Cerebrospinal fluid concentration of complement component 4A is increased in first episode schizophrenia

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Postsynaptic density is reduced in schizophrenia, and risk variants increasing complement component 4A (C4A) gene expression are linked to excessive synapse elimination. In two independent cohorts, we show that cerebrospinal fluid (CSF) C4A concentration is elevated in patients with first-episode psychosis (FEP) who develop schizophrenia (FEP-SCZ: median 0.41 fmol/ul [CI = 0.34 – 0.45], FEP-non-SCZ: median 0.29 fmol/ul [CI = 0.22 – 0.35], healthy controls: median 0.28 [CI = 0.24 – 0.33]). We show that the CSF elevation of C4A in FEP-SCZ exceeds what can be expected from genetic risk variance in the C4 locus, and in patient-derived cellular models we identify a mechanism dependent on the disease-associated cytokines interleukin (IL)–1beta and IL-6 to selectively increase neuronal C4A mRNA expression. In patient-derived CSF, we confirm that IL-1beta correlates with C4A controlled for genetically predicted C4A RNA expression (r = 0.39; CI: 0.01–0.68). These results suggest a role of C4A in early schizophrenia pathophysiology.

Schizophrenia (SCZ) is a highly heritable and polygenic brain disorder with the strongest associated SCZ locus located close to the complement component 4 (C4) genes. Distinct from mouse, human C4 is encoded by two closely related genes, C4A and C4B, then typically present in multiple copy numbers (CNs) per genome and gene isotype. SCZ risk attributed to this locus can largely be explained by genetically predicted C4A RNA expression, and elevated C4A expression has been confirmed in SCZ postmortem brain tissue2. During brain development, microglia utilize complement signaling for selective removal of supernumerary synapses by complement receptor 3 (C3R)-dependent phagocytosis3,5. In line with the observed decrease in synapse density in SCZ4,5, excessive microglial synapse elimination has been observed in SCZ-derived in vitro models6. C4A CNs also correlate with complement deposition in patient-derived models, as well as complement-dependent microglial engulfment of synaptic structures7. Notably, C4B CNs, not linked to SCZ risk8, do not influence neuronal complement deposition or synapse elimination in these models6. More recently, these in vitro findings were also confirmed in vivo using mouse models overexpressing mouse C4 or human C4A8.

Despite the accumulating evidence from experimental models linking C4A to excessive synapse removal in SCZ, data showing increased in vivo protein levels in patients has been lacking. One
reason is that these measurements have been limited by difficulties in distinguishing C4A and C4B as the peptide sequence only differ by a few amino acids. In the present study, we developed a targeted mass spectrometry method capable of detecting unique peptide sequences in C4A and C4B protein. We applied this method to human cerebrospinal fluid (CSF); collected from two independent cohorts of patients with first-episode psychosis (FEP), hypothesizing that patients with FEP who develop schizophrenia (FEP-SCZ) would display elevated CSF C4A concentrations as compared to healthy controls (HCs; n = 20) or patients with FEP who did not develop schizophrenia (FEP-nSCZ; n = 15) (HCs: 0.28 fmol/ul; 95% confidence interval [CI] = 0.24–0.33, FEP-nSCZ: 0.29 fmol/ul; CI = 0.22–0.35, FEP-SCZ: 0.41; CI = 0.34–0.45, adjusted P (FEP-SCZ vs. HCs) = 0.025, adjusted P (FEP-SCZ vs. FEP-nSCZ)=0.037); replication cohort (GRIP), patients with FEP-SCZ (n = 17) displayed significantly higher CSF C4A concentrations as compared to HCs (n = 23) or patients with FEP-nSCZ (n = 13) (HC: 0.34 fmol/ul; CI: 0.31–0.47, FEP-nSCZ: 0.28 fmol/ul; CI: 0.25–0.33, FEP-SCZ: 0.50 fmol/ul; CI: 0.41–0.56, adjusted P (FEP-SCZ vs. HCs) = 0.047, adjusted P (FEP-SCZ vs. FEP-nSCZ) = 0.001), while (e) CSF C4B concentrations were similar across groups (HCs [n = 21]: 0.51 fmol/ul; CI: 0.44–0.63, FEP-nSCZ [n = 14]: 0.56 fmol/ul; CI: 0.47–0.72, FEP-SCZ [n = 17]: 0.55 fmol/ul; CI: 0.41–0.65, adjusted P (FEP-SCZ vs. HCs) = 0.999, adjusted P (FEP-SCZ vs. FEP-nSCZ) = 0.708). Bar graphs represent medians and error bars represent 95% CIs. Data were analyzed using Kruskal-Wallis H tests followed by post-hoc tests. Significance was set to P < 0.05. All reported p-values are two-sided. Figure 1a was created with BioRender.com. Source data for graphs in Fig. 1b–e are provided in the Source Data file. 

Fig. 1a. CSF C4B concentrations were more similar across groups (HCs: 0.43 fmol/ul; CI: 0.36–0.57, FEP-nSCZ: 0.38 fmol/ul; CI = 0.30–0.49, FEP-SCZ [n = 28]: 0.46 fmol/ul; CI = 0.44–0.58, adjusted P (FEP-SCZ vs. HCs)=0.550, adjusted P (FEP-SCZ vs. FEP-nSCZ)=0.098). b) In the replication cohort (GRIP), patients with FEP-SCZ (n = 17) displayed significantly higher CSF C4A concentrations as compared to HCs (n = 23) or patients with FEP-nSCZ (n = 13) (HC: 0.34 fmol/ul; CI: 0.31–0.47, FEP-nSCZ: 0.28 fmol/ul; CI: 0.25–0.33, FEP-SCZ: 0.50 fmol/ul; CI: 0.41–0.56, adjusted P (FEP-SCZ vs. HCs) = 0.047, adjusted P (FEP-SCZ vs. FEP-nSCZ) = 0.001), while (e) CSF C4B concentrations were similar across groups (HCs [n = 21]: 0.51 fmol/ul; CI: 0.44–0.63, FEP-nSCZ [n = 14]: 0.56 fmol/ul; CI: 0.47–0.72, FEP-SCZ [n = 17]: 0.55 fmol/ul; CI: 0.41–0.65, adjusted P (FEP-SCZ vs. HCs) = 0.999, adjusted P (FEP-SCZ vs. FEP-nSCZ) = 0.708). Bar graphs represent medians and error bars represent 95% CIs. Data were analyzed using Kruskal-Wallis H tests followed by post-hoc tests. Significance was set to P < 0.05. All reported p-values are two-sided. Figure 1a was created with BioRender.com. Source data for graphs in Fig. 1b–e are provided in the Source Data file.

Results

Patients with schizophrenia display elevated CSF C4A concentrations

In the first cohort (Karolinska Schizophrenia Project; KaSP) we included 44 patients with FEP and 20 age- and sex-matched HCs. Patients with FEP were stratified depending on if they subsequently developed schizophrenia (FEP-SCZ; n = 29) or not (FEP-nSCZ; n = 15). The three groups (HCs and two patient groups) displayed no significant differences in terms of demographics and clinical characteristics (Supplementary Table 1). We observed significantly higher CSF C4A concentrations in patients with FEP-SCZ as compared to HCs or patients with FEP-nSCZ (HCS: 0.28 fmol/ul; 95% confidence interval [CI] = 0.24–0.33, FEP-nSCZ: 0.29 fmol/ul; CI = 0.22–0.35, FEP-SCZ: 0.41; CI = 0.34–0.45, adjusted P (FEP-SCZ vs. HCs) = 0.025, adjusted P (FEP-SCZ vs. FEP-nSCZ) = 0.037);
Elevation of CSF C4A exceeds genetically predicted C4A RNA expression

Despite a strong correlation between C4A CNs and C4A RNA expression in the brain, previous studies suggest that upregulation of C4A expression in SCZ exceeds what can be expected from genetic risk variance in the C4 locus. To test this at the protein level, we used droplet digital PCR (ddPCR) to measure C4A CNs and HERV insertions in patients with FEP that had available DNA and CSF C4A protein levels in the GRIP cohort (n = 22 FEP and n = 21 HCs). We observed a moderate correlation between C4A CNs and CSF C4A protein levels (r = 0.48; P = 0.012), as well as between genetically predicted C4A RNA expression and CSF C4A protein levels (r = 0.44; CI: 0.16–0.65; P = 0.003; Supplementary Fig. 3). The correlation between C4A CNs and CSF C4B protein levels was weaker and did not reach significance in this sample (r = 0.19; CI: 0.11–0.46; P = 0.205; Supplementary Fig. 3). As a sensitivity analysis, we then re-measured CSF concentrations of another C4B peptide and observed a similar and non-significant correlation to C4B CNs as in the original analysis (r = 0.22; CI: 0.08–0.49; P = 0.146; Supplementary Fig. 3). This suggests that CSF C4A protein levels are under a stricter genetic control from the C4 locus than CSF C4B protein levels. With available C4A CNs, as measured by ddPCR, we then adjusted the original analysis, comparing CSF C4A concentration across groups, for C4A CNs. CSF C4A

Table 1 | Correlations of CSF C4A and C4B concentration to the Positive and Negative Syndrome Scale (PANSS) scores, and performance in the key domains of the MATRICS Consensus Cognitive Battery (MCCB)

| Sub-scale | C4A | C4B |
|-----------|-----|-----|
| Positive sub-scale | r = 0.45, | r = 0.45, |
| &nbsp; | N = 15, 27 | N = 15, 27 |
| Negative sub-scale | r = 0.15, | r = 0.15, |
| &nbsp; | N = 15, 27 | N = 15, 27 |
| General sub-scale | r = 0.25, | r = 0.25, |
| &nbsp; | N = 15, 27 | N = 15, 27 |
| MATRICS Consensus Cognitive Battery (MCCB) | | |
| TMT | r = 0.15, | P = 0.32, |
| &nbsp; | N = 15, 27 | N = 15, 27 |
| BACS-SC | r = 0.15, | P = 0.71, |
| &nbsp; | N = 15, 27 | N = 15, 27 |
| HVLT-R | r = 0.15, | P = 0.56, |
| &nbsp; | N = 15, 27 | N = 15, 27 |
| WMS III SS | r = 0.15, | P = 0.32, |
| &nbsp; | N = 15, 27 | N = 15, 27 |
| LNS | r = 0.15, | P = 0.97, |
| &nbsp; | N = 15, 27 | N = 15, 27 |
| NAB-MAZES | r = 0.15, | P = 0.56, |
| &nbsp; | N = 15, 27 | N = 15, 27 |
| BVMT-R | r = 0.15, | P = 0.32, |
| &nbsp; | N = 15, 27 | N = 15, 27 |
| Fluency | r = 0.15, | P = 0.73, |
| &nbsp; | N = 15, 27 | N = 15, 27 |
| MSCEIT ME | r = 0.15, | P = 0.58, |
| &nbsp; | N = 15, 27 | N = 15, 27 |
| CPT-IT | r = 0.15, | P = 0.78, |
| &nbsp; | N = 15, 27 | N = 15, 27 |

Data were generated from a total of 44 patients in the KaSP cohort with available data (median age = 24; interquartile range: 22–33; 16 females and 28 males). Number of included subjects is given in the table for each analysis. Correlations were performed using Spearman correlation analyses (two-sided P values corrected for multiple testing using Benjamini-Hochberg false discovery rate correction).
protein levels per C4A CN (as well as predicted C4A RNA expression) then remained significantly elevated in patients with FEP-SCZ as compared to patients with FEP-nSCZ and HCs (HC: HCs: n = 21 median = 0.17, CI: 0.15–0.21 and median = 0.32, CI: 0.27–0.38; respectively, FEP-nSCZ: n = 10 median = 0.16, CI: 0.13–0.20 and median = 0.29, CI: 0.24–0.33; respectively, FEP-SCZ: n = 12 median = 0.23, CI: 0.19–0.28 and median = 0.46, CI: 0.35–0.56; respectively, P (FEP-SCZ vs. HCs) = 0.039 and 0.031 respectively, P (FEP-SCZ vs. FEP-nSCZ) = 0.029 and 0.020 respectively; Supplementary Fig. 4), then confirming that factors independent of genetic risk variance within the C4 locus contributes to the increase of CSF C4A protein levels in SCZ. However, CSF C4B protein levels were not significantly different when adjusting for copy numbers (as well as predicted C4B RNA expression) between FEP-SCZ and HC or FEP-nSCZ (Supplementary Fig. 4).

Disease-associated cytokines increases C4A mRNA expression in patient-derived cellular models

Recent brain co-expression network analyses42, using C4A as a seed gene, have revealed an enrichment of genes involved in immune processes, such as NFκB signaling, rather than other complement system genes, and experimental studies suggest that cytokines can modulate the expression of the C4 genes, although in a tissue-dependent fashion and with a variable effect on C4A versus C4B expression33,34. The pro-inflammatory cytokines, interleukin(IL)-beta and IL-6 both have repeatedly been shown to be elevated in CSF of patients with SCZ8–10, and we therefore explored their influence on neuronal C4A expression.

To this end, we generated stably inducible neurogenin 2 (NGN2) neural progenitor cells from induced pluripotent stem cell (iPSC) of four patients and differentiated these cells to cortical excitatory neurons. These derived cultures were then stimulated for 24 hours with either IL-1beta (1 ng/ml), IL-6 (1 ng/ml), or a combination of IL-1beta and IL-6 (Fig. 2a). We observed significantly increased neuronal mRNA expression of C4A as measured by qPCR in cultures stimulated with a combination of IL-1beta and IL-6 as compared to vehicle (VEH) stimulated cultures from the same patients (Fig. 2b), while no significant effect was evident on the relative C4B mRNA expression (Fig. 2c), then suggesting that the IL1beta/IL-6 combination foremost induces C4A mRNA expression. However, the relative increase in mRNA expression by IL-1beta/IL-6 induction could theoretically vary by CNs for the corresponding C4 gene. Thus, we specifically looked at the data from one of the patient-derived line (across three independent experiments) that had been genotyped for C4 haplotypes (ddPCR) and had equal CNs of C4A and C4B (two CNs for each gene). The significant increase in relative C4A mRNA expression after IL1beta/IL-6 stimulation remained when only analyzing cultures from this line (VEH: median (fold change) = 1.0, CI:0.66–1.5, IL-1beta: median = 0.98, CI:0.50–1.7, IL-6: median = 2.1, CI:1.24, IL1beta/IL-6: median = 2.5, CI:2.0–3.5, Kruskal-Wallis H test: adjusted P (VEH vs. IL-1beta) = 0.999, adjusted P (VEH vs. IL-6) = 0.285, and adjusted P (VEH vs. IL1beta/IL-6) = 0.004), while no significant increase was observed for C4B mRNA expression after IL1beta/IL-6 stimulation (VEH: median = 1.0, CI:0.63–1.5, IL-1beta: median = 1.2, CI: 0.71–2.1, IL-6: median = 1.9, CI: 0.85–3.0, IL1beta/IL-6: median = 2.4, CI: 1.2–3.7, Kruskal–Wallis H test: adjusted P (VEH vs. IL-1beta) = 0.999, adjusted P (VEH vs. IL-6) = 0.411, and adjusted P (VEH vs. IL1beta/IL-6) = 0.094).

IL-1beta correlates with C4A in CSF of patients with schizophrenia

To corroborate this observation in a clinical context, and to determine the relative importance of each cytokine in clinically relevant concentrations, we measured CSF IL-1beta and IL-6 in 26 patients with FEP and available CSF (Kasp cohort; Fig. 3a). Controlling for genetically predicted C4A RNA expression using whole genome sequencing data1, we observed a significant correlation between IL-1beta and CSF C4A concentration (r = 0.39; CI: 0.01–0.68; P = 0.047; Fig. 3b), while IL-6 did not significantly influence C4A levels (r = −0.047; CI: −0.42–0.34; P = 0.820; Fig. 3c). In contrast, there were no significant correlations between these two cytokines and C4B concentrations in CSF (r = 0.29; CI: −0.11–0.61; P = 0.150; Fig. 3d, and r = 0.32 CI: −0.09–0.64; P = 0.112; Fig. 3e, respectively). This suggests that an IL-1beta/IL-6 dependent mechanism, detected in our in vitro model and predominately influencing C4A mRNA expression, is also present in an in vivo context, with variance in vivo CSF levels of IL-1beta influencing C4A protein levels independent of C4A CNs (see Supplementary Fig. 5 for a comparison of the elevation of CSF IL-1beta and C4A in patients with FEP-SCZ as compared to patients to FEP-SCZ).

C4A correlates inversely with measurements of synapse density in CSF

As C4A CNs predict synaptic complement deposition and synaptic pruning in experimental patient-derived models8, we explored associations between C4A protein concentration and in vivo proxies of synapse density. CSF levels of neuronal pentraxins (1, 2, and the receptor) were recently shown to inversely correlate with prefrontal cortical thickness determined by Magnetic Resonance Imaging (MRI), as well as to predict synaptic loss in dementia and Alzheimer’s disease59–61. Thus, we measured these three markers in CSF from participants in the KaSP cohort (patients with FEP: n = 43, HCs: n = 19).

After adjusting for age, sex, and diagnosis, we observed significant negative correlations in CSF between C4A and neuronal pentraxin 1 (r = −0.31; (adjusted) = 0.026), neuronal pentraxin 2 (r = −0.27; (adjusted) = 0.026), and the neuronal pentraxin receptor (r = −0.34; (adjusted) = 0.040), see Fig. 4a–c.

Discussion

This study demonstrates that C4A protein concentrations are specifically elevated in the CSF of patients with FEP who subsequently develop SCZ, strengthening the notion of a role for this complement component in the early pathophysiological events of SCZ. Furthermore, in line with the effect of C4A CNs on microglial synapse engulfment in patient-derived models8, we show that C4A protein levels in patient-derived CSF inversely correlate with previously validated proxies of synapse density. This emphasizes the in vivo relevance of C4A for synapse density in SCZ and supports the notion from human experimental studies that C4A CNs influence SCZ risk by causing excessive synaptic pruning. Although the elevation of C4A protein levels in patients with FEP who develop SCZ was modest, it was still larger than expected from previous genetic studies. Thus, on protein level we corroborate previous findings from gene expression analyses that C4A CNs do not fully explain the observed C4A RNA upregulation in SCZ1,14. Using a patient-derived cellular model, we show that IL-1beta/IL-6 induces neuronal C4A mRNA expression. As patients with SCZ as a group display elevated levels of these cytokines in CSF123, the increase in C4A levels per C4A CNs can therefore be expected to be more pronounced in SCZ as compared to HCs and partly explain the remaining elevation in patients with FEP-SCZ after adjusting for C4A CNs. This hypothesis is supported by our CSF analyses, where we show that IL-1beta levels correlate with CSF C4A levels per C4A CNs, suggesting in vivo relevance. The results are in line with studies showing that immune activation in SCZ, including increased levels of pro-inflammatory cytokines such as IL-1beta and IL-6, are linked to a more pronounced cortical thinning and worsen cognitive performance24–26. Further, IL-1beta has been proposed as a biomarker also for several neurocognitive phenotypes beyond SCZ24,25, as well as in suicidal behavior27–29. Further studies are warranted to evaluate if a mediation by induction of C4A mRNA expression and excessive synaptic pruning contributes to these phenotypes.

Utilizing detailed clinical data regarding severity and symptom profiles we also study within-subject correlations to CSF C4A levels. We observe a clear inverse correlation between CSF C4A concentration...
Several previous studies have measured total C4 levels in plasma from patients with SCZ but with conflicting results32–35, and C4 levels in plasma do not significantly correlate with total C4 levels in CSF36. In the current study, we separate C4A and C4B levels in CSF and in line with previous experimental and genetic studies we observe that only C4A is increased in first episode patients with SCZ. A recent study reported elevated total C4 concentrations in 32 patients with chronic SCZ spectrum disorder (median duration of illness of 14 years) with ongoing antipsychotic medication37. Thus, we also measured a peptide corresponding to total C4 levels in CSF but unlike the previous study of chronic patients with schizophrenia we did not observe a significant elevation of total C4 levels in CSF of patients with FEP (Supplementary Fig. 6). Similar to this study, however, we observed an age effect on CSF total C4 levels (Supplementary Fig. 7), but by separating CSF C4A and C4B levels, we could show that this effect was driven by an age-dependent increase in C4B levels (Supplementary Fig. 8), with C4B...
levels per C4B CNs being significantly higher than C4A levels per C4A CNs in human CSF (Supplementary Fig. 9).

In conclusion, this study shows that SCZ risk variants that increase C4A RNA expression also led to an increase in C4A protein levels in CSF and a concomitant decrease in proxies for synapse density. In two independent cohorts, we observe higher CSF C4A concentrations in patients with FEP who go on to develop SCZ compared with those who get another diagnosis as well as in comparison to controls, and this also pertains to patients that were not on an antipsychotic agent. We show that this increase cannot be fully explained by genetic risk variants in the C4 locus, and in patient-derived models we discover a mechanism by which the SCZ-associated cytokines IL-1beta and IL-6, previously

Fig. 3 | Disease-associated cytokines associate with C4A protein levels in cerebrospinal fluid of patients with first-episode psychosis. a Overview of cytokine analyses. Cerebrospinal fluid (CSF) from patients with first-episode psychosis in the KaSP cohort (n = 26) were analyzed for interleukin (IL)–1beta, IL-6, and C4A levels. Median age in the cohort was 29 years (interquartile range: 23–32) and 18 participants were males. b CSF C4A protein levels (adjusted for genetically predicted C4A RNA expression) correlated with IL-1beta levels (r = 0.39; 95% confidence interval [CI]: 0.01–0.68, P = 0.046), c but not IL-6 levels (r = −0.05 CI: −0.44–0.36, P = 0.820). c C4B protein levels (adjusted for genetically predicted C4B RNA expression) did not correlate significantly with IL-1beta levels (r = 0.29 CI: −0.12–0.62, P = 0.150; c), or d IL-6 levels (r = 0.32 CI: −0.09–0.64, P = 0.112). Data were analyzed by creating CSF C4A (or C4B) levels adjusted for predicted RNA expression (by extracting unstandardized residuals from a linear regression model analyzing predicted RNA expression vs. measured CSF protein levels), and then used in Spearman correlation analyses against CSF cytokine levels. All reported P values are two sided. Intercepts and 95% CI bands are indicated in figure panel 3b–d. Source data for graphs in figure panels 3b–e are provided in the Source Data file. Figure 3a was created with BioRender.com.

Fig. 4 | Correlations of cerebrospinal fluid C4A and C4B protein concentration with markers of synapse density. Cerebrospinal fluid (CSF) from 19 healthy controls, 14 patients with first-episode psychosis (FEP) who developed schizophrenia and 29 patients with first-episode psychosis (FEP) who developed schizophrenia in the KaSP cohort were analyzed. Median age: 27 years (interquartile range: 22–32), 40 males and 22 females. CSF C4A levels (adjusted for age, sex, and diagnosis) were negatively correlated to CSF levels of neuronal pentraxin 1 [r(adjusted) = −0.30, P(adjusted) = 0.026; a], neuronal pentraxin 2 [r(adjusted) = −0.26; P(adjusted) = 0.026; b], and neuronal pentraxin receptor [r = −0.34; P(adjusted) = 0.040; c]. All correlations were analyzed using Spearman’s partial correlation analyses. All reported P values are two sided and adjusted for multiple testing using Benjamini-Hochberg correction. Intercepts and 95% confidence intervals are indicated in the graphs. Source data for the graphs are provided in the Source Data file.

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shown to be increased in SCZ, can selectively induce C4A mRNA expression. We then corroborate these results in patient-derived CSF analyses and show that IL-1beta correlates with C4A levels independent of C4A CNs. Taken together, these results confirm a role of C4A in early SCZ pathophysiology and suggest a targetable mechanism that can contribute to the increase in C4A levels and excessive synapse elimination.

**Methods**

**Ethical statement**

The study was approved by the Regional Ethics Committee in Stockholm (Sweden) and the Institutional Review Board of Partners HealthCare (MA, USA). Informed consent was obtained from all included subjects (CSF sampling and skin biopsies for deriving iPSCs).

**Study population and diagnostic assessments**

**KaSP Cohort:** Karolinska Schizophrenia Project (KaSP) is a Swedish multidisciplinary research consortium investigating the pathophysiology of FEP and SCZ. Patients who seek health care for psychotic symptoms for the first time are recruited at psychiatric emergency wards and in- or outpatient facilities at the psychiatric clinics located in Stockholm, Sweden. Exclusion criteria are ongoing or previous prescription of an antipsychotic for more than 30 days, severe somatic and neurological diseases, current substance abuse (except nicotine use), or autism spectrum disorder. These patients are excluded by clinical examination, medical history, routine laboratory tests, including screening for drugs and MRI scans. All KaSP study participants were white. A baseline diagnosis is established based on a structured clinical interview of the Diagnostic and Statistical Manual of Mental Disorders IV, DSM-IV (Structured Clinical Interview for DSM-IV-AXIS I Disorders, SCID-I), at the time of inclusion. For the subdivision of patients with FEP into a FEP-SCZ and a FEP-non-SCZ group we used data from a 1.5-year follow-up (median time to follow-up: 1.6 years). The same diagnostic procedure as at baseline was used. Patients who did not receive a SCZ diagnosis at follow-up received a diagnosis of delusional disorder, unspecified psychosis, acute and transient psychotic disorder, schizoaffective disorder, or major depressive disorder.

HCs were recruited by advertisement and matched on age and sex. Eligibility was determined by medical history, routine laboratory tests, clinical examination, and an MRI examination, as evaluated by an experienced neuroradiologist at the MR Centre, Karolinska University Hospital, Solna. The Mini International Neuropsychiatric Interview (MINI), performed by either a resident or a specialist in psychiatry, was used to exclude previous or current psychiatric illness. Further exclusion criteria were former or current use of illegal drugs, first-degree relatives with psychotic illness or bipolar disorder, as well as neurologic disease and/or severe somatic disease. All participants (patients and controls) were free from any form of substance use evaluated with Alcohol Use Disorders Identification Test (AUDIT) and the Drug Use Disorders Identification Test (DUDIT) at the time of the study. All eligible subjects with available data were included in the present study.

**GRIP cohort:** The Gothenburg research and investigation on psychosis (GRIP) is a study collecting data from patients with schizophrenia spectrum disorders at the psychosis clinic, Sahlgrenska University Hospital, Mölndal, Sweden. The initial diagnostic assessments are performed by treating clinicians at outpatient tertiary-care units, as well as the inpatient units, in Sahlgrenska University Hospital (Sweden). Further clinical information is obtained from interviews at tertiary-care units and investigation units, and when needed supplemented with information from clinical records. To secure diagnoses for research, a series of case conferences are then held (for this study in between October 2017–March 2018). A minimum of two board-certified psychiatrists participates in these case conferences where a consensus diagnosis is established according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). The diagnosis is based on structured interviews, medical records (also collected post-baseline), and when needed supplemented with information from the treating psychiatrist. The participating research clinicians are blinded to the results of the CSF analyses. The median time from inclusion in the study (when the initial diagnosis by the treating physician is made) to establishment of a research consensus diagnosis on the case conference was here 1.8 years. For patients with FEP, the median time from first contact with psychiatric care to referral for further investigation of psychotic symptoms at the tertiary unit (i.e., study baseline) was 2 years. This time period, as well as time to a SCZ diagnosis within the study period, was unrelated to CSF C4A (Spearman correlation: \( r = 0.15 \) CI: \(-0.25–0.50, P = 0.454\)) or C4B levels (Spearman correlation: \( r = 0.26 \) CI: \(-0.13–0.58, P = 0.170\), as well as to SCZ- vs. a non-SCZ-related diagnosis (Spearman correlation: \( r = 0.06 \) CI: \(-0.32–0.43, P = 0.746\)). Patients who did not receive a SCZ diagnosis at the diagnosis evaluation around 2 years after baseline received a diagnosis of bipolar disorder, brief psychotic disorder, substance-induced psychotic disorder (not known at the initial evaluation), or a psychotic disorder not otherwise specified.

As no matched HCs are recruited in GRIP, we used HCs from the larger “St. Göran projektet” research project in which GRIP is a sub-study focusing on psychosis. These HCs are randomly selected by Statistics Sweden and undergo the same clinical investigations as patients and do not fulfill the criteria for a DSM-IV-TR disorder. From this cohort, we then selected HCs to as closely as possible match the patients in GRIP dependent on sex and age. All eligible subjects with available data were included in the present study.

**Lumbar puncture and collection of cerebrospinal fluid**

**KaSP cohort:** Study participants fasted overnight, lumbar punctures were performed between 7.45 am–15.15 pm, using a non-cutting (atraumatic) 22 G spinal needle inserted at the L4–5 interspace with collection of 18 mL of CSF. Not more than 1 h after collection, CSF was centrifuged at 3500 rpm for 10 min divided into 10 aliquots and stored at \(-80^\circ\)C until analysis. All CSF samples were individually analyzed.

**GRIP cohort:** Subjects fasted overnight, and the lumbar puncture was performed between 10.45 and 11.15 am. A non-cutting (atraumatic) 22 G spinal needle was inserted into the L4/5 interspace and about 15 ml of CSF was collected, gently inverted to avoid gradient effects, centrifuged for 10 min at 3500 rpm at 20 degrees, not more than 30 min after sampling. CSF was then divided into 0.5 ml aliquots that were stored at \(-80^\circ\)C pending analysis. All CSF samples were individually analyzed.

**Targeted mass spectrometry (C4A and C4B)**

The protein concentration of the CSF samples was measured in triplicates using Micro BCA Protein Assay Kit (Thermo Fisher Scientific Inc, USA PN: 23235) prior to sample preparation. The measurements were performed in a 96-well plate (VWR International AB, Sweden PN: 738-0170) and fluorescence read by plate reader FLUOstar Omega. (BMG LABTECH, Germany). Sample preparation was performed on the Agilent AssayMAP Bravo Platform. Fifty \( \mu L \) protein of each CSF sample was used for analysis. Depending on the protein concentration of the samples, the appropriate volume of each sample was transferred to a 96-well plate (Greiner G682011) and 200 mM triethylammonium bicarbonate buffer (TEAB, Thermo Fisher Scientific, USA PN: 90114) manually added to a final volume of 50 \( \mu L \). The Bravo platform was used for the following steps; addition of 50 \( \mu L \) TEAB buffer and 50 \( \mu L \) 3.3% solution of sodium deoxycholate (SDC, Sigma-Aldrich, Sweden PN: L3771) for dissociation of proteins (final concentration of 1%). Proteins were reduced by the addition of 10 \( \mu L \) 80 mM tris(2-carboxyethyl) phosphine (TCEP, final concentration of 5 mM, Sigma-Aldrich, Sweden PN: C4706) and the reaction was carried out for 1 h at 35 °C. Alkylation was carried out by the addition of 15 \( \mu L \) 120 mM...
iodoacetamide (final concentration of 10 mM, Sigma-Aldrich, Sweden PN: I1149) for 30 min in the dark at room temperature. One μg trypsin (Sequencing Grade Modified, Promega, Sweden PN: VS111) was added to each sample and incubated overnight at 37 °C. The CSF samples were divided into four groups for sample preparation, and they were labeled with #1, #2, #3, and #4. In group #3, the protein concentration was lower than in the other groups and the starting volume for each sample was therefore more than 50 μL. To these samples, 200 mM triethylammonium bicarbonate buffer was manually added to a total volume of 100 μL and additional liquid handling steps were carried out on the Bravo platform. The trypsin digestion was quenched by the addition of 30 μL 10% FA (Thermo Fisher Scientific, USA PN: 94318) and the SDC pellet was removed by filtering the samples through a polypropylene filter plate (Agilent Technologies, Sweden, PN: 200931-100) with hydrophilic PVDF membrane (mean pore size 0.45 μm). The trypic peptide concentration was detected using the Pierce Quantitative Colorimetric Peptide Assay (Thermo Fisher Scientific, USA PN: 23275). Each sample was spiked with a mixture of heavy isotope-labeled peptide standards (AQUA Pro peptides from Thermo Fisher Scientific) before LC-MS analysis.

The LC-MS analysis was performed on a Tribrid mass spectrometer Fusion equipped with a Nanospray Flex ion source, coupled to an ESI-Q-TOF Ultra-high pressure liquid chromatography (UHPLC) system (Thermo Fisher Scientific, USA). The tryptic CSF peptides spiked with AQUA peptides, were loaded on to an Acclaim PepMap RSLC column (75 μm × 25 cm, nanoViper, C18, 2 μm, 100 Å) with the flow rate 300 nL/min. The column was kept at 40 °C using a Phoenix S&T Nano LC column heater (MS WiL GmbH, Zurich). Solvent A (0.1% FA, Sigma-Aldrich) and solvent B (0.1% FA in acetonitrile, Sigma-Aldrich) were used for the nonlinear gradient. The percentage of solvent B was 1% for the first 3 min and increased to 30% in 50 min and then to 90% in 5 min which was kept for 7 min to wash the column. The tryptic CSF peptides spiked with AQUA peptides, were introduced into the mass spectrometer via a stainless-steel Nano-bore emitter (OD 150 μm, ID 30 μm) with the spray voltage of 2 kV and capillary temperature 275 °C. The Orbitrap Fusion was operated in parallel reaction monitoring (PRM) mode. MS2 precursors were isolated with a quadrupole mass filter set to a width of 1.2 m/z. Precursors were fragmented by higher energy collision dissociation (HCD) and detected in Orbitrap detector with a resolution of 60,000. The normalized collision energy (NCE) for HCD was 25%. The values for the AGC target and maximum injection time were 5 × 105 and 118 ms, respectively. The peptide inclusion list is shown in Supplementary Table 4. Five μL (0.2 μg/μL) of trypic peptides (spiked with AQUA peptides) of each trypsin-digested CSF sample, was injected and run in triplicates. Pooled CSF samples were run as quality control samples throughout all the samples. The linear range of each heavy peptide was measured in pooled CSF digest. A mixture of heavy AQUA peptides was spiked at five different concentrations into pooled CSF samples. The concentration range was adjusted for each individual peptide according to its expected endogenous signal. Each sample was analyzed three times, and the peak areas of heavy peptides were plotted against the spiked heavy peptides concentrations (Fig. 5).

All the raw data generated on the Fusion MS were imported to Skyline v4.1 (MacCoss Lab Software, USA) for data analysis. Peak integration was done automatically by the software and was manually inspected to confirm correct peak detection. Peak identities were confirmed by measured transitions (dotp) and also between endogenous and corresponding heavy peptides (rdotp). The best 3–5 transitions were selected for the quantification and was performed by matching light and heavy peak area ratios. All calculations were performed using Microsoft Excel. For quality control, we also measured a peptide shared for C4A and C4B (tC4; GRIP). Finally, two subjects did not display detectable C4A (in the GRIP chort) and C4B (in the KaSP cohort) peptide levels. We decided to initially exclude these subjects (one sample from the KaSP cohort and one sample from the GRIP cohort) in the main analyses. However, elevated levels of CSF C4A in FEP-SCZ remained in the GRIP cohort when including all values (Kruskal-Wallis H test followed by post-hoc tests: adjusted P [FEP-SCZ vs. HCx] = 0.038, and adjusted P [FEP-SCZ vs. FEP-nSCZ] = 0.00003), while C4B levels in the KaSP cohort remained similar across groups (Kruskal-Wallis H test followed by post-hoc tests: adjusted P [FEP-SCZ vs. HCx] = 0.905, and adjusted P [FEP-SCZ vs. FEP-nSCZ] = 0.193). Similar results were also obtained by imputing values with half of the limit of detection in the GRIP cohort (Kruskal-Wallis H test followed by post-hoc tests: adjusted P [FEP-SCZ vs. HCx] = 0.039, and adjusted P [FEP-SCZ vs. FEP-nSCZ] = 0.00002) and in the KaSP cohort (Kruskal-Wallis H test followed by post-hoc tests: adjusted P [FEP-SCZ vs. HCx] = 0.859, and adjusted P [FEP-SCZ vs. FEP-nSCZ] = 0.151).

**Molecular analyses of C4 structural elements (ddPCR)**

Using ddPCR, CNs of C4 structural elements (C4A, C4B, C4-HERV CNs) in the GRIP cohort were measured from genomic DNA isolated from blood samples. C4AHERV, C4A-HERV, C4BHERV, and C4BHERV–CNs were determined based on imputations. A detailed protocol for imputations for can be found on Github: https://github.com/freesek/imput4c. For distributions of C4 CNs in the samples see Supplementary Fig. 10. C4A, or C4B, CNs displayed no association to age (C4A CN: r [Spearman correlation] = 0.008 CI: −0.30–0.31; P = 0.959, and C4B CN: r [Spearman correlation] = −0.03 CI: −0.33–0.28; P = 0.852) or sex (C4A CN: r [Spearman correlation] = 0.08 CI: −0.23–0.38; P = 0.602, and C4B CN: r [Spearman correlation] = 0.16 CI: −0.16–0.44; P = 0.315).

**Molecular analyses of C4 structural elements (imputation from WGS)**

CNs of C4 structural elements (C4AHERV, C4A-HERV, C4BHERV, and C4BHERV–CNs) in the KaSP cohort were inferred from MHC genotypes computed from whole genome sequencing (WGS) data. HapMap3 CEU reference haplotype panel, from Sekar et al., was used to impute pre-annotated C4 haplogroups, using Beagle (version 3.3), with subsets of SNPs in the extended MHC locus (chr6: 25–34 Mb).

**Cognitive testing**

The Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery was used to evaluate cognitive function in the KaSP cohort. This battery contains 10 tests that measure 7 cognitive domains: Speed of processing (Brief Assessment of Cognition in Schizophrenia: Symbol Coding, Category Fluency: Animal Naming, Trail Making Test: Part A); Attention/vigilance (Continuous Performance Test-Identical Pairs); working memory (Wechsler Memory Scale-3rd Edition: Spatial Span, Letter-Number Span); verbal learning (Hopkins Verbal Learning Test-Revised); Visual learning (Brief Visuospatial memory Test-Revised); reasoning and problem solving (Neuropsychological Assessment Battery: Mazes) and social cognition (Mayer–Salovey–Caruso Emotional Intelligence Test: Managing Emotions). One psychologist (HFB) administered all the tests.

**Sensorimotor gating**

Electromyography (EMG) was recorded from the orbicularis oculi using two 20-mm disk Ag/AgCl electrodes positioned below and lateral to the eye. A 40 × 50 mm ground electrode was implanted behind the ear on the bone. EMG activity was filtered (1- to 1000-Hz notch filter and 60-Hz notch filter) and digitized at a rate of 1 kHz. Acoustic stimuli were supplied via headphones using a PsyLab Stand Alone monitor and a tone generator (containing a digital white noise source and a digitally controlled sine wave generator with a range of 30–2000 Hz from Contact Precision Instruments). The EMG signal was amplified with a...
Grass A.C. Amplifier (model ICPS11, Astro-Med., Inc.) and acquired by using commercially available hardware/software (BioPac) on a laptop computer (Hewlet Pacard Compaq 6715b).

All subjects (KaSP) were tested using the same startle system. Subjects were instructed to relax and keep their eyes open and directed against a position marked on the wall, during the test. Throughout the session, background noise was set to 70 dB. The session consisted of 60 trials with two conditions: pulse-alone trials, consisting of a 40 ms, 115-dB pulse of white noise and prepulse-pulse trials, consisting of a 40 ms 115 dB pulse (white noise, as above) preceded by a 16-dB (above background) prepulse of 20 ms duration (white noise). The ISI between the prepulse and the pulse tested were 30, 60, and 120 ms, respectively. A 3-min acclimation period with 70 dB white noise started the session. The immediately following block consisted of 15 pulse-alone trials, used for the calculation of habituation using the latent curve modeling.

The primary outcome measures of PPI were calculated for 30, 60, and 120 ms ISI conditions, respectively, as percent change in response amplitudes [% PPI = (mean startle alone- mean prepulse-pulse condition)/mean startle alone × 100]. Startle response was calculated by comparing the mean startle magnitude of the middle five pulses during the first trial (that only contained pulse alone).

**iPSC generation and neuronal differentiation**

iPSCs from four chronic patients (all white males, 28 years old, 44 years old, 55 years old, and 58 years old, respectively) were used in this study. The patients were recruited from outpatient units at the Massachusetts General Hospital (MGH). All individuals signed a written informed consent before participating in the study, as approved by the Institutional Review Board of Partners HealthCare. All relevant ethical regulations were followed when performing the study. A structured psychiatric evaluation was performed using the Structured Clinical Interview for DSM-IV and the Mini-International Neuropsychiatric Interview by a single psychiatrist with at least 5 years of clinical experience.

Following subcutaneous injection of lidocaine 1%, a 3.0 or 4.0 mm punch tool was used to obtain a dermal biopsy sample from the non-dominant forearm. Biopsies were collected into a
transport medium of DMEM (Gibco) with 1% penicillin–streptomycin–glutamine (Corning Life Sciences, Tewksbury, MA, USA). Upon receipt of samples, they were washed twice in PBS with 10% penicillin–streptomycin–glutamine. Two 60-mm tissue culture-treated plates were used, one to cut out the biopsy and the other one to plate the pieces of biopsy. A scalpel was used to cut the biopsy into small pieces and then to grid the dish where the pieces of biopsy were plated (dermis side down). The dish was placed in an incubator at 37°C for 15 min in order for the skin to adhere to the dish. Medium of DMEM with 10% FBS and 1% penicillin–streptomycin–glutamine was finally gently added over the pieces of skin.

The collected fibroblasts were then reprogrammed (mRNA reprogramming) and the resulting iPSC colonies stabilized and expanded under xeno-free conditions by StemBioTics (San Diego, CA, USA). Briefly, stable iPSCs were expanded in NutriStem XF medium (Biological Industries) and on biofilm 521 LN-coated (Biolamina) plates to at least passage 3. iPSCs were then purified using MACS with anti-TRA1–60 MicroBeads (Miltenyi Biotec) on LS columns according to the manufacturer’s instructions. All fibroblasts and iPSCs were screened and found negative for Mycoplasma; they stained positive for ‘transfection control cells completely died.

We then generated Ngn2 expressing stable NPC lines using TALEN-based plasmids as previously described. Briefly, the doxycycline-inducible Ngn2 AAVS1 knock-in plasmid, based on an AAVS1 SA-2A-puro-pA donor (plasmid no. 22075; Addgene), was generated by replacing the puromycin resistance gene with the neomycin AA VS1 SA-2A-puro-pA donor (plasmid no. 22075; Addgene), was generated upon request. NPCs were collected with Accutase and counted. We were generated by golden gate assembly. Plasmid maps can be provided upon request. NPCS were collected with Accutase and counted.

For each transfection, 4 × 10⁶ cells were pelleted at 300 g and then resuspended in 100 μL prewarmed nucleofector solution (Human Stem Cell kit 1, Lonza, catalog no. VPH-5012); 4 μg of Ngn2 plasmid and 1.5 μg each of 1R and 1L plasmids were added directly to the resuspended cells, or negative control without plasmid, followed by nucleofection (Amaxa Nucleofector II; Lonza) according to the manufacturer’s protocol using program B-016. After nucleofection, cells were plated onto Geltrex-coated six-well plates in neural expansion medium (50% NBM, 50% advanced DMEM/F-12 with 1× NIS). Stable lines were expanded by selection with the addition of 125 μL/mL G418 (Genetec; Thermo Fisher Scientific) over 8–10 d or until the negative transfection control cells completely died.

NGN2 neurons were then generated as described earlier. Briefly, NPCs were plated as single cells on poly-L-ornithine/laminin coated 24-well polypropylene plates at 2.5 × 10⁵ cells/100 μL in 50% neurobasal medium (NBM; ThermoFisher Scientific) and 50% DMEM/F12 (ThermoFisher Scientific) with 1× neural induction supplement (Thermo Fisher Scientific). On the following day, the medium was replaced with neuronal differentiation medium (50% NBM and 50% DMEM/F12 supplemented with 1× MEM Non-essential Amino Acids (ThermoFisher Scientific), 1× N2 supplement (ThermoFisher Scientific), 10 ng/mL brain-derived neurotrophic factor (BDNF; Peprotech, Cat# 450-02) and 10 ng/mL recombinant human NT-3 (Peprotech, Cat# 450-03)). Day 2 cells were differentiated in neuronal differentiation medium enriched with 1× B-27 (ThermoFisher Scientific) and 2 μg/mL doxycycline (Sigma-Aldrich, Cat# D3073). This medium was maintained for the entire differentiation process, unless noted. On days 3–6, cells were incubated with the same medium with the addition of 5 μg/mL zeocin (ThermoFisher Scientific). For neuronal maturation, 11 days after seeding, astrocyte conditioned medium (ACM: ScienCell Research Laboratories, Cat#: SCI1811-sf) was added to the culture. Assays were then performed at day 25.

Immunocytochemistry

NGN2 neurons were cultured on cover slides coated with poly-L-ornithine/laminin. Cells were fixed in 4% paraformaldehyde in PBS for 15 min. Following permeabilization in PBS containing 0.1% Triton-X100 for 5 min, the slides were blocked with blocking solution (PBS containing 5% normal goat serum) for 1 h at room temperature. Primary antibodies against MAP-2 (1:500, Abcam, Cat# ab3392), Beta III Tubulin (1:2000, Promega, Cat# G7212), Oct4 (1:400, Cell signaling, Cat# 2840), TRA-1-60 (1:500, Abcam, Cat#Ab16288) in blocking solution were added and incubated overnight at 4 degrees C. Cells were then washed with PBS and incubated with the corresponding Alexa Fluor-conjugated secondary antibodies (1:500) for 1 h at room temperature. The following secondary antibodies were used: goat anti-chicken (ThermoFisher Scientific, Cat# A3933), goat anti-mouse (Abcam, Cat# ab105113) and goat anti-rabbit (Abcam, Cat# A32732). Images were obtained using a confocal microscope system (Zeiss, LSM800).

Cytokine stimulations of NGN2 neurons and C4A/C4B mRNA expression

After 25 days, mature NGN2 neurons were treated with either VEH, 1 ng/mL IL-1β (Thermo Fisher Scientific, cat# PHC0815) or 1 ng/mL IL-6 (Thermo Fisher Scientific, cat# PHC0063), alone or in combination for 24 hours. Following stimulations of cells, total RNA was extracted using Trizol® Reagent (Invitrogen) according to the manufacturer’s protocol. The quantity and purity of total RNA was measured using a NanoDrop® spectrophotometer (Thermo Scientific). Complementary DNA was synthesized using High-Capacity RNA-to-DNATM Kit (Applied Biosystems) following the manufacturer’s protocol. qPCR was performed with TaqMan Gene Expression Assays (Applied Biosystems) by using the StepOnePlus Real-time PCR system (Applied Biosystems). Each 20 μL PCR reaction contained 5 μL of cDNA, 300 nM of forward and reverse primer, 250 nM of probe and 10 μL of Taqman PCR master mix (Applied Biosystems). Primers for C4A and C4B reactions were as follows (forward: GCAGGAGACATCTAAGGGCTC and reverse: CCGCACCTGATGCTC). Specific probes for C4A (FAM-ACC CCT GTC CAG TGT TAG-MGB) and C4B (FAM-ACC TCT CCT CAG TAC-MGB) were used. ΔCt values were obtained by comparison to Ct values of the housekeeping gene (GAPDH, Assay ID. HS9999905_m1) for each technical replicate. Fold changes (2−ΔΔCt) were then generated by normalization to the average ΔCt value for VEH treated wells per plate. C4B mRNA could not be detected in non-stimulated cultures from one subject (not genotyped) and these cultures were excluded from quantification of C4B mRNA expression after stimulations. In one culture, C4A and C4B mRNA expression were also ~100× larger than in the other cultures and this well was excluded (see Source data). All other wells that passed QC (ICC, morphology) were analyzed by qPCR and included in the statistical analyses.

CSF cytokine analyses

IL-1β and IL-6 were previously measured as part of the Novo Human Utrasensitive Cytokine 10-Plex Panel (product number at that time: LHC6004; now distributed by Invitrogen) on the Luminex immunoassay platform. In contrast to the manual provided with the kit, an additional calibration curve point was included to extend the assay dynamic range in the low concentration range. 50 μL sample was diluted with 50 μL of sample diluent, the final sample dilution factor was 1:2. The assay was processed as recommended by the kit instructions. The measurement was performed on a FluorLM assay (operated under software version 4.0, Luminex, Austin, TX, USA). Data analysis was performed using MasterPlex QT 2010 (V 2.0, Hitachi Software.
Engineering, San Francisco, CA, USA). Standard curves were generated using weighted (1/Y²) 5-parameter logistics. The analyte concentrations in the samples were calculated based on the standard curves. In this study, only IL-1beta and IL-6 were initially analyzed (based on our in vitro data). Upon request from one of the reviewers, data on all cytokines were then extracted from the database for post-hoc analyses. Seven more cytokines were detected in more than 10 subjects (IL-1beta, IL-6, IL-10, IL-5, TNFα, GM-CSF, and IL-8) but none of them displayed a significant association with C4A (Supplementary Table S5).

**CSF neuronal pentraxins analyses**

Preparation of samples and LC-MS/MS analyses were performed as previously described using micro-high-performance liquid chromatography-mass-spectrometry (6495 Triple Quadrupole LC/MS system, Agilent Technologies). Briefly, in a flowrate of 0.3 mL/min, sample injection of 40 µL and a 30 min gradient was employed for separation on a Hypersil Gold reversed phase column (dim. 100 × 2.1 mm, particle size 1.9 µm, Thermo Fisher Scientific). The two mobile phases (A and B) were composed of 0.1% formic acid in water (v/v) and 0.1% formic acid/84% acetonitrile in water (v/v), respectively. Mobile phases (A and B) were used a delta retention time of 0.8 min for each peptide. Quality control samples, consisting of pooled CSF samples obtained from the Neurochemistry Laboratory at Sahlgrenska University Hospital, Mölndal, Sweden, were injected at regular intervals to monitor the performance of the assay over time. The collection and use are in accordance with the Swedish law on biobanks in healthcare (2002:297). Skyline 20.1 (MacCoss Lab Software) was used for peak visualization and inspection. Peak adjustment was performed if required for optimal peak area calculation. Relative peptide concentrations were obtained by summing all measured fragment peak areas for each peptide and dividing that by the sum of the fragment peak areas of the corresponding IS, followed by multiplication of the amount of IS added per µL of CSF.

**Statistics**

Available sample size for the initial analysis in the discovery cohort was deemed adequate based on the data presented by Sekar et al. (an estimated correlation coefficients of at least 0.5 for C4A or C4B) CNs versus corresponding RNA expression in 101 subjects, and an odds ratio of 1.4 (P = 2 × 10⁻²) comparing C4A RNA expression in 35 SCZ subjects and 70 HCs). According to the pre-defined analytical plan, a significant increase in C4A (or C4B) concentration in the discovery cohort (P < 0.05) was to be followed-up in an independent cohort then again measuring C4A, as well as C4B, with significance set at P < 0.05 (two-sided p-value). Potential confounders were to be tested on group levels (in each cohort). If this indicated any evidence of between group differences (on a liberal p-value threshold of 0.1), indicated variables were to be studied in relation to CSF C4A or C4B concentration. If this revealed an association with P < 0.05, we were to perform an adjusted analysis. The exception to this rule was (1) antipsychotics, here we before analyses decided to compare levels between medicated and non-medicated patients, and even if we observed no indication of altered levels in medicated vs. unmedicated we were to perform stratified/adjusted analyses, and (2) as post-hoc analyses, we also performed adjusted analyses (sex, age, and smoking) to control for possible residual confounding. For group comparisons, CSF C4A and C4B concentrations (or C4A and C4B RNA in experiments) in the main analyses were analyzed using Kruskal-Wallis H tests followed by post-hoc tests. Continuous variables were analyzed by Spearman’s or Pearson’s correlation analyses (or partial correlation analyses) as indicated. Experimental data were analyzed using Kruskal-Wallis H test followed by post-hoc tests. Data were analyzed using R for Mac OS (version 4.2.1), IBM SPSS statistics (version 28.0;0.0.0), and Graphpad prism 9 for macOS (version 9.3.1).

**Data availability**

Source data, necessary to interpret and verify the research in the article, are provided in the Source Data file. In accordance with the institutional regulations and the Swedish law, raw data containing sensitive information that can be used to identify individuals, cannot be shared publicly under the current data protection. Instead, such data can be made available upon request (to the corresponding author) and on a case-by-case basis as allowed by the legislation and ethical permits. Source data are provided with this paper.

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Author contributions

J. Gracias and C.M.S. contributed to the overall design, direction, analysis, and reporting of the study. F.O., J. Gracias, and C.S.M. designed in vitro experiments. J. Gracias, F.O., S.G., T.F., I.S.D., and N.K. derived neural cultures and performed the in vitro cytokine assay and qPCR. S.D.S. and R.H.P. provided the NGN2 stable neural progenitor cells for the in vitro experiments. C.P.G. performed the ddPCR for the GRIP cohort. S.M. extracted WGS data from KaSP cohort for C4A and C4B. J.Gr. imputed predicted C4A RNA levels from this data. M.S. and C.M.S. supervised the genetic analyses. J.H.L. performed shotgun mass spectrometry and helped to develop the method to detect C4A and C4B in CSF. L.S. helped collect and interpret the PPI and habitation data. L.S., S.E., G.E., S.C., F.P., H.F.B., and C.M.S. are part of the KaSP project and helped recruit and phenotype subjects and collect CSF. H.F.B. performed the cognitive testing of patients in the KaSP cohort. K.B., E.H., H.Z., M.L., A.P., A.G., K.A., and A.I. are part of the GRIP consortium and were involved in recruiting and phenotype subjects and collecting CSF. J.N. and A.B. analyzed KaSP CSF for neuronal pertuins. V.C. and J.C.O. analyzed CSF cytokine levels. All authors discussed the results and implications and commented on the manuscript at various stages.

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