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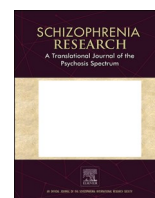
Complement component 4A protein levels are negatively related to frontal volumes in patients with schizophrenia spectrum disorders

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ABSTRACT

Background: Excessive *C4A*-gene expression may result in increased microglia-mediated synaptic pruning. As *C4A* overexpression is observed in schizophrenia spectrum disorders (SSD), this mechanism may account for the altered brain morphology (i.e. reduced volume and cortical thickness) and cognitive symptoms that characterize SSD. Therefore, this study investigates the association of C4A serum protein levels with brain morphology and cognition, and in particular whether this association differs between recent-onset SSD ($n = 69$) and HC ($n = 40$).
Methods: Serum C4A protein levels were compared between groups. Main outcomes included total gray matter volume, mean cortical thickness and cognitive performance. Regression analysis on these outcomes included C4A level, group (SSD vs. HC), and C4A*Group interactions. All statistical tests were corrected for age, sex, BMI, and antipsychotic medication dose. Follow-up analyses were performed on separate brain regions and scores on cognitive sub-tasks.
Results: The group difference in C4A levels was not statistically significant ($p = 0.86$). The main outcomes did not show a significant interaction effect ($p > 0.13$) or a C4A main effect ($p > 0.27$). Follow-up analyses revealed significant interaction effects for the left medial orbitofrontal and left frontal pole volumes ($p < 0.001$): C4A was negatively related to these volumes in SSD, but positively in HC.
Conclusion: This study demonstrated that C4A was negatively related to – specifically – frontal brain volumes in SSD, but this relation was inverse for HC. The results support the hypothesis of complement-mediated brain volume reduction in SSD. The results also suggest that C4A has a differential association with brain morphology in SSD compared to HC.

1. Introduction

Schizophrenia-spectrum disorders (SSD) are considered developmental disorders, with diagnosis typically at the debut of a first psychotic episode during (early) adulthood. Due to the considerable heterogeneity in both clinical and etiological factors in SSD patients, identifying the exact underlying mechanisms remains a challenge. The >100 associated risk genes suggest multiple underlying mechanisms that vary considerably between affected individuals. Yet, in accordance with the GWAS data of the psychiatric genomics consortium, the complement system is being increasingly studied as a promising pathophysiological factor in SSD.

The major histocompatibility complex (MHC) takes a prominent place in large genetic studies as the genetic locus most strongly associated with schizophrenia risk (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). The MHC locus contains several complement related genes, such as the complement component 4 (C4) gene isotypes *C4A* and *C4B*. The complement system is the first line of defence upon infections and is involved in opsonisation (i.e. tagging pathogens, cells and synapses for elimination), cell lysis and the promotion of inflammation (Hogenaar and van Bokhoven, 2021; Woo et al., 2020). C4A protein is associated with binding to immune complexes (i.e. antigen-bound antibody), and reduced levels skew towards development of immune complex mediated disease (Traustadottir et al., 2002). C4B protein, in contrast, is associated with binding to pathogens and deficiencies may increase vulnerability to bacterial infections (Jaatinen

et al., 2003). The level of *C4A* gene expression can be predicted based on gene copy number and structural genetic variant (i.e. short or long variants) (Sekar et al., 2016). Structural variations in alleles of the C4 gene were shown to result in increased *C4A* gene-expression in the brain which may drive the genetic association between the MHC locus and increased schizophrenia risk (Sekar et al., 2016). Predicted *C4A* expression also showed a positive relationship with positive symptom severity (Melbourne et al., 2018) and cognitive symptoms (Donohoe et al., 2018; Holland et al., 2020) in SSD. Post-mortem studies further corroborate the pathological role of complement, as brain tissue of individuals with SSD (*n* = 196) displayed an over-expression of total C4 in the stiatum, frontal and parietal cortex relative to healthy controls (HC) (Rey et al., 2020).

It is hypothesized that the role of complement in reduced synaptic spine density is mediated via microglia (i.e. resident immune-cells of the brain). Indeed, in an in vitro cell model with microglia-like cells and neurons of individuals with SSD, the *C4A* risk gene was associated with excessive synaptic pruning (Sellgren et al., 2019). Furthermore, recent preclinical studies reported that C4 over-expression in mouse brains results in increased microglial engulfment, decreased synaptic spine density, as well as the neurobiological (e.g. NMDA receptor hypofunction) and behavioural phenotypes (e.g. reduced working memory performance and social interaction) similar to SSD (Druart et al., 2021; Yilmaz et al., 2021). The most prominent neurobiological phenotypes of SSD include reduced brain volumes and cortical thinning (van Erp et al., 2018). These alterations precede the onset of psychosis and treatment with antipsychotics (Cannon et al., 2015; Ding et al., 2019). The reduction of volume and cortical thickness is associated with symptom severity, cognitive functioning, and disease outcome in SSD (Oomen et al., 2022; Walton et al., 2018).

The reduced brain volumes, cortical thinning, as well as cognitive symptoms in SSD may be linked to abnormal complement-mediated microglial synaptic pruning occurring during critical developmental periods (Germann et al., 2021; Schalbetter et al., 2022). The most recent genome wide association study also confirmed that schizophrenia was most strongly associated with genes that are involved in synaptic organization, differentiation and transmission (Trubetskoy et al., 2022). Other recent studies demonstrated significantly decreased levels of synaptic vesicle glycoprotein 2a (SV2a), which is indicative of lower synaptic density, in the frontal and anterior cingulate cortex of individuals with SSD compared to HC (Onwordi et al., 2020; Radhakrishnan et al., 2021). Reduced SV2a in frontal regions was also associated with positive symptoms and cognition in SSD (Radhakrishnan et al., 2021). Furthermore, genetic predisposition for high *C4A* expression has been shown to be related to high levels of a marker for microglial activity across the schizophrenia spectrum (*n* = 111) (Da Silva et al., 2021). These findings could be interpreted as excessive synaptic pruning in SSD as a result of abnormal microglia activity, which specifically affects the (frontal) cortex, but not the subcortical regions (Berdenis van Berlekom et al., 2019). Excessive synaptic pruning results in low synaptic density, which is considered to be reflected by reduced brain volumes and cortical thickness (Howes et al., 2023).

Only a handful of studies related *C4A* expression to brain morphology and cognition in HC (O'Connell et al., 2021) and SSD (Holland et al., 2020; Ji et al., 2021). In general, these studies conclude that high *C4A* expression and C4 protein levels are related to reduced cortical thickness and cognitive performance in HC and SSD (Ji et al., 2021; O'Connell et al., 2021). These prior studies were mainly performed in combined groups with HC and affected individuals (Da Silva et al., 2021; Holland et al., 2020; Ji et al., 2021), which obscures differentiation of the effects of C4 in each group. In addition, most studies were based on genetic predisposition for high *C4A* expression. However, environmental factors (e.g. infection, microbiome dysbiosis, trauma, obesity, smoking) influence the expression of complement genes and subsequent serum protein levels (Germann et al., 2021; Kim et al., 2021; Nimgaonkar et al., 2017; Severance et al., 2021). Studies that include

total C4 protein levels (i.e. combined C4A and C4B) are unable to assess the specific effects of the *C4A*-gene product (i.e. C4A protein).

Using isotype-specific enzyme-linked immunosorbent assays (ELISAs) (Wouters et al., 2009), we were able to specifically assess C4A protein levels in serum in SSD and HC. The current study aims to investigate the association of C4A serum protein levels with brain morphology (i.e. cortical thickness and brain volumes) and cognition, and specifically whether this association differs between SSD (*n* = 69) and HC (*n* = 40). Results from previous studies suggest that elevated C4A levels are reflective of SSD pathology and therefore we hypothesize that C4A levels will be negatively related to the main outcomes (overall cortical thickness, brain volumes and cognition), while this association will be less strong or even absent in HC. Follow-up analyses on individual brain regions and cognitive domains may show effects in the same direction. We hypothesized the strongest effects in the frontal cortex, as that region is associated with both C4 over-expression and lower synaptic density.

2. Methods

2.1. Participants

This study sample and data are part of the baseline measurements of a completed clinical trial (ClinicalTrails.gov:NCT01999309). Patients aged between 18 and 50 and diagnosed with schizophrenia, schizoaffective, schizophreniform disorder (295.x) or psychotic disorder not otherwise specified (298.9) within 3 years prior to screening were included. HCs had no prior or family history with psychiatric disorders. Participants without C4A protein levels or brain volumetric measurements were excluded from the analyses, which resulted in a dataset of 69 SSD patients and 40 HCs.

Table 1 shows demographic and clinical characteristics separately for SSD (*n* = 69) and HC (*n* = 40). Antipsychotic medication use at time of assessment was attained from patient report. Dose of antipsychotic medication intake (mg/day) was converted into an antipsychotic dose equivalent (i.e. chlorpromazine equivalent) for each patient (Gardner

Table 1
Demographic and clinical characteristics of the participants. BACS: Brief Assessment of Cognition in Schizophrenia; IQR: interquartile range; PANSS: Positive and Negative Syndrome Scale; SD: standard deviation; W: Wilcoxon test statistic. [1]Parental years of education was available for 55 SSD and all HC participants.

	SSD (*n* = 69)	HC (*n* = 40)	Statistics
Age, median (IQR), y	26 (10)	23 (5.25)	W = 1073, *p* = 0.053
Sex, F/M	15/54	8/32	$X^2(1) < 0.001$, *p* = 1.00
Parental education median (IQR)[1], y	13.5 (3.5)	14.5 (3)	W = 1275, *p* = 0.186
Education median (IQR), y	14 (3)	15 (3)	W = 1941.5, *p* = 0.0003
BMI median (IQR), kg/m²	23.9 (5.07)	23.0 (5.30)	W = 1258, *p* = 0.445
Antipsychotic dose equivalent, mean (SD), mg	310.6 (243.9)	–	
Duration of illness, mean (SD), y	1.16 (0.99)	–	
PANSS total score, mean (SD)	58.8 (13.2)	–	
BACS composite, standardized mean (SD)	-1.34 (1.15)	0.13 (1.15)	t(107) = 6.40, *p* = 4.1×10^{-9}
C4A, median (IQR), mg/L	312 (248)	357 (352.25)	W = 1429, p = 0.76
Total Gray matter volume, x10⁵ mm³	6.64 (0.68)	6.95 (0.58)	t(107) = 2.40, *p* = 0.018
Mean cortical thickness, mm	2.55 (0.10)	2.60 (0.09)	t(107) = 2.38, *p* = 0.019

et al., 2010). Educational attainment was provided in years of education, both from participants and the mean of both parents.

Cognitive performance was assessed with the Brief Assessment of Cognition in Schizophrenia (BACS). The BACS is comprised of six sub-tasks, which measure several cognitive domains most affected in schizophrenia: list learning (verbal memory), digit sequencing task (working memory), token motor task (motor speed), category instances and controlled oral word association test (verbal fluency), symbol coding (attention & information processing speed) and the tower of London (executive function). The scores of these subtasks were standardized and corrected for age and gender according to procedures described by Keefe et al. (Keefe et al., 2004). Standardized scores from all subtasks are averaged to calculate the composite BACS score, reflecting overall cognitive performance.

2.2. C4A protein measurements

Blood was drawn in the morning of the baseline measurements of the clinical trial (Begemann et al., 2015). Serum was prepared and stored at -80 °C in aliquots within 4 h after blood draw by the Central Biobank of the University Medical Center Utrecht. C4A levels were determined by Sanquin Diagnostic Services (Amsterdam, the Netherlands) using previously described isotype-specific enzyme-linked immunosorbent assays (Wouters et al., 2009). In short, the micotiterplates are coated with an antibody specific for C4A (clone C4-4; (Wouters et al., 2005)). Samples and controls are analyzed in three dilutions in duplo. Bound C4A is detected with a biotinylated sheep-anti-human C4c polyclonal antibody. Biotin is detected by streptavidin-HRP and the assay is developed with tetramethylbenzidine. Concentrations are determined using a standard line with known concentration and log-log transformation (Wouters et al., 2009). The quantified C4A concentrations (in mg/L) were log-transformed prior to further analyses.

2.3. MRI acquisition and processing

MRI data processing was described in a previous publication (Oomen et al., 2022). T1-weighted scans were acquired in the UMC Utrecht, using a 3 T Philips Ingenia CX and a 32-channel SENSE head-coil (3D T1-weighted Turbo Field Echo sequence; repetition time = 10 ms, echo-time = 4.6 ms, flip angle = 8°, reconstructed voxel size = $0.75 \times 0.75 \times 0.8$ mm^3, field of view = 240 mm $\times$ 240 mm $\times$ 160 mm). Brain volume and cortical thickness were calculated with the standard processing pipeline of FreeSurfer (version 6.0.1; http://surfer.nmr.mgh.harvard.edu/). T1 images were visually checked for errors in segmentation. We regarded the Euler number as a quantitative proxy of structural image quality. The Euler number reflects the topological complexity of the reconstructed cortical surface and is highly related to manual ratings of image quality (Rosen et al., 2018). FreeSurfer calculates the Euler number per hemisphere as part of the standard processing pipeline. The average Euler number of both hemispheres was used as a covariate for image quality in further analyses. Regions of interest (ROIs) included all regions from the Desikan-Killiany cortical atlas (Desikan et al., 2006) and FreeSurfer's subcortical atlas (Fischl et al., 2002). Brain volumes and cortical thickness of these areas were taken as a reflection of low synaptic density, since synaptic density is considered to be reflected more by brain volumes and cortical thickness, rather than cortical surface area or gyrification (Howes et al., 2023).

2.4. Statistical analyses

The difference in C4A levels between groups was analyzed using ANCOVA. This group comparison as well as all subsequent analyses included the following covariates: age, sex, BMI, and antipsychotic dose equivalent, as these factors have been previously related to C4A (Jaros et al., 2015; Kamitaki et al., 2020; Mohd Asyraf et al., 2022; Purves-Tyson et al., 2020; Ritchie et al., 2004).

Regression analyses included total gray matter volume, mean cortical thickness and BACS composite scores as main outcomes. Regression analyses on these three main outcome variables were performed including the covariates, C4A level, group (SSD vs. HC), and C4A*Group interaction terms. The interaction term was included to assess whether the relation between C4A and the outcomes differs between the groups (i.e. SSD vs. HC). Without a significant interaction, the main effect of C4A for both groups combined was reported. The regression analyses with the three main outcomes were evaluated at a significance threshold of α = 0.05.

For follow-up analyses, every ROI volume ($n = 103$), ROI thickness ($n = 70$), and score per cognitive task (6 BACS sub-scores), a separate regression analysis was performed with the same independent variables and covariates as the main analyses. Covariates for regression analysis on brain morphometry included handedness and mean Euler number. In addition, total intracranial volume was included as covariate in all volumetric analyses and mean cortical thickness was included as covariate in all thickness analyses. Analyses with cognitive outcomes included years of education as covariate. All follow-up regression analyses on subscores or ROIs were evaluated at a Bonferroni corrected significance threshold ($\alpha = 4.9 \times 10^{-4}$, 7.1×10^{-4} and 0.007, for brain volume, cortical thickness, and cognition, respectively).

3. Results

3.1. Group comparisons

The difference in log-transformed serum C4A levels between SSD (mean = 371 ± 217 mg/L) and HCs (mean = 373 ± 188 mg/L) was not statistically significant after controlling for the effect of covariates (F $(1,103) = 0.03$, $p = 0.86$). Fig. 1 shows the distribution of C4A between the groups. An non-parametric test on the untransformed C4A levels excluding covariates also showed no significant difference (W = 1429, $p = 0.76$; Table 1). C4A was not related to age (HC: r(38) = 0.13, $p = 0.40$, SSD: r(67) = 0.10, $p = 0.40$), sex (HC: t(10.3) = -0.38, $p = 0.71$, SSD: t (28.7) = 1.19, $p = 0.25$), BMI (HC: r(38) = 0.13, $p = 0.41$, SSD: r(67) = 0.15, $p = 0.23$), and antipsychotic dose equivalent (SSD: r(67) = 0.17, $p = 0.16$).
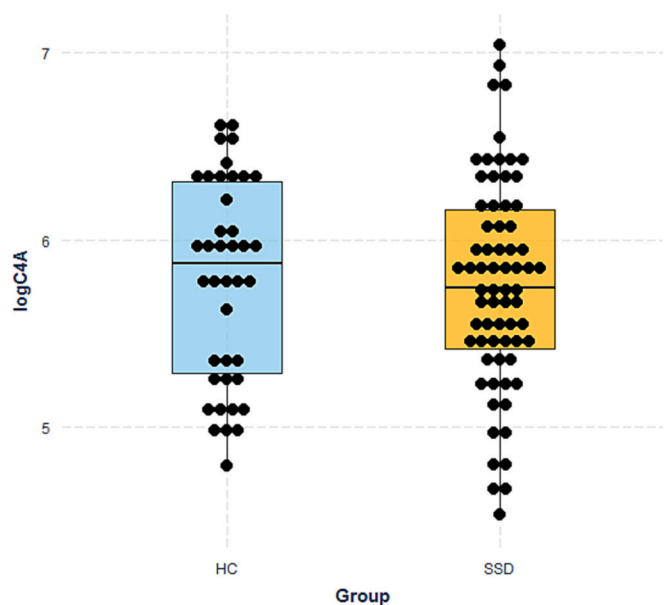

Fig. 1. Log-transformed C4A presence (mg/L) in serum of schizophrenia spectrum disorder (SSD) and healthy control (HC) groups.

3.2. Brain morphology

There was no significant C4A*Group interaction effect on either total gray matter volume (adj. $R^2 = 0.72$, $\beta = -0.16$, $p = 0.13$) or mean cortical thickness (adj. $R^2 = 0.24$, $\beta = -0.12$, $p = 0.49$). Main effect analyses of C4A on these two main outcome measurements did not reveal a significant effect as well (adj. $R^2 = 0.72$, $\beta = -0.03$, $p = 0.62$; adj. $R^2 = 0.24$, $\beta = 0.10$, $p = 0.27$, respectively).

The left medial orbitofrontal cortex (l-mOFC) volume ($R^2 = 0.51$, $\beta = -0.73$, $p = 1.7 \times 10^{-6}$; Fig. 2A) and left frontal pole volume ($R^2 = 0.41$, $\beta = -0.60$, $p = 2.5 \times 10^{-4}$; Fig. 2B) showed statistically significant C4A*Group interaction-effects (Bonferroni-corrected $\alpha = 4.9 \times 10^{-4}$). That is, while the relation between l-mOFC volume and C4A was negative for SSD ($\beta = -0.53$, $p = 0.001$), it was positive for HC ($\beta = 0.81$, $p = 2.1 \times 10^{-4}$). Similarly, whereas the relation between left frontal pole volume and C4A was negative for SSD ($\beta = -0.49$, $p = 0.007$), it was positive for HC ($\beta = 0.61$, $p = 0.01$). No other significant interaction effects ($p > 0.002$ and $p > 0.011$) or C4A main effects were observed for either volume or thickness analyses ($p > 0.005$ and $p > 0.006$, respectively).

The follow-up analyses resulted in effect sizes of the association between C4A and brain volumes (Fig. 3) and cortical thickness (Fig. 4), for HC (upper row), SSD (middle row) and both groups combined (lower row). It is interesting to note that C4A is mainly positively associated with brain volumes in HC (blue), but negatively associated with brain volumes in SSD (red). However, these effects did not reach statistical significance. Detailed statistical results for each ROI are presented in Supplementary Table 1 and 2.

3.3. Cognition

For the composite BACS score, there was no significant C4A *Group interaction (adj. $R^2 = 0.33$, $\beta = -0.02$, $p = 0.93$) or C4A main effect (adj. $R^2 = 0.30$, $\beta = 0.03$, $p = 0.73$). No significant C4A *Group interaction effect on executive functioning was found (adj. $R^2 = 0.12$, $\beta = 0.04$, $p = 0.85$). However, a positive C4A main effect on executive functioning in both groups combined was found (adj. $R^2 = 0.14$, $\beta = 0.26$, $p = 0.006$), yet the effect was not statistically significant considering bonferroni-corrected thresholds. Remaining individual cognitive subdomains assessed with the BACS did also not demonstrate significant interaction ($p > 0.15$) or C4A main effects ($p > 0.28$) (Supplementary Table 3). C4A does not moderate the relationship between relevant brain structures (i.e. total brain volume, mean cortical thickness, l-mOFC and the left frontal pole volume) and cognition, as three-way interactions between C4A, group and the brain structures were not statistically significant ($p > 0.43$). When taking both groups together, C4A also did not significantly moderate the relationship between the brain structures and composite BACS score (C4A*brain structure interaction; $p > 0.24$).

4. Discussion

The present study investigated the association between C4A serum protein levels, brain morphology (i.e. cortical thickness and brain volumes) and cognition as well as whether this association differs between SSD ($n = 69$) and HC ($n = 40$). First, there was no significant difference in C4A serum protein levels between the groups when controlling for age, sex and BMI. Next, C4A was not significantly related to cognition, total gray matter volume or cortical thickness. In ROI analyses, C4A levels showed a negative relation with mOFC and frontal pole volumes in SSD, but a positive relation in HC. These results suggest that peripheral levels of C4A may have a differential association with brain morphology in SSD compared to HC.

4.1. C4A group differences

The current study did not demonstrate a significant difference in C4A protein levels between SSD and HC participants. This finding is in accordance with a recent meta-analysis which reported no elevated total C4 protein levels in SSD compared to HC (Mongan et al., 2020). The

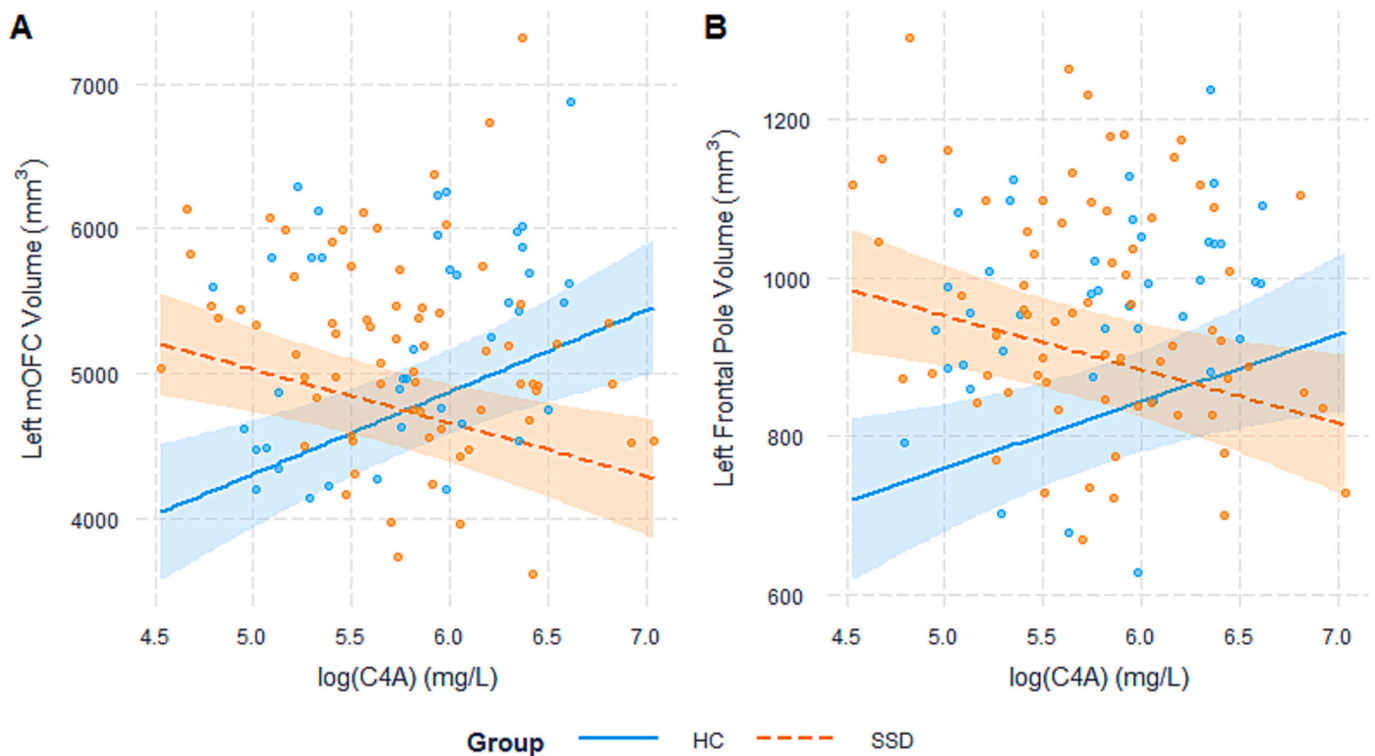**Fig. 2.** Interaction effects of C4A*Group for left medial orbitofrontal cortex (A) and left frontal pole (B). Estimated regression lines are corrected for covariates. The displayed datapoints are uncorrected. HC: healthy controls (blue); SSD: schizophrenia spectrum disorders (orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
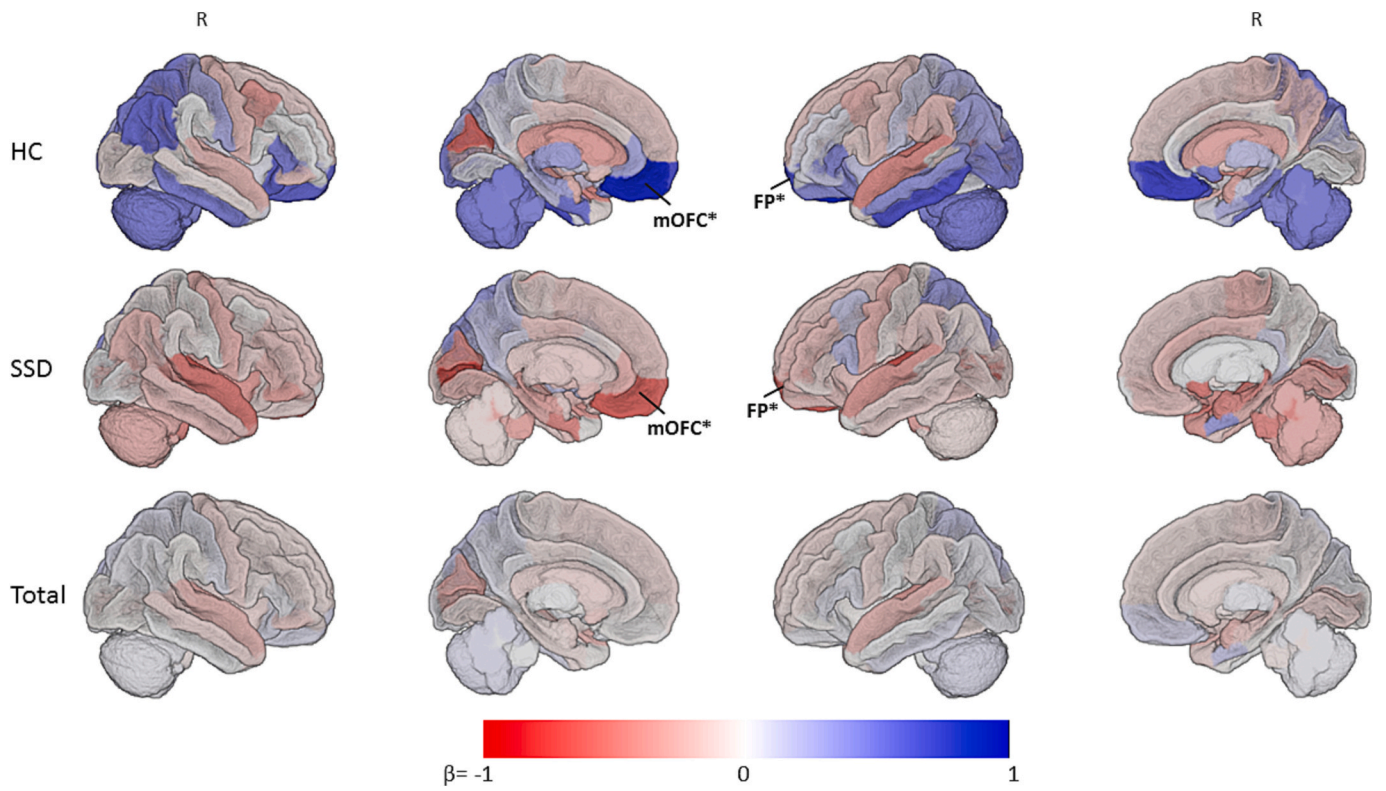
Fig. 3. Association between C4A and brain volumes for schizophrenia spectrum disorder (SSD), healthy control (HC), and both groups combined (Total). Negative effect sizes are indicated with red hues and positive effect sizes with blue hues. Brain regions with significant effects are indicated with an asterisk. FP: frontal pole; mOFC: lateral orbitofrontal cortex. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 4. Association between C4A and cortical thickness for schizophrenia spectrum disorder (SSD), healthy control (HC), and both groups combined (Total). Negative effect sizes are indicated with red hues and positive effect sizes with blue hues. No effect reached statistical significance after bonferroni correction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
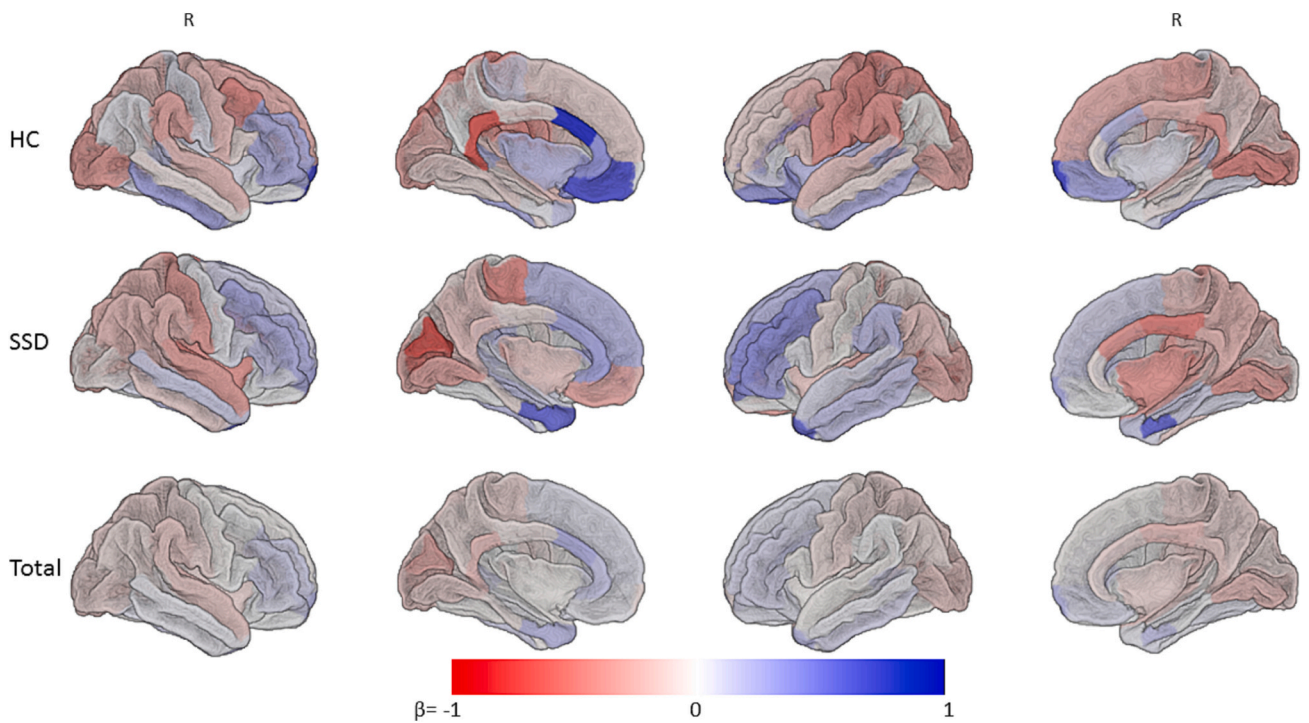
authors argue that this may potentially result from a lack of adequate control for confounding variables and a wide range of methods with insufficient sensitivity for the assessment of complement protein levels. The current investigation, however, accounted for these potential shortcomings by using methods specifically sensitive to C4A rather than total C4 protein levels, and by including relevant covariates in all analyses. Antipsychotics were not added as a covariate, since only the SSD group uses them and they did not correlate with C4A levels. Nevertheless, the potential effect that antipsychotics may have on the complement system are hard to ascertain, as studies are scarce and their results are conflicting (Woo et al., 2020). While there is some evidence that antipsychotics influence the complement system (Mongan et al., 2020), future (longitudinal) studies are required to determine the exact effects. Another important consideration is limited statistical power due to the naturally occurring wide range of C4 serum protein levels, as a result of genetic variation of the *C4* gene (Yang et al., 2003).

The absence of an overall group difference in C4 proteins may also be due to the heterogeneity of SSD. That is, C4 protein levels may not be elevated among first-episode psychosis FEP, but were significantly increased in chronic patients relative to HC (Laskaris et al., 2019). This may explain the findings of the current study, as we included recent-onset participants (mean time since diagnosis= 1.2 ± 0.99y). Furthermore, C4A protein levels were also elevated in a subgroup of SSD with elevated levels of peripheral cytokines (Ji et al., 2021), which further demonstrates increased complement levels in specific subgroups of SSD. Complement levels also showed to be predictive of treatment response (Mondelli et al., 2020; Susai et al., 2023) and transition from clinical high risk to first-episode psychosis (Mongan et al., 2021). Future studies should therefore further explore the clinical utility of complement proteins as biomarkers for transition and SSD disease course.

4.2. C4A and frontal volumes

The main finding of this study is the negative association between C4A serum protein levels and frontal volumes (i.e. mOFC and frontal pole) in SSD. According to recent preclinical and clinical studies, aberrant complement activity results in increased microglia-mediated synaptic pruning during critical developmental periods, which may underlie reduced brain volumes in SSD (Da Silva et al., 2021; Druart et al., 2021; Radhakrishnan et al., 2021; Sellgren et al., 2019; Yilmaz et al., 2021). In addition, a composite score of upregulated complement mRNA and complement protein was associated with reduced cortical thickness across SSD and HC (combined $n = 86$) (Ji et al., 2021). Based on these findings, a negative relation between C4A levels and brain volumes in SSD has been hypothesized. Although Fig. 3 of the current article suggests a negative association between C4A and volumes of regions across the brain in SSD, it only reached statistical significance for the mOFC and frontal pole and not for the total brain volume.

The frontal cortex is particularly affected by both SSD and complement pathology. For instance, post-mortem research in the dorsolateral prefrontal cortex shows C4 protein increases in the earliest stages of life (0-5y), which drops in teenagers (14-18y) and young adults (19-26y) (Sager et al., 2021). This drop of C4 levels also coincides with an increase in complement inhibitors (Ji et al., 2021; Sager et al., 2021). This C4 reduction may be present and possibly necessary for healthy neurodevelopment (Sager et al., 2021), whereas persistent C4 elevation could occur during a later developmental ages in SSD. Indeed, especially frontal regions display a higher rate of gray matter loss in clinical high risk who later convert to psychosis (Cannon et al., 2015).

C4A serum protein levels showed a statistically significant positive association with mOFC and frontal pole volumes in HC. However, O'Connell et al. (2021) reported a negative association between predicted C4A expression and mOFC thickness in undiagnosed UK biobank participants ($n > 10.000$). Yet, they also reported positive associations between predicted C4A expression and cortical surface area and cortical thickness in one third of their investigated brain regions (O'Connell

et al., 2021). The authors suggest that increased gray matter volume in combination with high C4A may be explained by altered cell migration and deficient synaptic pruning (O'Connell et al., 2021). As synaptic pruning in the frontal cortex persist throughout the third decade of life (Petanjek et al., 2011), altered or incomplete synaptic pruning in the young healthy adults in this study would indeed explain high levels of C4A that coincide with relatively large frontal cortical volumes. Alternatively, dependent on the isotype and structural form, C4 has both protective and deleterious effects (Sekar et al., 2016; Woo et al., 2020). In that case, the possible protective effects in HC (from the C4B gene and C4 short(*S*)-form) may outweigh the deleterious effects (from the C4A gene and the C4 long(L)-form). Support for this comes from findings demonstrating C4B/C4S to be protective of age-related white matter microstructural changes in undiagnosed UK biobank participants (Traylor et al., 2021). Ultimately, as C4B/C4S and C4A/C4L are inversely correlated (Woo et al., 2020), future efforts should be made to investigate the role of either one or the balance of both genotypes in SSD pathology.

4.3. C4A and cognition

While previous studies report a negative association between C4 expression and cognitive functioning, the current study did not find a significant relationship. In earlier work, elevated C4 expression was significantly related to poor fluid intelligence, visual memory, and processing speed in >80.000 HC (O'Connell et al., 2021) and to poor episodic memory in 141 HC and 503 SSD combined (Donohoe et al., 2018). However, the relation between episodic memory and C4 gene expression (Donohoe et al., 2018) was no longer significant when polygenic risk scores of other complement genes were included (Holland et al., 2020). Of these cognitive domains, only processing speed was part of the current study and demonstrated no associations with C4A levels in HC, SSD or in the groups combined.

Cognitive functioning and brain volumes of the present study sample were previously studied by Oomen et al. (2022). This study reported that 29 % of SSD patients in that sample ($n = 86$) showed a cognitive profile similar to HCs (Oomen et al., 2022). Especially executive functioning displayed the smallest differences between cognitive subgroups (Oomen et al., 2022). The present study also reported that C4A and executive functioning were positively related (C4A main effect), which did not remain after bonferroni-correction. This positive direction of this association may be a result of the relatively unaffected executive function in the group as a whole, since HC showed a positive relation between C4A and frontal volumes as well. As cognitive performance in SSD shows this heterogeneity, the absence of current significant effects in contrast to previous reports with large study samples may be due to a lack of sufficient statistical power.

4.4. Strengths and limitations

The use of C4A protein levels to investigate the relation between the complement system and the brain and cognition is novel. At the same time, this method could have formed a potential limitation since it is unknown to what extend peripheral proteins are a reflection of protein levels in the brain (Mongan et al., 2020) and no CSF was sampled for the current study to investigate this. The majority of studies used genetically predicted C4A levels instead of mRNA expression or protein levels of C4 (Da Silva et al., 2021; Donohoe et al., 2018; O'Connell et al., 2021). It is assumed that these predicted levels are reflective of C4A in the brain. Recently, however, genetically predicted C4A gene expression and C4A protein levels were found to be significantly related as well (Ji et al., 2021), which supports the use of either method. On the other hand, C4 protein in CSF and serum did not show a correlation (Gallego et al., 2021). Future studies on the most optimal, yet feasible, method to probe complement system activation in the brain would therefore be a great addition to the field.

Important to note is that the current as well as previous imaging studies assume reduced brain volume and thickness to be an indirect result of excessive synaptic pruning during the critical developmental period in SSD. Future studies may consider more specific proxies of synaptic pruning, such as molecular PET imaging of SV2a (Onwordi et al., 2020). Unsurprisingly, the contrasting effects of C4A on brain volume in SSD and HC in the present study resulted in no significant findings in the main C4A analyses with both groups combined. A previous study that combined SSD and HC ($n = 88$) also reported no significant associations between predicted C4A levels and brain morphometry (i.e. total brain volume, total cortical thickness or total surface area) (Holland et al., 2020). Furthermore, lack of statistical power is a limitation that prevented previous studies from stratifying their analyses based on clinical group. Results of the current study, however, demonstrated that the addition of the C4A *group interaction effect could yield interesting results.

As the morphological brain changes occur in the prodrome of psychosis (Cannon et al., 2015) and complement proteins could aid in the prediction of transition to psychosis (Mongan et al., 2021), associating the complement system in relation to brain morphology in SSD patients may not reflect the initial pathophysiological processes. However, as C4A protein levels are related to genotype (Ji et al., 2021), it is expected that SSD patients with relatively high C4A levels during adulthood, also showed this elevation in prodromal stages (Ji et al., 2021; Sager et al., 2021). The negative association between C4A and frontal volumes seen in SSD patients in this study, might thus be a reflection of earlier pathological processes. A strength of this study is that we included a sample with recent onset SSD, therefore the effects found are less obscured by the pathological processes that occur in chronic stages of SSD. Nevertheless, longitudinal studies with clinical high risk and SSD patients are warranted.

5. Conclusions

This study showed that C4A concentration in serum was negatively related to frontal brain volumes (i.e. mOFC and frontal pole volume) in SSD, but this relation was inverse for HC. These results suggest that peripheral levels of C4A may have a differential association with brain morphology in SSD compared to HC. The negative relation between C4A and frontal volumes support the hypothesis of complement-mediated microglial synaptic pruning as a pathological process in SSD.

Ethics approval statement (included in manuscript)

MRI acquisition and participant recruitment from Dutch mental healthcare settings were part of the Simvastatin and the Controls trial. These trials were approved by the research and ethics committee of the University Medical Center Utrecht (UMCU), the Netherlands, under protocol numbers: 13-249 and 14-572, respectively. These studies were conducted in compliance with the Declaration of Helsinki (2013).

Clinical trial registration (included in manuscript)

Simvastatin trial registration: ClinicalTrails.gov:NCT01999309; EudraCT-number:2013-000834-36

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Patient consent statement (included in manuscript)

All participants provided written informed consent prior to participation.

CRediT authorship contribution statement

S.S. Gangadin: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Project administration. **M. Germann:** Methodology, Validation, Writing – original draft. **L.D. de Witte:** Conceptualization, Resources, Writing – review & editing. **K.A. Gelderman:** Resources, Investigation, Writing – review & editing. **R.C.W. Mandl:** Methodology, Writing – review & editing, Supervision. **I.E.C. Sommer:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

The data that support the findings of this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2023.08.031.

References

Begemann, M.J.H., Schutte, M.J.L., Slot, M.I.E., Doorduin, J., Bakker, P.R., van Haren, N.E.M., Sommer, I.E.C., 2015. Simvastatin augmentation for recent-onset psychotic disorder: a study protocol. BBA Clin. 4, 52–58. https://doi.org/10.1016/j.bbacli.2015.06.007.

Berdenis van Berlekom, A., Muflihah, C.H., Snijders, G.J.L.J., MacGillavry, H.D., Middeldorp, J., Hol, E.M., Kahn, R.S., de Witte, L.D., 2019. Synapse pathology in schizophrenia: a meta-analysis of postsynaptic elements in postmortem brain studies. Schizophr. Bull. https://doi.org/10.1093/schbul/sbz060.

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 (2), 147–157. https://doi.org/10.1016/j.biopsych.2014.05.023.

Da Silva, T., Guma, E., Hafizi, S., Koppel, A., Rusjan, P., Kennedy, J.L., Chakravarty, M.M., Mizrahi, R., 2021. Genetically predicted brain C4A expression is associated with TSPO and hippocampal morphology. Biol. Psychiatry 90 (9), 652–660. https://doi.org/10.1016/j.biopsych.2021.06.021.

Desikan, R.S., Ségonne, F., Fischl, B., Quinn, B.T., Dickerson, B.C., Blacker, D., Buckner, R.L., Dale, A.M., Maguire, R.P., Hyman, B.T., Albert, M.S., Killiany, R.J., 2006. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. NeuroImage 31 (3), 968–980. https://doi.org/10.1016/j.neuroimage.2006.01.021.

Ding, Y., Ou, Y., Pan, P., Shan, X., Chen, J., Liu, F., Zhao, J., Guo, W., 2019. Brain structural abnormalities as potential markers for detecting individuals with ultra-

high risk for psychosis: a systematic review and meta-analysis. Schizophr. Res. 209, 22–31. https://doi.org/10.1016/j.schres.2019.05.015.

Donohoe, G., Holland, J., Mothersill, D., McCarthy-Jones, S., Cosgrove, D., Harold, D., Richards, A., Mantripragada, K., Owen, M.J., O'Donovan, M.C., Gill, M., Corvin, A., Morris, D.W., 2018. Genetically predicted complement component 4A expression: effects on memory function and middle temporal lobe activation. Psychol. Med. 48 (10), 1608–1615. https://doi.org/10.1017/S0033291717002987.

Druart, M., Nosten-Bertrand, M., Poll, S., Crux, N., Nebeling, F., Delhaye, C., Dubois, Y., Mittag, M., Leboyer, M., Tamouza, R., Fuhrmann, M., Le Magueresse, C., 2021. Elevated expression of complement C4 in the mouse prefrontal cortex causes schizophrenia-associated phenotypes. Mol. Psychiatry 26 (7), 3489–3501. https://doi.org/10.1038/s41380-021-01081-6.

Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., van der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., Dale, A.M., 2002. Whole brain segmentation. Neuron 33 (3), 341–355. https://doi.org/10.1016/S0896-6273(02)00569-X.

Gallego, J.A., Blanco, E.A., Morell, C., Lencz, T., Malhotra, A.K., 2021. Complement component C4 levels in the cerebrospinal fluid and plasma of patients with schizophrenia. Neuropsychopharmacology 46 (6), 1140–1144. https://doi.org/10.1038/s41386-020-00867-6.

Gardner, D.M., Murphy, A.L., O'Donnell, H., Centorrino, F., Baldessarini, R.J., 2010. International consensus study of antipsychotic dosing. Am. J. Psychiatr. 167 (6), 686–693. https://doi.org/10.1176/appi.ajp.2009.09060802.

Germann, M., Brederoo, S.G., Sommer, I.E.C., 2021. Abnormal synaptic pruning during adolescence underlying the development of psychotic disorders. Curr. Opin. Psychiatry 34 (3), 222–227. https://doi.org/10.1097/YCO.0000000000000696.

Hogenaar, J.T.T., van Bokhoven, H., 2021. Schizophrenia: complement cleaning or killing. Genes 12 (2). https://doi.org/10.3390/genes12020259.

Holland, J.F., Cosgrove, D., Whitton, L., Harold, D., Corvin, A., Gill, M., Mothersill, D.O., Morris, D.W., Donohoe, G., 2020. Effects of complement gene-set polygenic risk score on brain volume and cortical measures in patients with psychotic disorders and healthy controls. Am. J. Med. Genet. B Neuropsychiatr. Genet. 183 (8), 445–453. https://doi.org/10.1002/ajmg.b.32820.

Howes, O.D., Cummings, C., Chapman, G.E., Shatalina, E., 2023. Neuroimaging in schizophrenia: an overview of findings and their implications for synaptic changes. Neuropsychopharmacology 48 (1), 151–167. https://doi.org/10.1038/s41386-022-01426-x.

Jaatinen, T., Lahti, M., Ruuskanen, O., Kinos, R., Truedsson, L., Lahesmaa, R., Lokki, M.-L., 2003. Total C4B deficiency due to gene deletion and gene conversion in a patient with severe infections. Clin. Vaccine Immunol. 10 (2), 195–201. https://doi.org/10.1128/CDLI.10.2.195-201.2003.

Jaros, J.A., Rahmoune, H., Wesseling, H., Leweke, F.M., Ozcan, S., Guest, P.C., Bahn, S., 2015. Effects of olanzapine on serum protein phosphorylation patterns in patients with schizophrenia. PROTEOMICS Clin. Appl. 9 (9–10), 907–916. https://doi.org/10.1002/prca.201400148.

Ji, E., Boerrigter, D., Cai, H.Q., Lloyd, D., Bruggemann, J., O'Donnell, M., Gallety, C., Lloyd, A., Liu, D., Lenroot, R., Weickert, T., Shannon Weickert, C., 2021. Peripheral complement is increased in schizophrenia and inversely related to cortical thickness. Brain Behav. Immun. https://doi.org/10.1016/j.bbi.2021.11.014.

Kamitaki, N., Sekar, A., Handsaker, R.E., de Rivera, H., Tooley, K., Morris, D.L., Taylor, K.E., Whelan, C.W., Tombleson, P., Loohuis, L.M.O., Boehnke, M., Kimberly, R.P., Kaufman, K.M., Harley, J.B., Langefeld, C.D., Seidman, C.E., Pato, M.T., Pato, C.N., Ophoff, R.A., McCarroll, S.A., 2020. Complement genes contribute sex-biased vulnerability in diverse disorders. Nature 582 (7813), 577–581. https://doi.org/10.1038/s41586-020-2277-x.

Keefe, R.S.E., Goldberg, T.E., Harvey, P.D., Gold, J.M., Poe, M.P., Coughenour, L., 2004. The brief assessment of cognition in schizophrenia: reliability, sensitivity, and comparison with a standard neurocognitive battery. Schizophr. Res. 68 (2–3), 283–297. https://doi.org/10.1016/j.schres.2003.09.011.

Kim, M., Haney, J.R., Zhang, P., Hernandez, L.M., Wang, L., Perez-Cano, L., Loohuis, L.M.O., de la Torre-Ubieta, L., Gandal, M.J., 2021. Brain gene co-expression networks link complement signaling with convergent synaptic pathology in schizophrenia. Nat. Neurosci. https://doi.org/10.1038/s41593-021-00847-z.

Laskaris, L., Zalesky, A., Weickert, C.S., Di Biase, M.A., Chana, G., Baune, B.T., Bousman, C., Nelson, B., McGorry, P., Everall, I., Pantelis, C., Cropley, V., 2019. Investigation of peripheral complement factors across stages of psychosis. Schizophr. Res. 204, 30–37. https://doi.org/10.1016/j.schres.2018.11.035.

Melbourne, J.K., Rosen, C., Feiner, B., Sharma, R.P., 2018. C4A mRNA expression in PBMCs predicts the presence and severity of delusions in schizophrenia and bipolar disorder with psychosis. Schizophr. Res. 197, 321–327. https://doi.org/10.1016/j.schres.2018.01.018.

Mohd Asyraf, A.J., Nour El Huda, A.R., Hanisah, M.N., Norsidah, K.Z., Norlelawati, A.T., 2022. Relationship of selective complement markers with schizophrenia. J. Neuroimmunol. 363, 577793 https://doi.org/10.1016/j.jneuroim.2021.577793.

Mondelli, V., Di Forti, M., Morgan, B.P., Murray, R.M., Pariante, C.M., Dazzan, P., 2020. Baseline high levels of complement component 4 predict worse clinical outcome at 1-year follow-up in first-episode psychosis. Brain Behav. Immun. 88, 913–915. https://doi.org/10.1016/j.bbi.2020.01.014.

Mongan, D., Sabherwal, S., Susai, S.R., Föcking, M., Cannon, M., Cotter, D.R., 2020. Peripheral complement proteins in schizophrenia: a systematic review and meta-analysis of serological studies. Schizophr. Res. 222, 58–72. https://doi.org/10.1016/j.schres.2020.05.036.

Mongan, D., Föcking, M., Healy, C., Susai, S.R., Heurich, M., Wynne, K., Nelson, B., McGorry, P.D., Amminger, G.P., Nordentoft, M., Krebs, M.-O., Riecher-Rössler, A., Bressan, R.A., Barrantes-Vidal, N., Borgwardt, S., Ruhrmann, S., Sachs, G., Pantelis, C., van der Gaag, M., McGuire, P., 2021. Development of proteomic

prediction models for transition to psychotic disorder in the clinical high-risk state and psychotic experiences in adolescence. JAMA Psychiatry 78 (1), 77. https://doi.org/10.1001/jamapsychiatry.2020.2459.

Nimgaonkar, V.L., Prasad, K.M., Chowdari, K.V., Severance, E.G., Yolken, R.H., 2017. The complement system: a gateway to gene–environment interactions in schizophrenia pathogenesis. Mol. Psychiatry 22 (11), 1554–1561. https://doi.org/10.1038/mp.2017.151.

O'Connell, K.S., Sønderby, I.E., Frei, O., van der Meer, D., Athanasiu, L., Smeland, O.B., Alnæs, D., Kaufmann, T., Westlye, L.T., Steen, V.M., Andreassen, O.A., Hughes, T., Djurovic, S., 2021. Association between complement component 4A expression, cognitive performance and brain imaging measures in UK Biobank. Psychol. Med. 1–11 https://doi.org/10.1017/S0033291721000179.

Onwordi, E.C., Halff, E.F., Whitehurst, T., Mansur, A., Cotel, M.-C., Wells, L., Creeney, H., Bonsall, D., Rogdaki, M., Shatalina, E., Reis Marques, T., Rabiner, E.A., Gunn, R.N., Natesan, S., Vernon, A.C., Howes, O.D., 2020. Synaptic density marker SV2A is reduced in schizophrenia patients and unaffected by antipsychotics in rats. Nat. Commun. 11 (1), 246. https://doi.org/10.1038/s41467-019-14122-0.

Oomen, P.P., Gangadin, S.S., Begemann, M.J.H., Visser, E., Mandl, R.C.W., Sommer, I.E. C., 2022. The neurobiological characterization of distinct cognitive subtypes in early-phase schizophrenia-spectrum disorders. Schizophr. Res. 241, 228–237. https://doi.org/10.1016/j.schres.2022.02.006.

Petanjek, Z., Judaš, M., Šimić, G., Rašin, M.R., Uylings, H.B.M., Rakic, P., Kostović, I., 2011. Extraordinary neoteny of synaptic spines in the human prefrontal cortex. Proc. Natl. Acad. Sci. 108 (32), 13281–13286. https://doi.org/10.1073/pnas.1105108108.

Purves-Tyson, T.D., Robinson, K., Brown, A.M., Boerrigter, D., Cai, H.Q., Weissleder, C., Owens, S.J., Rothmond, D.A., Shannon Weickert, C., 2020. Increased macrophages and C1qA, C3, C4 transcripts in the midbrain of people with schizophrenia. Front. Immunol. 11 https://doi.org/10.3389/fimmu.2020.02002.

Radhakrishnan, R., Skosnik, P.D., Ranganathan, M., Naganawa, M., Toyonaga, T., Finnema, S., Hillmer, A.T., Esterlis, I., Huang, Y., Nabulsi, N., Carson, R.E., D'Souza, D.C., 2021. In vivo evidence of lower synaptic vesicle density in schizophrenia. Mol. Psychiatry 26 (12), 7690–7698. https://doi.org/10.1038/s41380-021-01184-0.

Rey, R., Suaud-Chagny, M.-F., Bohec, A.-L., Dorey, J.-M., D'Amato, T., Tamouza, R., Leboyer, M., 2020. Overexpression of complement component C4 in the dorsolateral prefrontal cortex, parietal cortex, superior temporal gyrus and associative striatum of patients with schizophrenia. Brain Behav. Immun. 90, 216–225. https://doi.org/10.1016/j.bbi.2020.08.019.

Ritchie, R.F., Palomaki, G.E., Neveux, L.M., Navolotskaia, O., Ledue, T.B., Craig, W.Y., 2004. Reference distributions for complement proteins C3 and C4: a practical, simple and clinically relevant approach in a large cohort. J. Clin. Lab. Anal. 18 (1), 1–8. https://doi.org/10.1002/jcla.10100.

Rosen, A.F.G., Roalf, D.R., Ruparel, K., Blake, J., Seelaus, K., Villa, L.P., Ciric, R., Cook, P. A., Davatzikos, C., Elliott, M.A., Garcia de La Garza, A., Gennatas, E.D., Quarmley, M., Schmitt, J.E., Shinohara, R.T., Tisdall, M.D., Craddock, R.C., Gur, R. E., Gur, R.C., Satterthwaite, T.D., 2018. Quantitative assessment of structural image quality. NeuroImage 169, 407–418. https://doi.org/10.1016/j.neuroimage.2017.12.059.

Sager, R.E.H., Walker, A.K., Middleton, F., Robinson, K., Webster, M.J., Weickert, C.S., 2021. Trajectory of change in brain complement factors from neonatal to young adult humans. J. Neurochem. 157 (3), 479–493. https://doi.org/10.1111/jnc.15241.

Schalbetter, S.M., von Arx, A.S., Cruz-Ochoa, N., Dawson, K., Ivanov, A., Mueller, F.S., Lin, H.-Y., Amport, R., Mildenberger, W., Mattei, D., Beule, D., Földy, C., Greter, M., Notter, T., Meyer, U., 2022. Adolescence is a sensitive period for prefrontal microglia to act on cognitive development. *Science*. Advances 8 (9), eabi6672. https://doi.org/10.1126/sciadv.abi6672.

Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014. Biological insights from 108 schizophrenia-associated genetic loci. Nature 511 (7510), 421–427. https://doi.org/10.1038/nature13595.

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 530 (7589), 177–183. https://doi.org/10.1038/nature16549.

Sellgren, C.M., Gracias, J., Watmuff, B., Biag, J.D., Thanos, J.M., Whittredge, P.B., Fu, T., Worringer, K., Brown, H.E., Wang, J., Kaykas, A., Karmacharya, R., Goold, C.P., Sheridan, S.D., Perlis, R.H., 2019. Increased synapse elimination by microglia in schizophrenia patient-derived models of synaptic pruning. Nat. Neurosci. 22 (3), 374–385. https://doi.org/10.1038/s41593-018-0334-7.

Severance, E.G., Leister, F., Lea, A., Yang, S., Dickerson, F., Yolken, R.H., 2021. Complement C4 associations with altered microbial biomarkers exemplify gene-by-environment interactions in schizophrenia. Schizophr. Res. 234, 87–93. https://doi.org/10.1016/j.schres.2021.02.001.

Susai, S.R., Föcking, M., Mongan, D., Heurich, M., Coutts, F., Egerton, A., Whetton, T., Winter-van Rossum, I., Unwin, R.D., Pollak, T.A., Weiser, M., Leboyer, M., Rujescu, D., Byrne, J.F., Gifford, G.W., Dazzan, P., Koutsouleris, N., Kahn, R.S., Cotter, D.R., McGuire, P., 2023. Association of complement and coagulation pathway proteins with treatment response in first-episode psychosis: a longitudinal analysis of the OPTiMiSE clinical trial. Schizophr. Bull. https://doi.org/10.1093/schbul/sbac201.

Traustadottir, K.H., Sigfusson, A., Steinsson, K., Erlendsson, K., 2002. C4A deficiency and elevated level of immune complexes: the mechanism behind increased susceptibility to systemic lupus erythematosus. J. Rheumatol. 29 (11), 2359–2366.

Traylor, M., Malik, R., Gesierich, B., Dichgans, M., 2021. The BS variant of C4 protects against age-related loss of white matter microstructural integrity. Brain. https://doi.org/10.1093/brain/awab261.

Trubetskoy, V., Pardiñas, A.F., Qi, T., Panagiotaropoulou, G., Awasthi, S., Bigdeli, T.B., Bryois, J., Chen, C.-Y., Dennison, C.A., Hall, L.S., Lam, M., Watanabe, K., Frei, O., Ge, T., Harwood, J.C., Koopmans, F., Magnusson, S., Richards, A.L., Sidorenko, J., van Os, J., 2022. Mapping genomic loci implicates genes and synaptic biology in schizophrenia. Nature 604 (7906), 502–508. https://doi.org/10.1038/s41586-022-04434-5.

van Erp, T.G.M., Walton, E., Hibar, D.P., Schmaal, L., Jiang, W., Glahn, D.C., Pearlson, G.D., Yao, N., Fukunaga, M., Hashimoto, R., Okada, N., Yamamori, H., Bustillo, J.R., Clark, V.P., Agartz, I., Mueller, B.A., Cahn, W., de Zwarte, S.M.C., Hulshoff Pol, H.E., Orhan, F., 2018. Cortical brain abnormalities in 4474 individuals with schizophrenia and 5098 control subjects via the enhancing neuro imaging genetics through meta analysis (ENIGMA) consortium. Biol. Psychiatry 84 (9), 644–654. https://doi.org/10.1016/j.biopsych.2018.04.023.

Walton, E., Hibar, D.P., van Erp, T.G.M., Potkin, S.G., Roiz-Santiañez, R., Crespo-Facorro, B., Suarez-Pinilla, P., van Haren, N.E.M., de Zwarte, S.M.C., Kahn, R.S., Cahn, W., Doan, N.T., Jørgensen, K.N., Gurholt, T.P., Agartz, I., Andreassen, O.A., Westlye, L.T., Melle, I., Berg, A.O., Ehrlich, S., 2018. Prefrontal cortical thinning links to negative symptoms in schizophrenia via the ENIGMA consortium. Psychol. Med. 48 (1), 82–94. https://doi.org/10.1017/S0033291717001283.

Woo, J.J., Pouget, J.G., Zai, C.C., Kennedy, J.L., 2020. The complement system in schizophrenia: where are we now and what's next? Mol. Psychiatry 25 (1), 114–130. https://doi.org/10.1038/s41380-019-0479-0.

Wouters, D., Wiessenberg, H.D., Hart, M., Bruins, P., Voskuyl, A., Daha, M.R., Hack, C.E., 2005. Complexes between C1q and C3 or C4: novel and specific markers for classical complement pathway activation. J. Immunol. Methods 298 (1–2), 35–45. https://doi.org/10.1016/j.jim.2004.12.018.

Wouters, D., van Schouwenburg, P., van der Horst, A., de Boer, M., Schooneman, D., Kuijpers, T.W., Aarden, L.A., Hamann, D., 2009. High-throughput analysis of the C4 polymorphism by a combination of MLPA and isotype-specific ELISA's. Mol. Immunol. 46 (4), 592–600. https://doi.org/10.1016/j.molimm.2008.07.028.

Yang, Y., Chung, E.K., Zhou, B., Blanchong, C.A., Yu, C.Y., Füst, G., Kovács, M., Vatay, Á., Szalai, C., Karádi, I., Varga, L., 2003. Diversity in intrinsic strengths of the human complement system: serum C4 protein concentrations correlate with C4 gene size and polygenic variations, hemolytic activities, and body mass index. J. Immunol. 171 (5), 2734–2745. https://doi.org/10.4049/jimmunol.171.5.2734.

Yilmaz, M., Yalcin, E., Presumey, J., Aw, E., Ma, M., Whelan, C.W., Stevens, B., McCarroll, S.A., Carroll, M.C., 2021. Overexpression of schizophrenia susceptibility factor human complement C4A promotes excessive synaptic loss and behavioral changes in mice. Nat. Neurosci. 24 (2), 214–224. https://doi.org/10.1038/s41593-020-00763-8.