Complement proteins are elevated in blood serum but not CSF in clinical high-risk and antipsychotic-naïve first-episode psychosis

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ABSTRACT

Alterations in the complement system have been reported in some people with psychotic disorder, including in pre-psychotic individuals, suggesting that complement pathway dysregulation may be a feature of the early psychosis phenotype. Measurement of complement protein expression in psychosis has been largely restricted to the blood from patients with established illness who were taking antipsychotic medication. The present study examined a range of complement proteins in blood and cerebrospinal fluid (CSF) derived from individuals at clinical high-risk for psychosis (CHR), antipsychotic-naïve first-episode psychosis (FEP) and healthy controls.

A panel of complement proteins (C1q, C3, C3b/iC3b, C4, factor B and factor H) were quantified in serum and matched CSF in 72 participants [n = 23 individuals at CHR, n = 24 antipsychotic-naïve FEP, n = 25 healthy controls] using a multiplex immunoassay. Analysis of covariance was used to assess between-group differences in complement protein levels in serum and CSF. Pearson's correlation was used to assess the relationship between serum and CSF proteins, and between complement proteins and symptom severity.

In serum, all proteins, except for C3, were significantly higher in FEP and CHR. While a trend was observed, protein levels in CSF did not statistically differ between groups and appeared to be impacted by BMI and sample storage time. Across the whole sample, serum and CSF protein levels were not correlated. In FEP, higher levels of serum classical and alternative grouped pathway components were correlated with symptom severity.

Our exploratory study provides evidence for increased activity of the peripheral complement system in the psychosis spectrum, with such elevations varying with clinical severity. Further study of complement in CSF is warranted. Longitudinal investigations are required to elucidate whether complement proteins change peripherally and/or centrally with progression of psychotic illness.

1. Introduction

The complement system is a central component of innate immunity, involved in the inflammatory response (Merle et al., 2015) and has been implicated as a risk factor for schizophrenia (Sekar et al., 2016) and the development of psychotic disorder (Heurich et al., 2022). Accumulating research has investigated components of the peripheral complement cascade in people at various stages of a psychotic illness. Although serological studies of complement protein levels in psychosis are mixed (Mongan et al., 2020), there is evidence for alterations in blood protein levels in clinical-high risk individuals prior to the onset of psychosis (English et al., 2018; Föcking et al., 2021; Madrid-Gambin et al., 2019; Mongan et al., 2021), as well as in individuals with first-episode and established schizophrenia (e.g. Jiang et al., 2019; Laskaris et al., 2019; Ramsey et al., 2013; Sória et al., 2012).

Whilst this research points to a systemic dysregulation of complement pathways (Heurich et al., 2022), the role of the complement system in the brain in relation to psychotic illness pathophysiology remains unclear. Complement components are found in the fluid phase of the blood or bound to cells with specific functions in the promotion or

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regulation of complement activation (Merle et al., 2015). Complement components can also be locally produced in the brain (Veerhuis et al., 2011), and are involved in neurodevelopmental processes (Schafer et al., 2012; Stephan et al., 2012; Stevens et al., 2007). For example, components of the classical pathway have been implicated in microglial-mediated synaptic elimination in an activity-dependent manner (Schafer et al., 2012; Stephan et al., 2012). This process is not only associated with normal postnatal CNS development but, if dysregulated, is thought to contribute to synaptic dysfunction in disease (Schafer and Stevens, 2010). A role for complement in schizophrenia has also been described, with evidence for a greater number of the complement component 4A (C4A) alleles and C4A RNA expression levels in schizophrenia (Sekar et al., 2016). As such, alterations of the complement pathway in the CNS, either independently or inter-dependently of the peripheral system, may be implicated in the pathogenesis of psychosis.

Investigation of cerebrospinal fluid (CSF) is one approach to index the CNS environment more closely in living people with psychotic disorders, given the close contact CSF has with the brain, enabling the diffusion of molecules from brain cells into the CSF. To our knowledge, only three studies have investigated complement components in the CSF in people with psychosis. Ishii et al. (2018), examined complement C5 in people with major psychiatric disorders and reported increased levels in schizophrenia and major depressive disorder. Although complement C5 level was not associated with clinical symptoms, it was associated with chlorpromazine-equivalent dose. In a study examining the C4 component, Gallego et al. (2021) found higher levels of C4 in the CSF, but not plasma, in people with schizophrenia compared to controls, and no correlation between CSF and plasma C4 levels. In this study, C4 levels were not associated with total symptom severity or chlorpromazine-

equivalents. Most recently, Gracias et al. (2022) examined the C4 isotypes, C4A and C4B, in first-episode psychosis (FEP) patients and reported higher C4A protein level in those who received a schizophrenia diagnosis ~ 1.5 year later but not in FEP patients who did not. These studies suggest that some complement components may be altered in the CSF of people with schizophrenia. Nevertheless, it remains unclear the extent of complement dysregulation in the CSF, whether complement components are altered at earlier and antipsychotic-naive stages of the psychosis spectrum, and whether complement components in CSF and blood co-vary or are independent, and correlate with clinical symptoms.

Complement comprises three activation pathways: the classical pathway (CP), the lectin pathway (LP), and the alternative pathway (AP) that are each activated by distinct protein recognition molecules in response to a trigger (Merle et al., 2015). To this end, the present study examined complement protein levels relevant to classical pathway (CP) activation (C1q, C4), alternative pathway (AP) amplification (C3, C3b/iC3b, factor B; CFB) or regulation (factor H; CFH) in matched blood (serum) and CSF in people with clinical high-risk for psychosis (CHR), antipsychotic-naïve first-episode psychosis (FEP) and healthy controls (HC) (Fig. 1). Our aims were to ascertain: i) whether there were differences in complement protein levels in CSF and serum in people with CHR and FEP, and when compared to healthy controls, ii) whether CSF and serum levels were associated, and iii) whether complement proteins were associated with the severity of clinical symptoms.
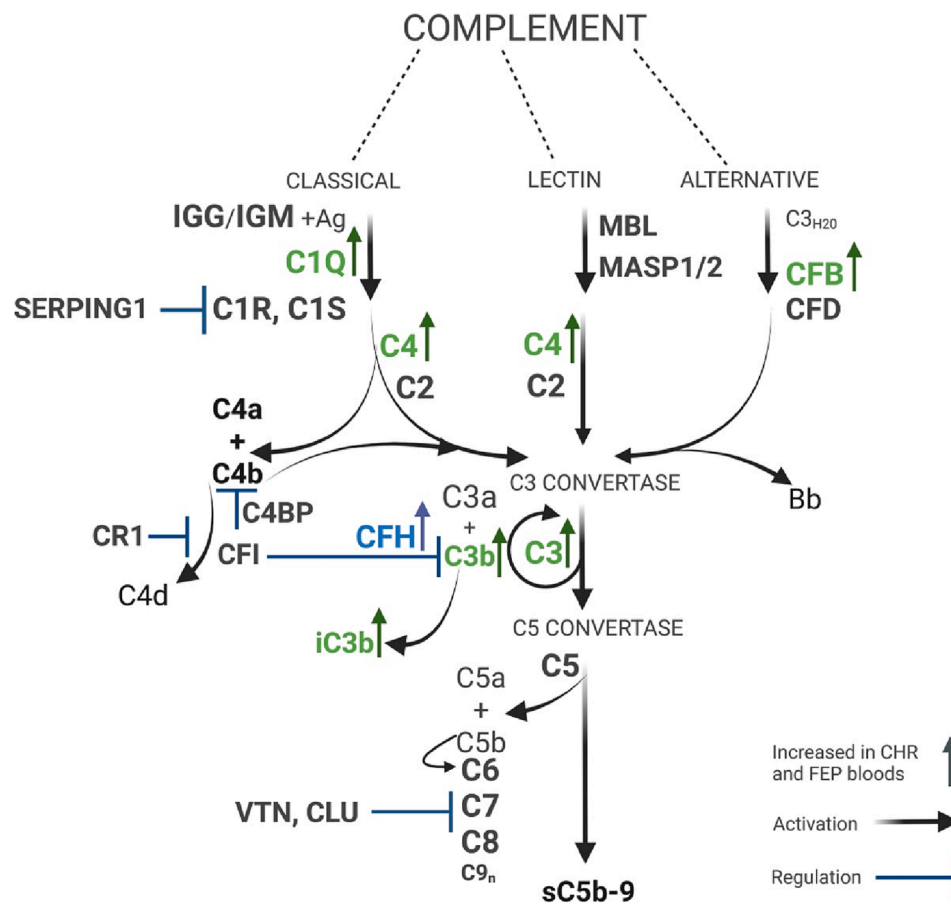

Fig. 1. Schematic of the complement pathway. Overview of the complement pathways showing components of the complement marker panel in green (C1Q, C4, CFB, C3, C3b/iC3b) or blue (CFH) for regulatory components. Significantly increased levels were observed for components C1Q, C4, CFB and CFH and activation fragments C3b/iC3b in CHR and FEP blood, but not CSF, as indicated by an arrow. Figure created with BioRender.com.

2. Material and methods

2.1. Subjects and methods

We used biological and clinical data from a total of 72 participants: 23 individuals at clinical high-risk for psychosis (CHR), 24 antipsychotic-naïve first episode psychosis (FEP) patients, and 25 healthy controls (HC) previously recruited from the catchment areas of Cologne and Mannheim, Germany, for inclusion in the Cologne-Mannheim CSF II database. Samples and clinical data from the database have been used in previous publications (e.g. Bien et al., 2021; Coughlin et al., 2017; Giuffrida et al., 2004; Huang et al., 2007; Koethe et al., 2009; Kranaster et al., 2011; Leweke et al., 2007; Schwarz et al., 2012; Xiao et al., 2021).

FEP and CHR participants were recruited from help-seeking early recognition for psychosis units that provided inpatient services for diagnostic assessment. FEP patients had a first-time clinical diagnosis of schizophrenia or schizophreniform psychosis according to the Diagnostic and Statistical Manual IV edition (DSM-IV). Patients with drug-induced psychosis were excluded. CHR participants met criteria for clinical high-risk for psychosis as determined by the Scale of Prodromal Symptoms (SOPS) (Miller et al., 1999), and the Schizophrenia Prediction Instrument for Adults (SPI–A) (Schultze-Lutter et al., 2016). CHR was assumed if any of ten cognitive–perceptive basic symptoms or any attenuated or transient psychotic symptoms were present. All FEP and CHR participants were antipsychotic-naïve (no previous or current treatment with antipsychotic medication) at the time of assessments. Treatment with low-dose lorazepam was allowed but rarely used. Healthy controls had no family history of schizophrenia or other detected medical, psychiatric, or neurological disturbance. Healthy volunteers were screened for psychiatric disorders using the SCID-I and the SCID-II clinical interview and a neurological and physical examination was carried out by a trained neurologist. All participants (FEP, CHR and controls) reported a negative urine drug screening for illicit drugs.

Samples and clinical data used in this study were previously approved by the Medical Faculty of the University of Cologne and the ethics committee II of the Medical Faculty Mannheim, Heidelberg University, including the procedures for sample collection, retrospective use of samples, and analysis. All participants provided written informed consent.

2.2. Biological fluids

The serum and CSF samples were collected between 1999 and 2006 as part of the Cologne-Mannheim CSF 2 Collection Biobank and stored in these locations. Serum and CSF samples were collected on the same day. Blood samples were processed within 30 min to separate serum. CSF samples were collected using a lumbar puncture procedure in the morning between 10:00 and 12:00 hrs using previously published methods (Giuffrida et al., 2004). For patients, lumbar punctures occurred within the routine diagnostic process with remaining CSF agreed for use in research studies. For controls, all lumbar punctures were performed for the purpose of research only. Up to 15 mL CSF was collected using a sprotte needle 22G with introducer. Routine testing (glucose, proteins and albumin) was performed on both CSF and blood samples. Collection, handling and storage of biospecimen samples followed a highly standardized operation procedure. All samples were biobanked in aliquots at −80° C in specialized cryotubes and stored under similar controlled conditions until subsequent analysis. All samples in the current study underwent one freeze–thaw cycle and were shipped once on dry ice.

Samples for analysis were thawed in batches and centrifuged in a prechilled centrifuge at 3,000 RCF for 10 min and subsequently stored on ice. Specimens were diluted with the recommended reagent diluent according to the manufacturer's instructions before subsequent analyses. A dilution factor of 1:40,000 was chosen for serum samples and 1:30 for CSF samples. All samples were prepared and measured in duplicate. The HUMAN Complement Magnetic Bead Panel 2 HCMP2MAG-19 K analysis kit (lot number: HCM 2–105 and HCM 2–205, EMD Millipore Corporation - Merck KGaA, Darmstadt, Germany) was used. Antibody beads with the following targets were applied: C1q, C3, C3b/iC3b (assay detects both activation fragments indistinguishably), C4, factor B (CFB) and factor H (CFH). Blanks, controls and standards were prepared according to the manufacturer's instructions. The Luminex 200 TM system with xPONENT software (version 3.1; build 917; both EMD Millipore Corporation - Merck KGaA, Darmstadt, Germany) was applied as detection device. Test performance and analysis were performed according to the manufacturer's protocol with an initial incubation of 2 h at room temperature under light protection and continuous orbital shaking at 400 rpm (TPM-2, Sarstedt™, Nümbrecht, Germany). All washing and pipetting procedures were performed manually.

2.3. Clinical measures

Clinical symptoms in FEP and CHR were assessed with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Positive, negative, and general psychopathology scores were computed by summing the relevant component items.

2.4. Statistical analyses

Statistical analyses and plots were performed using R-Studio Version 3.0.386.

2.4.1. Demographics and data screening

Differences in demographic and routine laboratory variables between groups were analyzed with analysis of variance (ANOVA) or Pearson's $\chi 2$ statistics for continuous and categorical variables, respectively, with non-parametric tests being used if appropriate. Differences in PANSS total, positive, negative, and general symptoms between FEP and CHR were analyzed with independent t-tests. Missing data for BMI (n = 11), and serum levels of C1q, C3, CFB, C3b/iC3b (each n = 2) and CFH (n = 3) were imputed with the *mice* package (version 3.13). As raw complement data was positively skewed it was transformed with the rank-based inverse normal transform using the *RNOmni* package to ensure normality. A rank-based inverse normal transformation was selected as it resulted in the best distribution (based on Shapiro-Wilk and visual inspection of Q-Q plots) after testing several potential transformations.

2.4.2. Between-group analyses of complement components

A series of analyses of covariance (ANCOVA) were used to test the null hypothesis of equality in complement proteins across the healthy control, FEP and CHR groups, adjusting for age, sex, BMI, and sample storage time (days). The assumptions of homogeneity of variances and normality were supported for all models. Six models (corresponding to each complement protein) were run for CSF and serum samples separately, with the false discovery rate (FDR) used to control for the Type I error rate related to multiple comparisons. In the event of a main effect of group, post-hoc comparisons with Tukey's Honest Significant Difference (HSD) were performed.

2.4.3. Association between serum and CSF complement components

The associations between complement proteins in serum and CSF were examined by computing Pearson correlation coefficient matrices in the entire sample using the *corrplot* (version 0.92) package.

2.4.4. Association of complement components with clinical symptoms

The associations between complement protein levels and symptoms (PANSS positive, negative and general sub-scores) were examined using Pearson correlations in FEP and CHR separately. To reduce the number

of comparisons, proteins were combined in each biofluid according to their general function within the complement cascade. Specifically, C1q and C4 proteins were combined to represent a 'classical pathway' index, CFB, CFH and C3b/iC3b were combined to represent an 'alternative pathway' index and C3 was examined separately as the component central to the amplification of all pathways. In supplementary analyses, CFB and C3b/iC3b were combined to represent a 'positive index', and CFH and C3b/iC3b were combined to represent a 'negative index' and complement proteins were examined separately.

Relevant proteins, which were z-score normalized, were summed to create the index score. Proteins were combined for serum and CSF separately. FDR (across 9 tests corresponding to 3 protein scores by 3 symptom scores in each group separately) was implemented for multiple comparison correction.

3. Results

3.1. Demographics

Demographic and clinical characteristics of participants are shown in Table 1. Age ($F = 2.5$, $p = 0.09$) and BMI ($F = 0.2$, $p = 0.82$) did not differ between groups. The distribution of sex significantly differed between groups ($\chi^2 = 9.7$, $p = 0.008$), with males being more predominant in the FEP and CHR groups in comparison to HC. Storage sample time also significantly differed between groups ($\chi 2 = 14.3$, $p < 0.001$), with post-hoc comparisons revealing that the samples were stored for a shorter time in FEP compared to HC ($W = 5.7$, $p < 0.001$). Although a similar proportion of participants in each group were current smokers, a larger proportion of HC and CHR participants had a history of cannabis use compared to FEP ($W = 7.35$, $p < 0.027$). History of other illicit drugs was minimal and similar across the groups (ranging from 8 to 12%). Fifty and 30% of FEP and CHR participants, respectively, were taking benzodiazepine medication. There were no differences between groups in total protein and albumin in each biofluid, nor a difference in the serum/CSF albumin ratio. Glucose was elevated in FEP compared to HC in both serum and CSF.

No associations existed between age and complement component levels. BMI was not associated with complement levels in serum but was positively associated with C3 ($r = 0.35$, $p = 0.003$), CFB ($r = 0.35$, $p = 0.0026$), CFH ($r = 0.28$, $p = 0.016$) and C3b/iC3b ($r = 0.33$, $p = 0.005$) levels in CSF. Likewise, sample storage time was not associated with complement levels in serum but was negatively associated with all proteins in CSF (r: $-0.26 - -0.53$; all p's < 0.05). With the exception of CSF C1q ($t = 2.1$, $p = 0.04$), complement levels did not differ between

smokers and non-smokers in either biofluid. For C1q CSF, smokers had higher levels than non-smokers. There was a significant effect of sex on C4 ($t = 2.4$, $p = 0.02$), CFH ($t = 2.4$, $p = 0.02$) and C3b/iC3b ($t = 2.1$, $p = 0.04$) protein levels in CSF, with males having higher protein levels than females. Sex was not significantly associated with protein levels in serum.

3.2. Between-group comparisons in complement

A schematic of the complement components examined in the current study is shown in Fig. 1. We observed generally raised complement protein levels in both CSF and blood serum in CHR and FEP groups compared to HC. In CSF, these differences did not significantly differ between diagnostic groups. In serum, all proteins except for C3 were significantly higher in FEP and CHR compared to HC (Fig. 2, Table 2). Estimated marginal means of the proteins from each model adjusted for covariates are reported in Supplementary Table 1. Statistical inference without inclusion of covariates is presented in Supplementary Table 2.

3.3. Associations between serum and CSF protein levels

Across the whole sample (i.e. CHR, FEP and HC groups combined), individual proteins in their biological fluid were highly correlated with each other, but were not significantly correlated across biological fluids (all *p-values* > 0.05, Fig. 3). That is, individual proteins in serum were significantly and highly correlated with each other, and individual proteins in CSF were significantly and highly correlated with each other, but proteins in serum were not significantly correlated with proteins in CSF.

3.4. Associations between protein levels and clinical symptoms

Total ($t = 2.7$, $p = 0.009$), positive ($t = 3.9$, $p = 0.0003$), negative ($t = 2.3$, $p = 0.02$) and general ($t = 2.0$, $p = 0.048$) PANSS scores were significantly higher in FEP compared to CHR. There were no associations between CSF protein levels and symptoms in FEP and CHR. Serum complement levels were not associated with symptoms in CHR, but were positively associated in FEP (Supplementary Table 4, Fig. 4). Associations in FEP were significant for negative and general symptoms with the classical and alternative pathway grouped components ($r = 0.48 - 0.52$; p's 0.02–0.01) which remained significant after FDR adjustment. Similar effect sizes were observed for positive psychotic symptoms, but these did not meet statistical significance (uncorrected). Supplementary Tables 2 and 3 report associations with the 'negative' and 'positive' index

Table 1
Clinical cohort characteristics.

Characteristic	HC	CHR	FEP	F/χ^2	p	Posthoc
	N = 25	N = 23	N = 24			
Age, mean (sd) years	28.9 (9.8)	24.8 (5.3)	30.3 (10.1)	2.5	0.09	
Sex, % (n) male	48 (12)	83 (19)	83 (20)	9.7	0.008	FEP/CHR > HC
BMI, mean (sd)	22.3 (3.1)	22.8 (2.5)	22.6 (3.3)	0.20	0.82	
Current smoker, % (n)	36 (9)	48 (11)	46 (11)	0.8	0.67	
History of cannabis use, % (n)	64 (16)	70 (16)	33 (8)	7.35	0.027	HC/CHR > FEP
Sample storage time (days), median (IQR)	7244 (36)	6627 (1417)	6060 (948)	14.3	0.0008	HC > FEP***
CSF Glucose, median (IQR)	56 (2.0)	68 (4.5)	70.5 (9.5)	37.3	<0.00001	FEP > HC***
CSF Protein, median (IQR)	34 (12.0)	32 (23.0)	30 (14.5)	2.5	0.28	
CSF Albumin, median (IQR)	18.1 (9.7)	17.1 (8.6)	19.2 (8.6)	0.15	0.929	
Serum Glucose, median (IQR)	87 (22.0)	93 (17.0)	99 (16.25)	19.3	0.00006	FEP > HC***
Serum Protein, median (IQR)	7.5 (0.6)	7.45 (0.5)	7.7 (0.5)	3.89	0.14	
Serum Albumin, median (IQR)	4568 (508)	4841 (956)	4711 (484)	0.39	0.82	
CSF/Serum Albumin ratio, median (IQR)	0.004 (0.002)	0.003 (0.002)	0.004 (0.002)	0.31	0.86	
Total psychopathology (PANSS Total), mean (sd)	–	66.8 (22.4)	86.4 (25.4)			
Positive symptom subscore (PANSS P), mean (sd)	–	14.9 (5.17)	21.5 (6.02)			
Negative symptom subscore (PANSS N), mean (sd)	–	16.1 (6.37)	21.7 (9.4)			
General symptom subscore (PANSS G), mean (sd)	–	35.8 (12.2)	43.1 (11.9)			

HC = Healthy controls, CHR = Clinical high risk, FEP = First episode psychosis, BMI = body mass index, PANSS = positive and negative syndrome scale.
Posthoc tests conducted in event of overall group effect. *Significant at p < 0.05, **Significant at p < 0.01, ***Significant at p < 0.001.
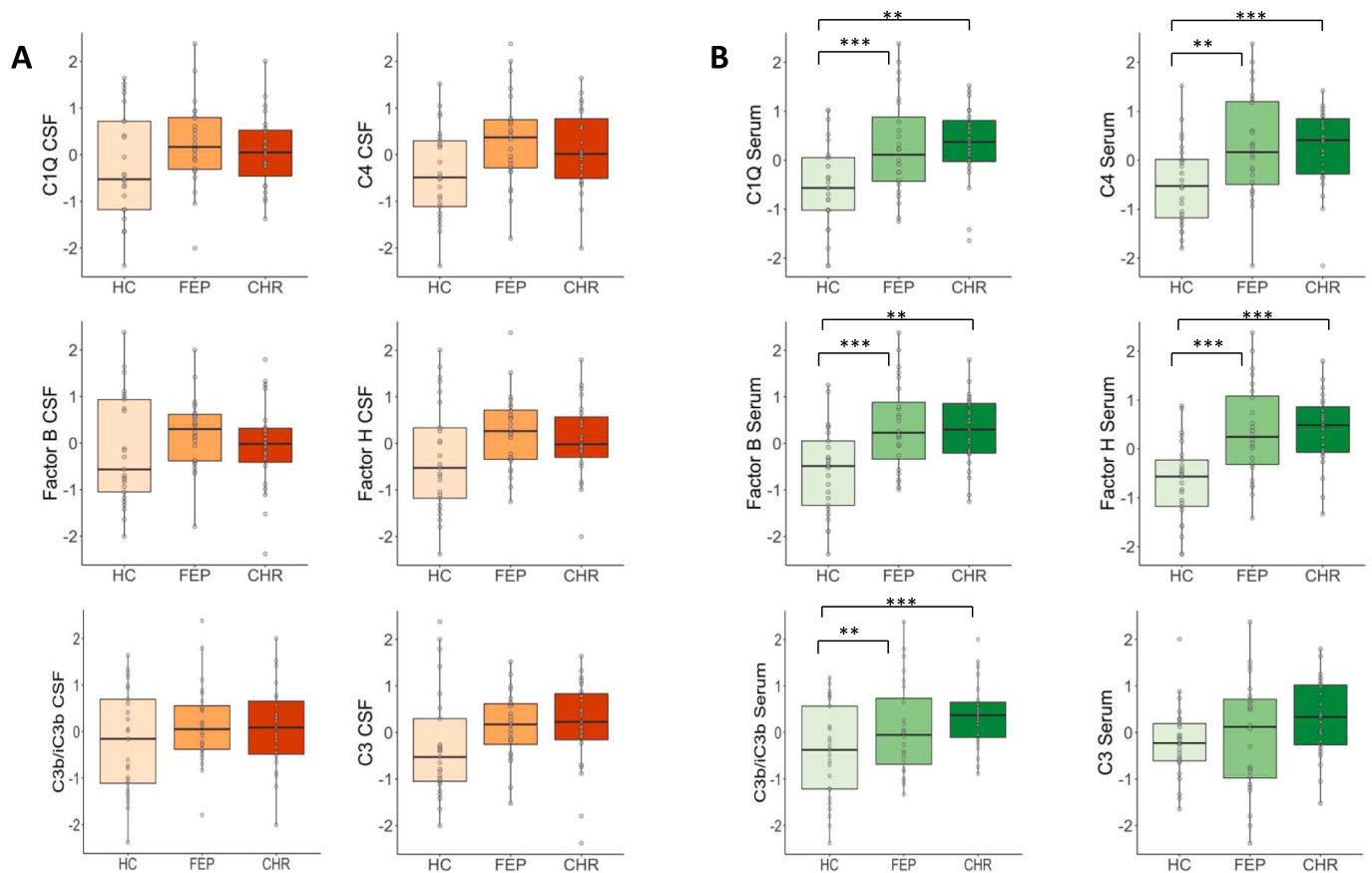
Fig. 2. Boxplots showing distribution of complement proteins across the groups. A) Protein level in cerebrospinal fluid; B) Protein level in serum. HC = healthy controls, FEP = first-episode psychosis, CHR = Clinical high-risk for psychosis. Protein values are rank transformed to ensure normality. Significance based on post-hoc comparisons with Tukey's Honest Significant Distance. *Significant at p < 0.05, **Significant at p < 0.01, ***Significant at p < 0.001.

Table 2
Between-group comparisons of complement protein levels in healthy controls, clinical high risk and first-episode psychosis groups.

	HC $N = 25$	CHR $N = 23$	FEP $N = 24$	Comparisons		
	M (SD)	M (SD)	M (SD)	F	p	Post-hoc[a]
CSF [ng/mL]						
Complement C1Q	54.5 (47.3)	64.2 (46.0)	75.4 (54.8)	0.012	0.99	
Complement C3	14,266 (31000)	9671 (9418)	8467 (8253)	0.108	0.898	
Complement C4	280 (249)	393 (264)	490 (348)	0.076	0.927	
Complement Factor B	166 (191)	160 (143)	186 (125)	0.526	0.594	
Complement Factor H	191 (182)	222 (138)	242 (157)	0.093	0.911	
C3b/iC3b	8.65 (8.67)	10.3 (9.99)	12.4 (19.6)	0.0219	0.978	
Serum [ug/mL]						
Complement C1Q	55.24 (44.01)	106.88 (53.69)	130.89 (158.85)	8.605	**0.0005**	FEP > HC***, CHR > HC**, FEP = CHR
Complement C3	670.14 (687.42)	1177.59 (815.33)	995.57 (979.65)	2.375	0.101	
Complement C4	101.62 (126.26)	217.22 (144.59)	330.6 (466.54)	7.861	**0.0009**	FEP > HC**, CHR > HC***, FEP = CHR
Complement Factor B	100.47 (103.86)	205.96 (136.09)	234.39 (191.37)	8.090	**0.0007**	FEP > HC***, CHR > HC**, FEP = CHR
Complement Factor H	109.05 (114.22)	296.68 (193.83)	329.62 (314.76)	12.838	**< 0.0001**	FEP > HC***, CHR > HC***, FEP = CHR
C3b/iC3b	12.19 (12.62)	20.85 (17.31)	20.84 (26.15)	9.874	**0.0002**	FEP > HC**, CHR > HC***, FEP = CHR

HC = Healthy controls, CHR = Clinical high risk, FEP = First episode psychosis.
All models included the covariates age, sex, BMI and sample storage time. All proteins were rank transformed for analysis purposes to ensure normality of residuals.
Raw mean (sd) values shown for interpretation. P-values in bold are significant after false discovery rate correction.
[a] Post-hoc tests were corrected with Tukey's Honest Significant Difference; *Significant at p < 0.05, **Significant at p < 0.01, ***Significant at p < 0.001.

components and with each complement protein separately. These showed similar associations in serum but no associations were found in CSF.

4. Discussion

To our knowledge, this is the first report examining the levels of a range of complement proteins in both serum and CSF in people at risk for psychosis and antipsychotic-naïve first-episode psychosis. We found higher complement protein levels in both CHR and FEP in serum, but not CSF. Across the whole sample there was no evidence for an association between complement components in serum and CSF. Complement proteins in serum were positively associated with symptom severity in FEP but not CHR. Male sex and greater BMI were associated with higher
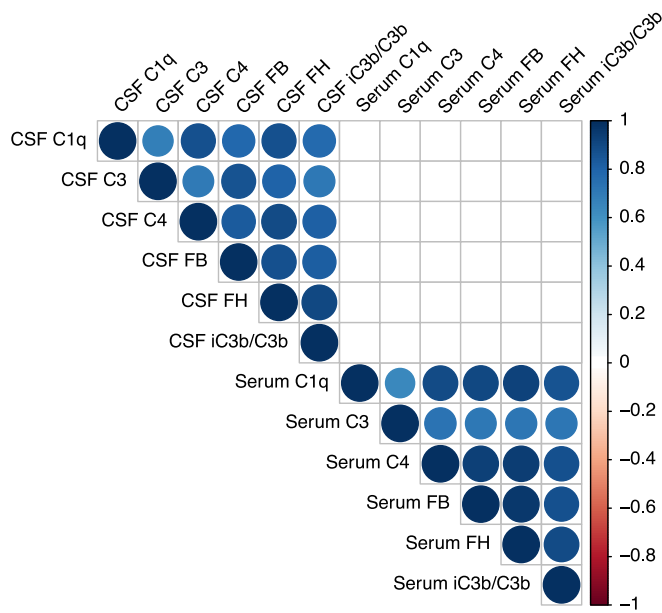
Fig. 3. Correlations between complement protein levels in serum and cerebrospinal fluid. A). Correlation matrix in the entire sample. Colour bar represents Pearson correlation coefficient. Blank squares indicate a non-significant (p > 0.05) correlation between the proteins. Results indicate that complement proteins are highly correlated with each other in their biofluid (i.e., CSF and serum) but are not correlated across biofluids (i.e., serum protein levels are not correlated with CSF protein levels). Colour bar represents the correlation coefficient. HC = healthy controls, FEP = first-episode psychosis, CHR = Clinical high-risk for psychosis. Protein values are rank normalized.

component levels, and longer sample storage time was associated with lower component levels, in CSF, but not serum. In contrast with previous studies (Gallego et al., 2021; Gaya da Costa et al., 2018; Kamitaki et al., 2020), age was not associated with protein levels in either biofluid. The reason for this discrepancy is unclear but could be due to the younger age of the sample in the current study, as well as the possibility for sex differences in age-dependent associations as previously reported (Kamitaki et al., 2020). Given the small sample, we were unable to examine sex differences as a function of age. Smoking status did not affect complement levels, in contrast with previous studies reporting that exposure to cigarette smoke affects complement activation (Kew et al., 1985), and proteins regulating the complement system (Kokelj et al., 2021).

In serum, complement components were higher in both FEP and CHR. Protein levels were higher across all complement components tested (albeit C3 was not statistically significant), suggesting that complement dysregulation is not pathway-specific and implicates at least some components in both the classical and alternative pathways. Although some serological studies examining the peripheral complement system in psychosis are contradictory (Mongan et al., 2020), a recent review of the literature suggests that altered peripheral complement proteins in psychosis are most evident in the early psychosis phenotype, such as individuals with psychotic experiences and individuals who transition from the CHR state to psychotic disorder (Heurich et al., 2022). This includes longitudinal population-based studies examining individuals who later developed a psychotic disorder or psychotic experiences (English et al., 2018; Föcking et al., 2021; Madrid-Gambin et al., 2019) and clinical high-risk studies examining transition status (Mongan et al., 2021; Perkins et al., 2015). According to their review (Heurich et al., 2022), the top-most implicated proteins in these studies include those also tested in our study, such as upregulated CFH, but also C1Q and CFB. In case-control studies of drug-free or drug-naïve schizophrenia (predominantly first-episode), findings are less consistent, although elevated levels of C3 (Ali et al., 2017; Ramsey et al.,

2013), C3c (Maes et al., 1997) and C4 (Maes et al., 1997) have been reported in targeted protein studies, as well as a range of components and regulatory proteins in a study using a comprehensive proteomic approach (Jiang et al., 2019). In contrast, a previous study in serum of a large sample of medicated FEP patients that examined 11 complement analytes found significantly elevated terminal complement complex, which was not measured in this study, but no significant difference in C3 or C4 (Kopczynska et al., 2019).

To our knowledge we are the first to measure C3b/iC3b in the psychosis spectrum. As C3b/iC3b are activating fragments of C3, the higher serum levels observed in FEP and CHR in the current study suggest that C3 is activated into C3b, which is readily inactivated into iC3b via CFH/CFI. As such, we provide evidence for both higher component levels and increased activation of complement, together representing a general amplification of the peripheral complement pathway in the psychosis spectrum. Of note, this amplification or enhanced 'activating capacity' of complement is not the result of antipsychotic medication or illness chronicity, as these alterations in complement were observed in anti-psychotic-naïve FEP and CHR, and after adjustment for confounding factors such as BMI and sex. The higher levels of complement in FEP as well as CHR, point to elevated complement levels and activation prior to psychosis onset or in individuals vulnerable to the development of a psychotic disorder. Data pertaining to CHR transition status was not available in the current study; this will be important to examine in future prospective studies to elucidate the temporal nature of complement dysregulation across the psychosis spectrum.

Our study contrasts with three previous studies that measured single complement components in the CSF in people with schizophrenia. While we found no significant differences in CSF complement levels, Ishii et al. (2018) reported higher C5 levels, whereas Gallego et al. (2021) and Gracias et al. (2022) reported higher C4/C4A, in people with schizophrenia. Differences in the patient populations could contribute to the discrepancy between these and the current findings. For example, the patient cohorts in Ishii et al. (2018) and Gallego et al. (2021) were older, had a more chronic illness profile and were taking antipsychotic medication. In contrast, the current samples were not exposed to antipsychotic treatment and were at the earliest stages of the psychosis spectrum. Interestingly, in the proteomics study by Gracias et al. (2022), elevated C4A, but not C4B, proteins were specific to FEP patients who subsequently maintained a diagnosis of schizophrenia. Our study, which also comprised FEP with a diagnosis of schizophrenia or schizophreniform disorder, contrasts with this finding, although we measured total C4 rather than C4 isotypes. It is thus possible that higher levels of complement manifest with illness chronicity or antipsychotic exposure and, in the case of C4, is specific to schizophrenia and C4A, which may have been obscured with measurement of total protein level.

Alternatively, discrepancies with other studies may also be due to demographic and methodological factors. In CSF but not serum, we found higher complement levels in males and in individuals with higher BMI and shorter sample storage times, with these factors being adjusted for in the between-group analyses. Of note, there was also a wide spread of values in the healthy control group, possibly due in part to storage conditions and other confounding factors, which likely contributed to the absence of group differences. Males have been reported to have higher complement levels, though this has been observed in both blood (Gaya da Costa et al., 2018) and CSF (Gallego et al., 2021; Ishii et al., 2018; Kamitaki et al., 2020). In a previous study that examined concomitant peripheral and CSF samples in the same participants, males showed higher C4 protein level in CSF but not plasma (Gallego et al., 2021), similar to the current study. This suggests potential sex differences in the complement system in the brain, aligned with the higher incidence or earlier age of onset of schizophrenia in males (Ochoa et al., 2012). Correlations between BMI and complement components in CSF have likewise been reported in one study (Ishii et al., 2018). Of note, the CSF and serum samples in the current study were, on average, stored for longer than 15 years, with the negative correlation between storage time

PANSS Positive Ratings

PANSS Negative Ratings

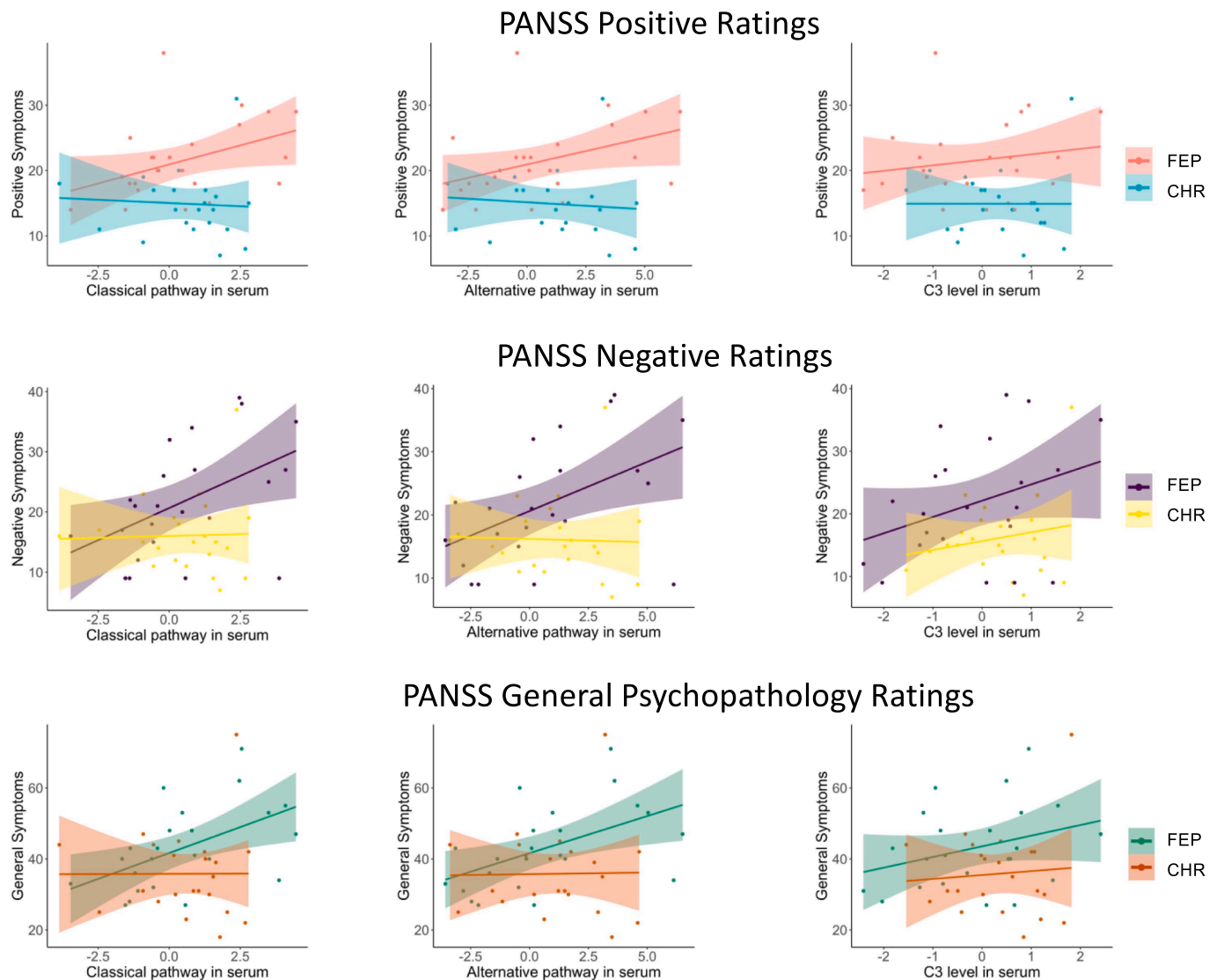## PANSS General Psychopathology Ratings

Fig. 4. Scatterplots showing correlation of serum complement proteins with clinical symptoms in first-episode psychosis (FEP) and clinical high-risk (CHR) participants Associations of symptoms are shown with a 'classical pathway' complement index (combined C1q and C4 proteins), an 'alternative pathway' complement index (combined factor B, factor H and C3b/iC3b proteins) and C3 levels.

and CSF (but not serum) protein level indicating a degradation of complement proteins over time. The concentration of CSF complement proteins, especially C4, was also very low, being an order of magnitude lower than previous studies (Gallego et al., 2021; Kamitaki et al., 2020). Clinical biofluid samples are often stored for several years in the freezer prior to analysis. In plasma, previous studies have reported non-significant (Yang et al., 2015), positive (Morgan et al., 2017) and negative (Morgan et al., 2017) associations between storage time and a range of complement factors, suggesting there may be some degree of activation or degradation of biomarkers during storage. However, the impact of storage time on complement analytes in CSF has previously remained unclear, and thus our findings highlight the need for future studies to consider storage time as an important co-variable. It is likely that the lower detection of complement in CSF in our study was due to the relative lower concentration of these analytes in CSF compared to serum, combined with challenges associated with measuring these markers in CSF as well as possible degradation of complement over time. As such, it remains possible that complement is higher in CSF in the psychosis spectrum. Indeed, inspection of the plots across groups (see Fig. 2) reveal that most analytes show higher mean levels in FEP and CHR compared with controls, although not reaching statistical

significance. We also observed significantly higher C4 in FEP relative to controls ($t = 2.7$, $p = 0.009$, Cohen's $d = 0.76$) in models unadjusted for the covariates. Although we cannot draw a clear conclusion regarding the CSF findings due to the limitations cited above, our study provides valuable insight that these limitations affect the CSF measurements more so than serum. Further research examining complement components in the CSF in the unmedicated, early stage of the psychosis spectrum is warranted.

Our finding of elevated complement in blood, but not CSF, in FEP and CHR at a group level, points to potential independence of complement mechanisms in each biofluid. This was supported in the correlation analyses, where we observed a poor correlation between complement components in serum and CSF across the whole sample. This finding is consistent with Gallego et al. (2021), who did not find a correlation between CSF and plasma levels of C4; our study extended this finding to other components of the complement pathway and to associations of each component to all others. Our findings also concur with a recent study reporting no correlation between CSF and plasma C1q in genetic frontotemporal dementia (van der Ende et al., 2022) and a recent *meta*-analysis by Gigase et al. (2023) showing poor correlation between paired blood and CSF measurements of cytokines. The overall absence of

a correlation between serum and CSF complement proteins suggests that peripheral markers are a poor reflection of the CSF profile and questions the use of serum as a surrogate for CSF complement activity. Although further studies are needed, our findings indicate that the central and peripheral complement pathways are independent or differentially regulated and that systemic complement abnormalities are present at the early stages of the psychosis spectrum.

A notable finding of our study was the relationship between serum complement expression and clinical symptoms in FEP, but not CHR. Higher protein levels were associated with higher symptom scores across all components, including when combined into indices of classical and alternative pathway expression, positive and negative expression as well as single components. Higher expression level was significantly associated with higher negative and general symptoms, although a similar trend was seen for positive symptoms. Few studies have examined the relationship between peripheral complement levels and symptom severity and of these, results have been mixed. For example, studies have reported associations between increases in C3 and C4 (Morera et al., 2007), decreases in C3 (Li et al., 2016) and an imbalance between C4 and C3 (Laskaris et al., 2019), and symptom severity. Our finding is consistent with the work of Morera et al (2007) but extends it to other complement components, suggesting that the overall amplification of the peripheral complement system in FEP is associated with clinical severity. The presence of this relationship in FEP, but not CHR, in the context of higher complement concentration in both cohorts, might indicate that higher complement expression leads to increased symptom severity as the illness progresses to frank psychosis. Nevertheless, this notion remains speculative and requires longitudinal studies in CHR to elucidate the temporal relationships between complement and clinical severity. Alternatively, interactions between stage of illness and unrelated demographic or illness-related factors may also explain the different relationships observed in FEP and CHR.

Strengths of the current study include the measurement of complement in both serum and CSF, as well as assessment of these in antipsychotic-naïve and CHR and FEP cohorts. Nevertheless, there are several limitations to be noted. First, the serum and CSF samples were stored for lengthy periods and the multiplex assay may have been suboptimal for detecting low protein concentrations. Therefore, further studies of complement proteins are required in CSF. Second, the sample size of each cohort was small, necessitating replication efforts or the creation of a consortium to ensure larger samples that are sufficiently powered. Third, we did not measure complement isotypes (e.g., C4A and C4B) and lacked genetic data to measure C4 copy number. Such information would be valuable given the association of the C4A isotype with schizophrenia diagnosis (Gracias et al., 2022; Sekar et al., 2016), and with increased synaptic engulfment in patient-derived cellular (Sellgren et al., 2019) and mouse (Yilmaz et al., 2021) models. Lastly, a further limitation of the study was the slightly higher than expected male distribution. We observed that this may affect C4, CFH and C3b/iC3b levels in CSF, but not serum.

In summary, we observe significant complement changes in serum when comparing HC with CHR and FEP, providing evidence for increased activity of the peripheral complement system in the psychosis spectrum. Activation of the peripheral complement system may have clinical implications, varying with clinical severity. These changes in serum are somewhat reflected by a trend observed in paired CSF samples. However, while the CSF findings are interesting, these cannot be critically assessed any further in relation with the serum findings due to several limitations of the study, including differences in storage times and lack of power between groups. Further longitudinal studies of the complement cascade in larger samples that measure a wider range of components, including regulatory proteins and activating fragments (such as with discovery or proteomic approaches), along with concurrent biological and neuroimaging measures of immune and neuronal-glia function, are needed to elucidate the temporal and pathway-specific relationships and pathophysiological mechanisms underlying complement dysregulation in the psychosis spectrum.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2023.07.004.

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