conceptualization, Funding acquisition, Project administration, Formal analysis, Data curation.

Acknowledgements

We thank Roxanne Cadiz for providing training and assistance in protein analysis. We also thank Steven Tahtalian, Eleni Ganella and Cassandra Wannan for assisting with clinical data collection and Dr. Phassouliotis for study coordination.

References

Andreasen, N.C., Nopoulos, P., Magnotta, V., Pierson, R., Ziebell, S., Ho, B.C., 2011. Progressive brain change in schizophrenia: a prospective longitudinal study of first-episode schizophrenia. Biol. Psychiatry 70, 672–679. https://doi.org/10.1016/j.biopsych.2011.05.017.

Arakelyan, A., Zakharyan, R., Khoyetsyan, A., Poghosyan, D., Aroutiounian, R., Mrazek, F., Petrek, M., Boyajyan, A., 2011. Functional characterization of the complement receptor type 1 and its circulating ligands in patients with schizophrenia. BMC Clin. Pathol. 11, 10. https://doi.org/10.1186/1472-6890-11-10.

Benros, M.E., Eaton, W.W., Mortensen, P.B., 2014. The epidemiologic evidence linking autoimmune diseases and psychosis. Biol. Psychiatry 75 (4), 300–306.

Bilimoria, P.M., Stevens, B., 2014. Microglia function during brain development: new insights from animal models. Brain Res., 1–11 https://doi.org/10.1016/j.brainres.2014.11.032.

Blanchard, J.J., Cohen, A.S., 2006. The structure of negative symptoms within schizophrenia: implications for assessment. Schizophr. Bull. 32, 238–245. https://doi.org/10.1093/schbul/sbj013.

Boyajyan, A.S., Khoyetsyan, A.G., Hakobyan, S.S., Tsakanova, G.V., Ayvazyan, V.A., 2008. Hyperactivation of the alternative complement cascade in schizophrenia. Dokl. Biochem. Biophys. 419, 56–57. https://doi.org/10.1134/S1607672908020026.

Boyajyan, A., Khoyetsyan, A., Chavushyan, A., 2010. Alternative complement pathway in schizophrenia. Neurochem. Res. 35, 894–898. https://doi.org/10.1007/s11064-010-0126-2.

Brown, A.S., Derkits, E.J., 2010. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. Am. J. Psychiatry 167, 261–280. https://doi.org/10.1176/appi.ajp.2009.09030361.

Cannon, T.D., 2015. How schizophrenia develops: cognitive and brain mechanisms underlying onset of psychosis. Trends Cogn. Sci. 19, 744–756. https://doi.org/10.1016/j.tics.2015.09.009.

Cannon, T.D., Chung, Y., He, G., Sun, D., Jacobson, A., van Erp, T.G.M., McEwen, S., Addington, J., Bearden, C.E., Cadenhead, K., Cornblatt, B., Mathalon, D.H., McGlashan, T., Perkins, D., Jeffries, C., Seidman, L.J., Tsuang, M., Walker, E., Woods, S.W., Heinssen, R., 2015. Progressive reduction in cortical thickness as psychosis develops: a multisite longitudinal neuroimaging study of youth at elevated clinical risk. Biol. Psychiatry 77, 147–157. https://doi.org/10.1016/j.biopsych.2014.05.023.

Catts, V.S., Fung, S.J., Long, L.E., Joshi, D., Vercammen, A., Allen, K.M., Fillman, S.G., Rothmond, D.A., Sinclair, D., Tiwari, Y., Tsai, S.-Y., Weickert, T.W., Shannon Weickert, C., 2013. Rethinking schizophrenia in the context of normal neurodevelopment. Front. Cell. Neurosci. 7, 60. https://doi.org/10.3389/fncel.2013.00060.

Di Biase, M.A., Zalesky, A., O'Keefe, G., Laskaris, L., Baune, B.T., Weickert, C.S., Olver, J., McGorry, P.D., Amminger, G.P., Nelson, B., Scott, A.M., Hickie, I., Banati, R., Turkheimer, F., Yaqub, M., Everall, I.P., Pantelis, C., Cropley, V., 2017. PET imaging of putative microglial activation in individuals at ultra-high risk for psychosis, recently diagnosed and chronically ill with schizophrenia. Transl. Psychiatry 7, e1225. https://doi.org/10.1038/tp.2017.193.

Dickerson, F., Stallings, C., Origoni, A., Schroeder, J., Katsafanas, E., Schweinfurth, L., Savage, C., Khushalani, S., Yolken, R., 2015. Inflammatory markers in recent onset psychosis and chronic schizophrenia. Schizophr. Bull., sbv108 https://doi.org/10.1093/schbul/sbv108.

Ellman, L.M., Deicken, R.F., Vinogradov, S., Kremen, W.S., Poole, J.H., Kern, D.M., Tsai, W.Y., Schaefer, C.A., Brown, A.S., 2010. Structural brain alterations in schizophrenia following fetal exposure to the inflammatory cytokine interleukin-8. Schizophr. Res. 121, 46–54. https://doi.org/10.1016/j.schres.2010.05.014.

Faludi, G., Mirnics, K., 2011. Synaptic changes in the brain of subjects with schizophrenia. Int. J. Dev. Neurosci. 29, 305–309. https://doi.org/10.1016/j.ijdevneu.2011.02.013.

Feinberg, I., 1982. Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? J. Psychiatr. Res. 17, 319–334. https://doi.org/10.1016/0022-3956(82)90038-3.

Fillman, S.G., Weickert, T.W., Lenroot, R.K., Catts, S.V., Bruggemann, J.M., Catts, V.S., Weickert, C.S., 2015. Elevated peripheral cytokines characterize a subgroup of people with schizophrenia displaying poor verbal fluency and reduced Broca's area volume. Mol. Psychiatry, 1–9 https://doi.org/10.1038/mp.2015.90.

Hakobyan, S., Boyajyan, A., Sim, R.B., 2005. Classical pathway complement activity in schizophrenia. Neurosci. Lett. 374, 35–37. https://doi.org/10.1016/j.neulet.2004.10.024.

Idonije, O.B., Akinlade, K.S., Ihenyen, O., Arinola, O.G., 2012. Complement factors in newly diagnosed Nigerian schizoprenic patients and those on antipsychotic therapy. Niger. J. Physiol. Sci. 27, 19–21.

Jacob, A., Alexander, J.J., 2014. Complement and blood-brain barrier integrity. Mol. Immunol. 61, 149–152. https://doi.org/10.1016/j.molimm.2014.06.039.

Kay, S.R., Fiszbein, A., Opler, Lewis A., 1987. The Positive and Negative Syndrome Scale for schizophrenia. Schizophr. Bull. 13, 261–276. https://doi.org/10.1093/schbul/13.2.261.

Kopczynska, M., Zelek, W., Touchard, S., Gaughran, F., Di Forti, M., Mondelli, V., Murray, R., O'Donovan, M.C., Morgan, B.P., 2017. Complement system biomarkers in first episode psychosis. Schizophr. Res., 1–7 https://doi.org/10.1016/j.schres.2017.12.012.

Leucht, S., Rothe, P., Davis, J.M., Engel, R.R., 2013. Equipercentile linking of the BPRS and the PANSS. Eur. Neuropsychopharmacol. 23, 956–959. https://doi.org/10.1016/j.euroneuro.2012.11.004.

Li, Y., Zhou, K., Zhang, Z., Sun, L., Yang, J., Zhang, M., Ji, B., Tang, K., Wei, Z., He, G., Gao, L., Yang, L., Wang, P., Yang, P., Feng, G., He, L., Wan, C., 2012. Label-free quantitative proteomic analysis reveals dysfunction of complement pathway in peripheral blood of schizophrenia patients: evidence for the immune hypothesis of schizophrenia. Mol. BioSyst. 8, 2664. https://doi.org/10.1039/c2mb25158b.

Li, H., Zhang, Q., Li, N., Wang, F., Xiang, H., Zhang, Z., Su, Y., Huang, Y., Zhang, S., Zhao, G., Zhou, R., Mao, L., Lin, Z., Cai, W., Fang, Y., Xie, B., Zhao, M., Hong, W., 2016. Plasma levels of Th17-related cytokines and complement C3 correlated with aggressive behavior in patients with schizophrenia. Psychiatry Res. 246, 1–7. https://doi.org/10.1016/j.psychres.2016.10.061.

Maes, M., Delange, J., Ranjan, R., Meltzer, H.Y., Desnyder, R., Cooremans, W., Scharpé, S., 1997. Acute phase proteins in schizophrenia, mania and major depression: modulation by psychotropic drugs. Psychiatry Res. 66, 1–11. https://doi.org/10.1016/S0165-1781(96)02915-0.

Markiewski, M.M., Lambris, J.D., 2007. The role of complement in inflammatory diseases from behind the scenes into the spotlight. Am. J. Pathol. 171, 715–727. https://doi.org/10.2353/ajpath.2007.070166.

Mayilyan, K.R., Arnold, J.N., Presanis, J.S., Soghoyan, A.F., Sim, R.B., 2006. Increased complement classical and mannan-binding lectin pathway activities in schizophrenia. Neurosci. Lett. 404, 336–341. https://doi.org/10.1016/j.neulet.2006.06.051.

Mayilyan, K.R., Dodds, A.W., Boyajyan, A.S., Soghoyan, A.F., Sim, R.B., 2008. Complement C4B protein in schizophrenia. World J. Biol. Psychiatry 9, 225–230. https://doi.org/10.1080/15622970701227803.

Miller, B.J., Buckley, P., Seabolt, W., Mellor, A., Kirkpatrick, B., 2011. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. Biol. Psychiatry 70, 663–671. https://doi.org/10.1016/j.biopsych.2011.04.013.

Morera, A.L., Henry, M., García-Hernández, A., Fernandez-López, L., 2007. Proteínas de fase aguda como marcadores biológicos de la psicopatología negativa en la esquizofrenia paranoide. Actas Esp. Psiquiatr. 35, 249–252.

Moser, D.A., Doucet, G.E., Ing, A., Dima, D., Schumann, G., Bilder, R.M., Frangou, S., 2017. An integrated brain–behavior model for working memory. Mol. Psychiatry, 1–7 https://doi.org/10.1038/mp.2017.247.

Olabi, B., Ellison-Wright, I., McIntosh, A.M., Wood, S.J., Bullmore, E., Lawrie, S.M., 2011. Are there progressive brain changes in schizophrenia? A meta-analysis of structural magnetic resonance imaging studies. Biol. Psychiatry 70, 88–96. https://doi.org/10.1016/j.biopsych.2011.01.032.

Overall, J.E., Gorham, D.R., 1962. The Brief Psychiatric Rating Scale. Psychol. Rep. 10, 799–812. https://doi.org/10.2466/pr0.1962.10.3.799.

Presumey, J., Bialas, A.R., Carroll, M.C., 2017. Complement System in Neural Synapse Elimination in Development and Disease. https://doi.org/10.1016/bs.ai.2017.06.004.

Ripke, S., Neale, B.M., Corvin, A., Walters, J.T.R., Farh, K.-H., Holmans, P.A., Lee, P., Bulik-Sullivan, B., Collier, D.A., Huang, H., Pers, T.H., Agartz, I., Agerbo, E., Albus, M., Alexander, M., Amin, F., Bacanu, S.A., Begemann, M., Belliveau Jr., R.A., Bene, J., Bergen, S.E., Bevilacqua, E., Bigdeli, T.B., Black, D.W., Bruggeman, R., Buccola, N.G., Buckner, R.L., Byerley, W., Cahn, W., Cai, G., Campion, D., Cantor, R.M., Carr, V.J., Carrera, N., Catts, S.V., Chambert, K.D., Chan, R.C.K., Chen, R.Y.L., Chen, E.Y.H., Cheng, W., Cheung, E.F.C., Ann Chong, S., Robert Cloninger, C., Cohen, D., Cohen, N., Cormican, P., Craddock, N., Crowley, J.J., Curtis, D., Davidson, M., Davis, K.L., Degenhardt, F., Del Favero, J., Demontis, D., Dikeos, D., Dinan, T., Djurovic, S., Donohoe, G., Drapeau, E., Duan, J., Dudbridge, F., Durmishi, N., Eichhammer, P., Eriksson, J., Escott-Price, V., Essioux, L., Fanous, A.H., Farrell, M.S., Frank, J., Franke, L., Freedman, R., Freimer, N.B., Friedl, M., Friedman, J.I., Fromer, M., Genovese, G., Georgieva, L., Giegling, I., Giusti-Rodríguez, P., Godard, S., Goldstein, J.I., Golimbet, V., Gopal, S., Gratten, J., de Haan, L., Hammer, C., Hamshere, M.L., Hansen, M., Hansen, T., Haroutunian, V., Hartmann, A.M., Henskens, F.A., Herms, S., Hirschhorn, J.N., Hoffmann, P., Hofman, A., Hollegaard, M.V., Hougaard, D.M., Ikeda, M., Joa, I., Julià, A., Kahn, R.S., Kalaydjieva, L., Karachanak-Yankova, S., Karjalainen, J., Kavanagh, D., Keller, M.C., Kennedy, J.L., Khrunin, A., Kim, Y., Klovins, J., Knowles, J.A., Konte, B., Kucinskas, V., Ausrele Kucinskiene, Z., Kuzelova-Ptackova, H., Kähler, A.K., Laurent, C., Lee Chee Keong, J., Hong Lee, S., Legge, S.E., Lerer, B., Li, M., Li, T., Liang, K.-Y., Lieberman, J., Limborska, S., Loughland, C.M., Lubinski, J., Lönnqvist, J., Macek Jr., M., Magnusson, P.K.E., Maher, B.S., Maier, W., Mallet, J., Marsal, S., Mattheisen, M., Mattingsdal, M., McCarley, R.W., McDonald, C., McIntosh, A.M., Meier, S., Meijer, C.J., Melegh, B., Melle, I., Mesholam-Gately, R.I., Metspalu, A., Michie, P.T., Milani, L., Milanova, V., Mokrab, Y., Morris, D.W., Mors, O., Murphy, K.C., Murray, R.M., Myin-Germeys, I., Müller-Myhsok, B., Nelis, M., Nenadic, I., Nertney, D.A., Nestadt, G., Nicodemus, K.K., Nikitina-Zake, L., Nisenbaum, L., Nordin, A., O'Callaghan, E., O'Dushlaine, C., O'Neill, F.A., Oh, S.-Y., Olincy, A., Olsen, L., Van Os, J., Endophenotypes International Consortium, Pantelis, C., Papadimitriou, G.N., Papiol, S., Parkhomenko, E., Pato, M.T., Paunio, T., Pejovic-Milovancevic, M., Perkins, D.O., Pietiläinen, O., Pimm, J., Pocklington, A.J., Powell, J., Price, A., Pulver, A.E., Purcell, S.M., Quested, D., Rasmussen, H.B., Reichenberg, A., Reimers, M.A., Richards, A.L., Roffman, J.L., Roussos, P., Ruderfer, D.M., Salomaa, V., Sanders, A.R., Schall, U., Schubert, C.R., Schulze, T.G., Schwab, S.G., Scolnick, E.M., Scott, R.J., Seidman, L.J., Shi, J., Sigurdsson, E., Silagadze, T., Silverman, J.M., Sim, K., Slominsky, P., Smoller, J.W., So, H.-C., Spencer, C.A., Stahl, E.A., Stefansson, H., Steinberg, S., Stogmann, E., Straub, R.E., Strengman, E., Strohmaier, J., Scott Stroup, T., Subramaniam, M., Suvisaari, J., Svrakic, D.M., Szatkiewicz, J.P., Söderman, E., Thirumalai, S., Toncheva, D., Tosato, S., Veijola, J., Waddington, J., Walsh, D., Wang, D., Wang, Q., Webb, B.T., Weiser, M., Wildenauer, D.B., Williams, N.M., Williams, S., Witt, S.H., Wolen, A.R., Wong, E.H.M., Wormley, B.K., Simon Xi, H., Zai, C.C., Zheng, X., Zimprich, F., Wray, N.R., Stefansson, K., Visscher, P.M., Trust Case-Control Consortium, Adolfsson, R., Andreassen, O.A., Blackwood, D.H.R., Bramon, E., Buxbaum, J.D., Børglum, A.D., Cichon, S., Darvasi, A., Domenici, E., Ehrenreich, H., Esko, T., Gejman, P.V., Gill, M., Gurling, H., Hultman, C.M., Iwata, N., Jablensky, A.V., Jönsson, E.G., Kendler, K.S., Kirov, G., Knight, J., Lencz, T., Levinson, D.F., Li, Q.S., Liu, J., Malhotra, A.K., McCarroll, S.A., McQuillin, A., Moran, J.L., Mortensen, P.B., Mowry, B.J., Nöthen, M.M., Ophoff, R.A., Owen, M.J., Palotie, A., Pato, C.N., Petryshen, T.L., Posthuma, D., Rietschel, M., Riley, B.P., Rujescu, D., Sham, P.C., Sklar, P., St Clair, D., Weinberger, D.R., Wendland, J.R., Werge, T., Daly, M.J., Sullivan, P.F., O'Donovan, M.C., 2014. Biological insights from 108 schizophrenia-associated genetic loci. Nature https://doi.org/10.1038/nature13595.

Santos Soria, L., Moura Gubert, C., Cereser, K.M., Gama, C.S., Kapczinski, F., 2012. Increased serum levels of C3 and C4 in patients with schizophrenia compared to eutymic patients with bipolar disorder and healthy. Rev. Bras. Psiquiatr. 34, 119–120. https://doi.org/10.1590/S1516-44462012000100022.

Sekar, A., Bialas, A.R., de Rivera, H., Davis, A., Hammond, T.R., Kamitaki, N., Tooley, K., Presumey, J., Baum, M., Van Doren, V., Genovese, G., Rose, S.A., Handsaker, R.E., Daly, M.J., Carroll, M.C., Stevens, B., McCarroll, S.A., 2016. Schizophrenia risk from complex variation of complement component 4. Nature, 1–17 https://doi.org/10.1038/nature16549.

Severance, E.G., Gressitt, K.L., Halling, M., Stallings, C.R., Origoni, A.E., Vaughan, C., Khushalani, S., Alaedini, A., Dupont, D., Dickerson, F.B., Yolken, R.H., 2012. Complement C1q formation of immune complexes with milk caseins and wheat glutens in schizophrenia. Neurobiol. Dis. 48, 447–453. https://doi.org/10.1016/j.nbd.2012.07.005.

Severance, E.G., Gressitt, K.L., Buka, S.L., Cannon, T.D., Yolken, R.H., 2014. Maternal complement C1q and increased odds for psychosis in adult offspring. Schizophr. Res. 159 (1), 14–19. https://doi.org/10.1016/j.schres.2014.07.053.

Sheehan, D.V., Lecrubier, Yves, Harnett Sheehan, K., Amorim, Patricia, Janavs, Juris, Weiller, Emmanuelle, Hergueta, Thierry, Baker, Roxy, Dunbar, Geoffrey C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 22–23.

Spitzer, R.L., Williams, J.B., Gibbon, M., First, M.B., 1992. The Structured Clinical Interview for DSM-III-R (SCID) - history, rationale, and description. Arch. Gen. Psychiatry 49, 624–629. https://doi.org/10.1001/archpsyc.1992.01820080032005.

Stephan, A.H., Barres, B.A., Stevens, B., 2012. The complement system: an unexpected role in synaptic pruning during development and disease. Annu. Rev. Neurosci. 35, 369–389. https://doi.org/10.1146/annurev-neuro-061010–113810.

Stevens, B., Allen, N.J., Vazquez, L.E., Howell, G.R., Christopherson, K.S., Nouri, N., Micheva, K.D., Mehalow, A.K., Huberman, A.D., Stafford, B., Sher, A., Litke, A.M., Lambris, J.D., Smith, S.J., John, S.W.M., Barres, B.A., 2007. The classical complement cascade mediates CNS synapse elimination. Cell 131, 1164–1178. https://doi.org/10.1016/j.cell.2007.10.036.

Stolp, H.B., Dziegielewska, K.M., 2009. Review: role of developmental inflammation and blood-brain barrier dysfunction in neurodevelopmental and neurodegenerative diseases. Neuropathol. Appl. Neurobiol. 35, 132–146. https://doi.org/10.1111/j.1365-2990.2008.01005.x.

Upthegrove, R., Manzanares-Teson, N., Barnes, N.M., 2014. Cytokine function in medication-naive first episode psychosis: a systematic review and meta-analysis. Schizophr. Res. 155, 101–108. https://doi.org/10.1016/j.schres.2014.03.005.

Vasic, N., Connemann, B.J., Wolf, R.C., Tumani, H., Brettschneider, J., 2012. Cerebrospinal fluid biomarker candidates of schizophrenia: where do we stand? Eur. Arch. Psychiatry Clin. Neurosci. 262, 375–391. https://doi.org/10.1007/s00406-011-0280-9.

Veerhuis, R., Nielsen, H.M., Tenner, A.J., 2011. Complement in the brain. Mol. Immunol. 48, 1592–1603. https://doi.org/10.1016/j.molimm.2011.04.003.

Volk, D.W., Chitrapu, A., Edelson, J.R., Roman, K.M., Moroco, A.E., Lewis, D.A., 2015. Molecular mechanisms and timing of cortical immune activation in schizophrenia. Am. J. Psychiatry 172, 1112–1121. https://doi.org/10.1176/appi.ajp.2015.15010019.

Yang, X., Sun, J., Gao, Y., Tan, A., Zhang, H., Hu, Y., Feng, J., Qin, X., Tao, S., Chen, Z., Kim, S.T., Peng, T., Liao, M., Lin, X., Zhang, Z., Tang, M., Li, L., Mo, L., Liang, Z., Shi, D., Huang, Z., Huang, X., Liu, M., Liu, Q., Zhang, S., Trent, J.M., Zheng, S.L., Xu, J., Mo, Z., 2012. Genome-wide association study for serum complement C3 and C4 levels in healthy Chinese subjects. PLoS Genet. 8. https://doi.org/10.1371/journal.pgen.1002916.

Yung, A.R., Yuen, H.P., McGorry, P.D., Phillips, L.J., Kelly, D., Dell'Olio, M., Francey, S.M., Cosgrave, E.M., Killackey, E., Stanford, C., Godfrey, K., Buckby, J., 2005. Mapping the onset of psychosis: the comprehensive assessment of at-risk mental states. Aust. N. Z. J. Psychiatry 39, 964–971. https://doi.org/10.1111/j.1440-1614.2005.01714.x.