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Full-length Article

Plasma complement and coagulation proteins as prognostic factors of negative symptoms: An analysis of the NAPLS 2 and 3 studies

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ABSTRACT

Introduction: Negative symptoms impact the quality of life of individuals with psychosis and current treatment options for negative symptoms have limited effectiveness. Previous studies have demonstrated that complement and coagulation pathway protein levels are related to later psychotic experiences, psychotic disorder, and functioning. However, the prognostic relationship between complement and coagulation proteins and negative symptoms is poorly characterised.

Methods: In the North American Prodrome Longitudinal Studies 2 and 3, negative symptoms in 431 individuals at clinical high-risk for psychosis (mean age: 18.2, SD 3.6; 42.5 % female) were measured at multiple visits over 2 years using the Scale of Psychosis-Risk Symptoms. Plasma proteins were quantified at baseline using mass spectrometry. Four factors were derived to represent levels of proteins involved in the activation or regulation of the complement or coagulation systems. The relationships between standardised protein group factors and serial measurements of negative symptoms over time were modelled using generalised least squares regression. Analyses were adjusted for baseline candidate prognostic factors: negative symptoms, positive symptoms, functioning, depressive symptoms, suicidal ideation, cannabis use, tobacco use, antipsychotic use, antidepressant use, age, and sex.

Results: Clinical and demographic prognostic factors of follow-up negative symptoms included negative, positive, and depressive symptoms, functioning, and age. Adjusting for all candidate prognostic factors, the complement

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regulators group and the coagulation regulators group were identified as prognostic factors of follow-up negative symptoms (β: 0.501, 95 % CI: 0.160, 0.842; β: 0.430, 95 % CI: 0.080, 0.780 respectively. The relationship between complement regulator levels and negative symptoms was also observed in NAPLS2 alone (β: 0.501, 95 % CI: −0.037, 1.039) and NAPLS3 alone, additionally adjusting for BMI (β: 0.442, 95 % CI: 0.127, 0.757).
Conclusion: The results indicate that plasma complement and coagulation regulator levels are prognostic factors of negative symptoms, independent of clinical and demographic prognostic factors. These results suggest complement and coagulation regulator levels could have potential utility in informing treatment decisions for negative symptoms in individuals at risk.

1. Introduction

Positive and negative symptoms are conceptualised as core features of psychotic disorders (Maj et al., 2021; Fernandez-Egea et al., 2023). Positive symptoms include hallucinations and delusions, while negative symptoms include reduced emotional expression, emotional experience, and motivation (Lyne et al., 2018; Howes et al., 2023); symptoms which partly overlap with symptoms of depression but are considered a distinct construct (Marder and Galderisi, 2017). Negative symptoms often go unrecognised, and are considered an unmet therapeutic need (Fernandez-Egea et al., 2023; Lyne et al., 2018; Howes et al., 2023; Cella et al., 2023; Leucht et al., 2017; Fusar-Poli et al., 2015; Lutgens et al., 2017). Moreover, negative symptoms are some of the most disabling symptoms in those with psychotic disorders. They are detrimental to social and personal relationships, prevent independent living and restrict opportunities for employment and further education (Lyne et al., 2018; Andrianarisoa et al., 2017; Harvey et al., 2012; Lang et al., 2013; Montemagni et al., 2014; Rabinowitz et al., 2013; Savill et al., 2016). Studies indicate that negative symptoms can have a greater impact on functioning than positive symptoms (Cotter et al., 2014; Rabinowitz et al., 2012).

To aid early detection and prevention of psychotic disorders, clinical-high risk (CHR) paradigms have been proposed which aim to capture mental states which precede psychotic disorders (Fusar-Poli et al., 2013). CHR states are largely defined by brief or subclinical positive symptoms (Fusar-Poli et al., 2013). Negative symptoms are frequently reported to precede a first episode of psychosis (Lyne et al., 2017) and previously have been observed among individuals at clinical high-risk, with over half experiencing symptoms of at least moderate severity and as many as 80 % experiencing at least one negative symptom (Addington et al., 2015; Piskulic et al., 2012; Salazar de Pablo et al., 2023). Meta-analyses have confirmed that the relationship between negative symptoms and social or occupational functioning persists among those at CHR (Devoe et al., 2020).

Prognostic factors of important clinical outcomes in psychosis can inform patient management and treatment decisions, as well as become potential targets for new interventions (Riley et al., 2013). Potential candidates include immune system-related biomarkers. Studies have shown that IL-6 (Khandaker et al., 2014; Perry et al., 2021) and complement proteins (Föcking et al., 2021; English et al., 2018; Staines et al., 2022) are longitudinally associated with psychotic experiences and psychotic disorders. A wide range of immune-related analytes have been proposed as potential prognostic factors of transition to psychosis among individuals at risk (Chan et al., 2015; Mongan et al., 2021; Perkins et al., 2015), with transition defined by time-sensitive cut-offs on positive symptom scales. Few studies have investigated prognostic factors of positive symptom severity measured on a continuous scale (van Os et al., 2009; Johns and van Os, 2001).

Studies have investigated clinical prognostic factors of negative symptoms largely among those with a first-episode of psychosis (Mezquida et al., 2017; Üçok and Ergül, 2014; Díaz-Caneja et al., 2015). A recent systematic review summarised associations of inflammatory cytokines with negative symptoms in first-episode psychosis, indicating mixed results to date (Dunleavy et al., 2022). Among those at risk of psychosis, certain clinical prognostic factors of negative symptoms have been identified including more severe positive symptoms, depression and poorer functioning (Tran et al., 2023). A study of 37 individuals investigated cytokines as prognostic factors of negative symptoms, suggesting Tumour Necrosis Factor and Interleukin-6 for further research as they associated with deficits in emotional experience (positively and inversely, respectively) (Goldsmith et al., 2019). Otherwise however, our understanding of blood-based prognostic factors of negative symptoms in individuals at risk of psychosis is poor.

The complement and coagulation networks are aspects of the immune system which have received increasing attention in psychosis research. Several studies have analysed relationships between individual complement and coagulation proteins and psychotic experiences or psychotic disorder (Föcking et al., 2021; English et al., 2018; Chan et al., 2015; Mongan et al., 2021; Zhang et al., 2023; Heurich et al., 2022). However, different complement and coagulation proteins have been identified in the studies, leading us to hypothesise that there is a broader dysfunction of the complement and coagulation pathways, or their regulation, which predicts future negative or positive symptoms. To focus on broader functional roles of the complement and coagulation pathways in this study, we grouped proteins in the complement and coagulation pathways based on their intrinsic function, as either regulators or activating components of the pathways, respectively (Sarma and Ward, 2011; Merle et al., 2015; Zipfel and Skerka, 2009; Davie et al., 1991; Esmon, 2000; Smith et al., 2015; Grover et al., 2022). In a sample of individuals at CHR for psychosis, we aimed to measure the prognostic value of complement and coagulation protein groups for negative symptoms. Furthermore, we aimed to compare prognostic associations of complement and coagulation protein groups with negative symptoms to their prognostic association with positive symptoms. As a secondary aim, we investigated clinical and demographic prognostic factors of negative symptoms in individuals at CHR for psychosis.

2. Methods

2.1. Participants and studies

Participants were part of the North American Prodrome Longitudinal Study (NAPLS) 2 (Addington et al., 2012), and NAPLS3, a later wave of the same study (Addington et al., 2020). NAPLS2 and NAPLS3 are multisite studies from North America and include prospective cohort data on individuals at CHR across 8 and 9 sites, respectively (Addington et al., 2012; Addington et al., 2020). NAPLS2 participants were recruited between 2008 and 2013. NAPLS3 participants were recruited between 2015 and 2018. Individuals with CHR symptoms were referred from health care providers, educators, or social service agencies or self-referred as a result of community outreach (Addington et al., 2012). Individuals, aged between 12 and 30 years, were screened for suitability, and then undertook the Structured Interview for Psychosis-Risk Syndromes (SIPS) (McGlashan et al., 2001), to determine if they met Criteria of Psychosis-Risk Syndromes. Baseline and follow-up interviews were conducted to assess various clinical outcomes, including negative symptoms.

CHR participants who: a) provided a blood sample, b) had at least one follow-up measure of negative symptoms and c) either converted to psychosis or did not convert to psychosis and were followed for a

minimum of two years were included in this investigation (total n = 438, NAPLS2 n = 214, NAPLS3 n = 224). Characteristics of participants who did and did not provide a blood sample are detailed in Supplementary Table 1. Blood samples used for the purpose of this investigation were drawn at baseline into EDTA plasma tubes. Processing time varied with an interquartile range of 40–79 min. Samples were stored in aliquots at −80 °C. NAPLS2 and NAPLS3 samples underwent two and one freeze–thaw cycles, respectively, prior to analysis.

2.2. Mass spectrometry and bioinformatics

Detailed description of the sample preparation and mass spectrometry methods used in this investigation are described in previous publications (Susai et al., 2022; Byrne et al., 2024). Briefly, samples were randomised following a block randomisation design (Burger et al., 2020). Sample processing and mass spectrometry analysis were carried out blind to the outcome data. Plasma samples were prepared for mass spectrometry (MS) using PreOmics kits (PreOmics GmbH, Munich, Germany). Samples were then transferred to Evosep tips (Evosep, Odense, Denmark) and eluted. Samples were analysed in a Bruker timsTof Pro mass spectrometer (Bruker, Massachusetts, United States) connected to an Evosep One liquid chromatography system which injected the samples. Internal standards were included at regular intervals between samples (for details see Supplementary Methods).

MaxQuant (Cox and Mann, 2008) was used to analyse the raw MS files and label-free quantification (LFQ) values. Proteins of the complement or coagulation pathways (soluble activating or regulating components) that were quantified in more than 70 % of samples were brought forward for analysis. Thirty proteins met these criteria, as described in further detail below.

2.3. Outcomes

The primary outcome was repeated measures of total negative symptom scores. Prognostic associations between protein groups and negative symptoms were compared to associations with positive symptoms. In NAPLS2 and NAPLS3, negative and positive symptoms were measured with the Scale of Psychosis-Risk Symptoms (SOPS) (Woods et al., 2019) at month 6, 12, 18, 24 follow-up visits, as well as at an additional visit after transition to psychosis, if this occurred. In NAPLS3, there were additional follow-up visits at month 2, 4, and 8. Outcome data from these visits were included in analyses, and analyses were adjusted for study when combining data from both studies (NAPLS2 or NAPLS3).

SOPS negative symptoms include social anhedonia, avolition, expression of emotion, experience of emotions and self, ideational richness, and occupational functioning. The total SOPS negative symptom score has a range of 0–36. SOPS positive symptoms include unusual thought content/delusional ideas, suspiciousness/persecutory ideas, grandiose ideas, perceptual abnormalities/hallucinations, and disorganised communication. The total SOPS positive symptom score has a range of 0–30 (Woods et al., 2019).

Partial missing repeated measures outcome data were addressed through use of a maximum-likelihood model estimation method (Harrell, 2015) (further details below). Participants had a median of four follow-up measures for both negative and positive symptoms (interquartile range: 3, 6). The median follow-up visit for all observations was the 12-month follow-up visit.

2.4. Data analysis

2.4.1. Missing protein data and pre-processing

Data were pre-processed as previously described (Byrne et al., 2024; Healy et al., 2024). LFQ values for each protein were log2 transformed. Missing LFQ values were treated as left-censored missing data (missing not-at-random; assumed to be below the limit of detection). Using a left-censored imputation approach, missing values were replaced with values drawn from a normal distribution centred at the first percentile of a protein's overall distribution. The normal distribution centred at the first percentile had its own variation in a ratio of 0.5 times the standard deviation of values for a protein. Values for each protein were subsequently standardised and winsorised at 4 standard deviations above and below the mean.

2.4.2. Protein groups and factor derivation

Proteins of the complement or coagulation pathways can be described according to their role as activators (proteases or members of functional complexes) or regulators (inhibitors and cofactors) of the complement and coagulation pathways, respectively (Sarma and Ward, 2011; Merle et al., 2015; Zipfel and Skerka, 2009; Davie et al., 1991; Esmon, 2000).

We use the terms *complement activators* for all complement components contributing to the activation and amplification of the cascade and *complement regulators* for all proteins that control or inhibit different steps of the cascade. Soluble complement components and regulators in the blood were measured through mass spectrometry of plasma samples. The soluble classical and lectin pathway regulators include C1 inhibitor (SERPING1) and C4b-binding protein (C4BP). The alternative pathway inhibitors include complement factor H (CFH), as well as the only positive complement regulator, properdin (CFP). The soluble inhibitors of the terminal pathway include clusterin (CLU), and vitronectin (VTN) (Sarma and Ward, 2011; Merle et al., 2015; Zipfel and Skerka, 2009).

The coagulation cascade is initiated by activation of the intrinsic or extrinsic pathway and involves the activation of a series of clotting factors or *coagulation factors* (Davie et al., 1991). It is controlled by *coagulation regulators*, or anticoagulants. Anticoagulants include the serine protease inhibitors (SERPINs) antithrombin (SERPINC1), heparin co-factor II (SERPIND1), protein Z dependent protease inhibitor (SERPINA10), protease nexin 1 (SERPINE2) and C1-inhibitor (SERPING1). Notably, the complement and coagulation systems share the regulator SERPING1, which not only inhibits coagulation factors PK, FXIIa, FXIa, and FIIa, but also fibrinolytic plasmin (PLN), as well as complement serine proteases C1r, C1s, and MASP1 and 2. Non-SERPIN anticoagulants include tissue factor pathway inhibitor (TFPI), and activated protein C (APC) and its cofactor protein S (PROS) of the anticoagulant pathway (Davie et al., 1991; Esmon, 2000; Smith et al., 2015; Grover et al., 2022).

Proteins which were quantified by mass spectrometry were included in the following groups, as defined by the literature: complement components (C1R, C1S, C1QC, C2, C3, C4A, C4B, CFB, C5, C6, C7, C8A, C8B, C9), complement regulators (C4BPA, SERPING1, CFI, CFH, VTN, CLU) (Sarma and Ward, 2011; Merle et al., 2015; Zipfel and Skerka, 2009), coagulation factors (F2, F12, FGA, FGB, KLKB1, PLG, KNG1), and coagulation regulators (SERPINC1, SERPIND1, PROS1, SERPING1) (Davie et al., 1991; Esmon, 2000; Smith et al., 2015; Grover et al., 2022). To represent variation in protein levels in each group with a single variable, the first principal component of each protein group was derived using the eigenvalue decomposition method with the *princomp* function in R (R Core Team R, 2013).

2.4.3. Prognostic factor evaluation

Complement and coagulation protein groups were examined as prognostic factors of follow-up positive and negative symptoms. Following previous guidelines, the association between the prognostic factor of interest and the outcome was adjusted for other candidate prognostic factors, to ascertain the independent prognostic value of the factor of interest (Riley et al., 2013). Analyses were adjusted for the following candidate prognostic factors: total SOPS negative symptom scores, total SOPS positive symptom scores, Global Assessment of Functioning scores (Hall, 1995), total Calgary Depression Scale for Schizophrenia (CDSS) scores (Addington et al., 1993), suicidal ideation (CDSS question 8), cannabis use and tobacco use (determined with the

Alcohol and Drug Use Scale (Drake et al., 1996)), antipsychotic use, antidepressant use, age, and sex. The relative contribution of these candidate clinical and demographic prognostic factors to the prediction of follow-up negative symptoms was also investigated.

All analyses in the combined sample were additionally adjusted for study (NAPLS2 or NAPLS3). Analyses were then repeated within each cohort (NAPLS2 and NAPLS3) separately. In NAPLS3, where body mass index (BMI) data were available, analyses were further adjusted for BMI. Further sensitivity analyses were carried out in the overall sample adjusting for sample storage time and processing parameters; number of days since blood collection and number of hours before sample freezing after collection. Finally, analyses were repeated excluding participants taking: a) antipsychotics, b) antidepressants or c) either medication.

Linear regression models were fit to repeated measures outcome data using the generalised least squares method, where generalised least squares is an extension of ordinary least squares used to address correlation between model residuals. Models were fit with the *Gls* function from the *rms* package for R (Harrell et al., 2017). Negative and positive symptoms were modelled with a continuous autoregressive correlation structure (Pinheiro and Bates, 2000), under the assumption that correlations between follow-up symptom scores would decrease as time between observations increased. Analysis of variance was used to assess the relative contribution of prognostic factors in the models to the prediction of follow-up symptoms. Prognostic factors were compared with Wald chi-square statistics minus degrees of freedom for each variable. Relative explained variation describes the proportion of explained variation in outcome data attributed to each prognostic factor in a multivariable model. Relative explained variation was calculated as the ratio of the partial Wald chi-square statistic to the total Wald chi-square statistic (Harrell, 2015; Harrell et al., 2017).

Table 1
Baseline participant characteristics.

		Overall (n = 431)	NAPLS2 (n = 210)	NAPLS3 (n = 221)	P Value[c]
Age [$\tilde{x}$(IQR)]		18.0 (15.5–20.0)	17.1 (15.0–20.0)	18.0 (16.0–21.0)	0.068
Sex [N (%)]	Female	183 (42.5)	91 (43.3)	92 (41.6)	0.795
	Male	248 (57.5)	119 (56.7)	129 (58.4)	
Ethnicity [N (%)]	African	61 (14.2)	32 (15.2)	29 (13.1)	0.037
	Central or South American	19 (4.4)	7 (3.3)	12 (5.4)	
	East Asian or Southeast Asian	27 (6.3)	7 (3.3)	20 (9.0)	
	European	240 (55.7)	130 (61.9)	110 (49.8)	
	Interracial	60 (13.9)	22 (10.5)	38 (17.2)	
	South Asian	12 (2.8)	6 (2.9)	6 (2.7)	
	Other	12 (2.8)	6 (2.9)	6 (2.7)	
Antipsychotic Use [N (%)]	No	346 (80.3)	166 (79.0)	180 (81.4)	0.614
	Yes	85 (19.7)	44 (21.0)	41 (18.6)	
Antidepressant Use [N (%)]	No	296 (68.7)	145 (69.0)	151 (68.3)	0.954
	Yes	135 (31.3)	65 (31.0)	70 (31.7)	
Tobacco Use [N(%)]	None	341 (79.1)	159 (75.7)	182 (82.4)	0.089
	Occasionally	45 (10.4)	21 (10.0)	24 (10.9)	
	< 10 a day	23 (5.3)	15 (7.1)	8 (3.6)	
	> 10 a day	22 (5.1)	15 (7.1)	7 (3.2)	
Cannabis Use[a] [N (%)]	No	322 (74.7)	157 (74.8)	165 (74.7)	0.999
	Yes	109 (25.3)	53 (25.2)	56 (25.3)	
BMI[b] [$\tilde{x}$(IQR)]		–	–	23.4 (20.8–26.4)	–
SOPS Positive [$\tilde{x}$(IQR)]		13.0 (11.0–15.0)	12.0 (9.2–15.0)	13.0 (11.0–15.0)	0.003
SOPS Negative [$\tilde{x}$(IQR)]		12.0 (8.0–16.0)	11.0 (7.0–16.0)	13.0 (8.0–17.0)	0.082
CDSS [$\tilde{x}$(IQR)]		6.0 (2.0–10.0)	5.0 (2.0–9.0)	6.0 (3.0–10.0)	0.015
GAF [$\tilde{x}$(IQR)]		50.0 (42.0–58.0)	48.0 (41.0–55.0)	51.0 (43.0–60.0)	0.026

CDSS: Calgary Depression Scale for Schizophrenia, GAF: Global Assessment of Functioning, SOPS: Scale of Prodromal Symptoms, $\tilde{X}$: median, IQR: interquartile range. [a]Cannabis use defined by a frequency of at least monthly. [b]BMI data were only available in NAPLS3. [c]Differences in participant characteristics by study were compared using the Wilcoxon–Mann–Whitney test or Pearson's Chi-squared test.

2.5. Ethics

Ethics committee approval was obtained for the NAPLS2 and NAPLS3 studies at each individual site. Ethical approval for the plasma biomarker analysis in this study was obtained from the Royal College of Surgeons in Ireland research ethics committee (REC No. 202211009).

3. Results

3.1. Participant characteristics

Participant characteristics are detailed in Table 1. At baseline, participants had a median SOPS negative symptom score of 12 (interquartile range [IQR]: 8–16) and a median SOPS positive symptom score of 13 (IQR: 11–15). Seven participants (1.6 %) were excluded from the adjusted analyses due to missing information for non-proteomic independent variables, giving a sample size of n = 431. Characteristics of included and excluded participants are presented in Supplementary Table 1.

3.2. Clinical and demographic prognostic factors of negative symptoms

The following clinical and demographic prognostic factors were included in all regression models: SOPS Negative symptom scores, SOPS Positive symptom scores, Global Assessment of Functioning scores, Calgary Depression Scale for Schizophrenia scores, suicidal ideation, cannabis use, tobacco use, antipsychotic use, antidepressant use, age, and sex.

Baseline negative symptoms were a strong prognostic factor for follow-up negative symptoms (β: 0.555, 95 % CI: 0.488, 0.621) and contributed to 52 % of explained variation in follow-up negative symptoms. Other prognostic factors for follow-up negative symptoms in the CHR sample included Global Assessment of Functioning scores, SOPS Positive symptom scores, Calgary Depression Scale for Schizophrenia scores, and age, such that older age, less severe depressive symptoms, more severe positive symptoms and worse functioning predicted more severe follow-up negative symptoms (see Supplementary Table 3 for predictor coefficient estimates and Supplementary Fig. 1 for the relative contribution of prognostic factors to the prediction of follow-up negative symptoms).

3.3. Complement and coagulation protein groups

There were thirty proteins of the complement or coagulation pathways quantified in more than 70 % of samples which were brought forward for analysis. To represent plasma levels of complement components, complement regulators, coagulation factors and coagulation regulators, the first principal component of each protein group was derived (see Supplementary Table 4 for correlations between individual proteins and the first principal components in each protein group; Supplementary Fig. 2 for the cumulative proportion of variance in protein groups captured by principal components).

3.4. Complement and coagulation protein groups as prognostic factors of negative symptoms

Adjusting for all clinical and demographic prognostic factors, there was no evidence that complement components and coagulation factors group levels were associated with follow-up negative symptoms (β: 0.156, 95 % CI: −0.187, 0.498; β: 0.068, 95 % CI: −0.280, 0.416 respectively). However, the complement regulators group and the coagulation regulators group were identified as prognostic factors of negative symptoms (β: 0.501, 95 % CI: 0.160, 0.842; β: 0.430, 95 % CI: 0.080, 0.780 respectively, see Table 2), such that higher complement and coagulation regulator levels predicted more severe negative symptoms over the follow-up period. The relative contribution of complement regulators and clinical and demographic prognostic factors to the prediction of follow-up negative symptoms are presented in Fig. 1. Complement regulators and coagulation regulators had a relative explained variation of 1.6 % and 1.1 % for follow-up negative symptoms respectively, compared to functioning (1.6 %), depressive symptoms (1.7 %) and positive symptoms (1.8 %).

3.4.1. Sensitivity Analyses: Complement and coagulation regulators as prognostic factors of negative symptoms

The prognostic value of complement regulators for negative symptoms was consistent in NAPLS2 alone (β: 0.501, 95 % CI: −0.037, 1.039) and NAPLS3 alone, additionally adjusting for BMI in NAPLS3 (β: 0.442, 95 % CI: 0.127, 0.757). The prognostic value of coagulation regulators remained in NAPLS3 adjusting for BMI (β: 0.410, 95 % CI: 0.114, 0.706) but was weaker in NAPLS2 (β: 0.344, 95 % CI: −0.215, 0.903). The relationship between complement or coagulation regulators and negative symptoms was also consistent when additionally adjusting for sample storage time and sample processing time in the overall sample (complement regulators β: 0.535, 95 % CI: 0.195, 0.875 and coagulation regulators β: 0.408, 95 % CI: 0.059, 0.756; Supplementary Table 5). Results of sensitivity analyses excluding individuals with antipsychotic or antidepressant medication use were broadly consistent (Supplementary Tables 6–8), but in addition suggested that coagulation factors could have prognostic value for negative symptoms in individuals not taking antidepressants (β: 0.368, 95 % CI: 0.083, 0.654).

3.5. Complement and coagulation protein groups as prognostic factors of positive symptoms

Adjusting for clinical and demographic prognostic factors, there was no evidence that complement components, complement regulators and coagulation regulators groups were of prognostic value for follow-up positive symptoms (β: 0.103, 95 % CI: −0.245, 0.451; β: 0.052, 95 % CI: −0.298, 0.401; β: 0.038, 95 % CI: −0.316, 0.391 respectively).

Table 2
Unadjusted and adjusted associations between complement and coagulation protein groups and follow-up negative symptoms.

	Unadjusted [a]					*Adjusted* [b]				
Protein group	**Beta coefficient**	**S.E.**	**LCI**	**UCI**	**P Value**	**Beta coefficient**	**S.E.**	**LCI**	**UCI**	**P Value**
Complement components	0.081	0.174	−0.261	0.422	0.644	0.156	0.175	−0.187	0.498	0.373
Complement regulators	0.440	0.175	0.097	0.782	0.012	0.501	0.174	0.160	0.842	0.004
Coagulation factors	0.110	0.173	−0.228	0.449	0.523	0.068	0.178	−0.280	0.416	0.701
Coagulation regulators	0.335	0.177	−0.013	0.682	0.059	0.430	0.179	0.080	0.780	0.016

Protein group members: complement components (C1R, C1S, C1QC, C2, C3, C4A, C4B, CFB, C5, C6, C7, C8A, C8B, C9); complement regulators (C4BPA, SERPING1, CFI, C4BPA, CFH, VTN, CLU); coagulation factors (F2, F12, FGA, FGB, KLKB1, PLG, KNG1); coagulation regulators (SERPINC1, SERPIND1, PROS1, SERPING1). Standard Error: S.E.; Lower Confidence Interval: LCI; Upper Confidence Interval: UCI. [a] Adjusted only for baseline SOPS negative symptoms, i.e., the association with follow-up SOPS negative symptoms taking into account baseline negative symptoms. [b] Adjusted for baseline SOPS negative symptoms, SOPS positive symptoms, Global Assessment of Functioning, Calgary Depression Scale for Schizophrenia, suicidal ideation, cannabis use, tobacco use, antipsychotic use, antidepressant use, age, sex, and study.

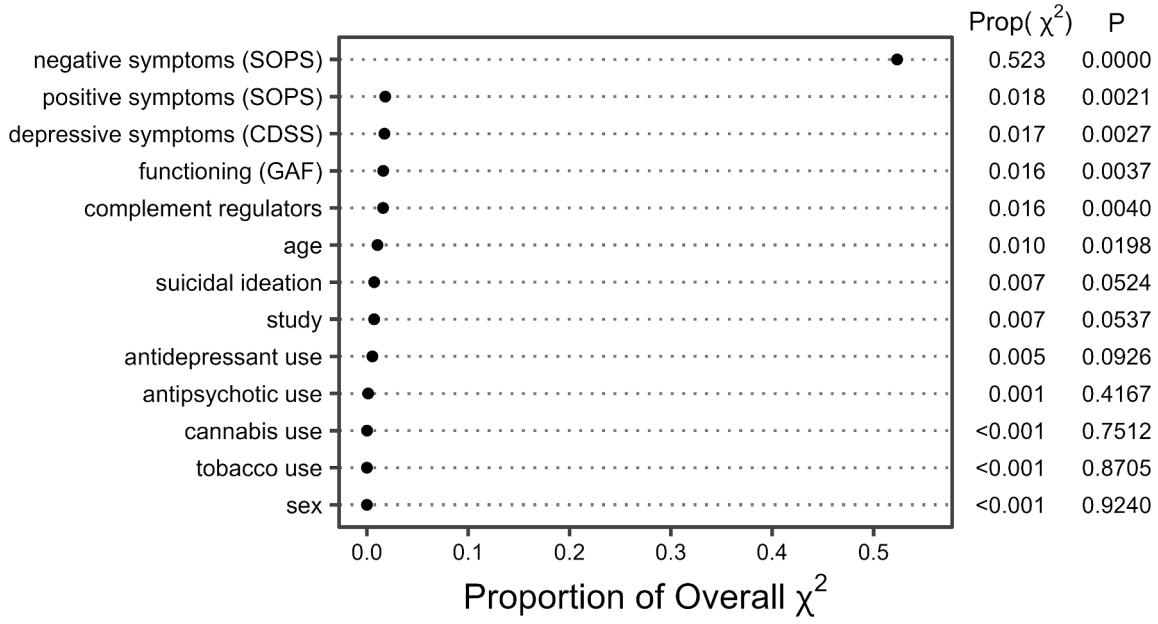

Fig. 1. Relative explained variation in follow-up negative symptoms by complement regulators and clinical and demographic prognostic factors. The proportion of explained variation in follow-up negative symptoms attributed to each prognostic factor in the multivariable model. Relative explained variation was calculated as the ratio of the partial Wald chi-square statistic to the total Wald chi-square statistic. P-values corresponding to Wald chi-square statistics are listed for each variable.

Coagulation factors were the only protein group to have a prognostic association with follow-up positive symptoms (β: 0.370, 95 % CI: 0.014, 0.726; Supplementary Table 9).

3.5.1. Sensitivity Analyses: Coagulation factors as prognostic factors of positive symptoms

There was less evidence for coagulation factors as prognostic factors of positive symptoms in NAPLS2 participants alone (β: 0.295, 95 % CI: −0.296, 0.886), or when additionally adjusting for BMI in NAPLS3 only (β: 0.375, 95 % CI: −0.090, 0.840). Sensitivity analyses additionally adjusting for sample storage time and sample processing time in the overall sample had minimal effect on the results (Supplementary Table 10), as did analyses which were repeated excluding individuals taking antidepressants or antipsychotics (Supplementary Tables 11–13).

4. Discussion

The current investigation found that plasma complement pathway regulator levels were a modest prognostic factor for future negative symptoms in two samples of participants at clinical high-risk for psychosis, NAPLS2 and NAPLS3. This prognostic association was robust, as it was observed independently of several clinical and demographic prognostic factors and remained in sensitivity analyses. Coagulation regulator levels also had a prognostic association with negative symptoms; however, the results were not as robust in sensitivity analyses in comparison to complement regulator levels. There was no evidence that complement and coagulation regulators were prognostic factors of future positive symptoms, although levels of coagulation pathway components were weakly associated with future positive symptoms. Clinical and demographic prognostic factors of future negative symptoms included current negative symptoms, positive symptoms, depressive symptoms, functioning and age.

Prognostic factors can be used to help inform treatment decisions (Riley et al., 2013). For example, interventions which can reduce negative symptoms could potentially be recommended for individuals at clinical high-risk with elevated levels of complement regulators, particularly interventions which have a low-harm potential such as behavioural interventions (Cella et al., 2023). Complement regulator levels could also be included as variables in a prognostic model of future negative symptoms. However, our results indicated that current negative symptoms predict most of the variation in future negative symptoms. Beyond baseline negative symptoms, we observed that positive symptoms, depressive symptoms, functioning, and age were also prognostic factors, such that more severe positive symptoms and functioning, less severe depressive symptoms and older age predicted higher negative symptoms at follow-up. This is largely in agreement with a previous analysis of trajectories of follow-up negative symptoms in the NAPLS3 study (Tran et al., 2023). Of note, the contribution of complement regulator levels to the prediction of follow-up negative symptoms in the present study was similar to that of current positive symptoms, depression, and functioning.

In contrast to previous investigations of associations between proteins and outcomes in psychosis (Dunleavy et al., 2022; Heurich et al., 2022) or individuals at risk of psychosis (Byrne et al., 2023; Heurich et al., 2022); this investigation considered levels of protein groups with a similar function, either directly related to complement and coagulation activation or involved in the regulation of these cascades. Previous studies have repeatedly highlighted the relevance of complement and coagulation proteins to psychosis. Nevertheless, the individual complement and coagulation proteins that have been highlighted have often differed between studies (Byrne et al., 2023; Heurich et al., 2022). In line with this, a systematic review of studies describing the relationship between cytokines and negative symptoms in first-episode psychosis found that six of the eligible studies each reported a relationship between a different cytokine and negative symptoms (Dunleavy et al., 2022). Plasma proteins have great interdependency and redundancy (Miyajima et al., 1992; Nicola, 1994; Sim and Tsiftsoglou, 2004), and therefore investigating the relationship between single proteins and outcomes in psychosis, though more practical, may be an overly simplistic approach. The data reduction approach employed in the current investigation addresses the possibility that broader dysfunction of biological pathways may be related to risk of specific symptom severity.

This investigation took a symptom-based approach rather than a diagnostic approach. It has been highlighted previously that research regarding the relevance of biological markers for individual psychiatric diagnoses may have limited utility, given that different psychiatric diagnoses are unlikely to reflect distinct biological processes, and that

comorbidities are common (Berk, 2023). By adjusting for other psychiatric symptoms, as we have done, the results represent the prognostic value of functional protein groups of interest for negative or positive symptoms, over the prognostic value provided by other symptoms. Moreover, the results relating to complement and coagulation regulators were robust to other potential demographic and lifestyle prognostic factors (age, sex, BMI, smoking, medication use), were not influenced sample processing and storage times (Berk, 2023; Yatham, 2023), and were consistent in NAPLS2 and NAPLS3 when analysed separately.

Currently, there is a lack of effective treatments for negative symptoms of psychosis (Cella et al., 2023; Fusar-Poli et al., 2015; Lutgens et al., 2017). Mechanisms relating to early negative symptoms could be used to inform potential treatment targets for negative symptoms in individuals with a first episode of psychosis. While prognostic factors could be used to guide the development of new interventions (Riley et al., 2013), our analysis did not take a causal approach. We hypothesise that a persistent elevation in regulatory proteins may be due to a lack of immune activation resolution. However, further research using causal frameworks is needed to determine if any mechanistic relationship exists between complement regulator levels and negative symptoms, or if blood complement regulator levels reflect part of a causal process. Nevertheless, our results contribute novel information to research to date on biological correlates of negative symptoms in the broad psychosis phenotype. Aberrant complement and coagulation regulator levels may be related to the association between inflammatory markers and negative symptoms observed in a population-based study (Perry et al., 2021), or analyses suggesting that negative symptoms have a greater genetic basis than positive symptoms do (Ahangari et al., 2023). The present results may also be relevant to a previously observed inverse relationship between gene expression levels of the complement regulator, SERPING1, and cortical thickness (Allswede et al., 2018).

This study has several limitations. Firstly, while several proteins involved in each function were measured, not all components and regulators of the complement and coagulation pathways were measured. The replicability of the individual protein loadings on to each functional group, using different methods of protein quantification, is unclear. Furthermore, while the broad grouping of activating and regulatory components gives an overview of the relative the association of each group with negative symptoms, it does not, however, reflect on the pathway-specific contribution of these regulators. Secondly, by targeting a population with increased positive symptoms, baseline positive symptoms had a truncated distribution and lower variance than baseline negative symptoms. Despite the distribution of follow-up positive symptoms being unconstrained, the lower baseline variance of positive symptoms could have impacted power to detect prognostic factors of positive symptoms, compared to negative symptoms. Thirdly, in this study, negative symptoms were measured using the SOPS. It has been suggested that new studies should employ second-generation negative symptom measurement scales, which measure severity in the five consensus domains of negative symptoms: anhedonia, asociality, avolition, blunted affect and alogia (Fernandez-Egea et al., 2023; Howes et al., 2023). These are measured in the SOPS, but in addition ideational richness and occupational functioning are included in the SOPS negative symptoms. Furthermore, negative symptoms are not part of CHR criteria. However, it is estimated that over half of individuals meeting CHR criteria experience negative symptoms of at least moderate severity and up to 80 % experience at least one negative symptom (Addington et al., 2015; Piskulic et al., 2012; Salazar de Pablo et al., 2023). Moreover, a previous study identified avolition as one of the primary symptoms associated with self-reported reasons for help-seeking in individuals with meeting CHR criteria (Falkenberg et al., 2015).

In conclusion, this study has provided novel insight into biological characteristics that are predictive of future negative symptoms, highlighting the particular relevance of plasma complement pathway and coagulation pathway regulator levels. This information could be used as starting point from which to investigate new potential therapeutic interventions for negative symptoms. The results suggest that complement and coagulation regulators are modest and robust prognostic factors of negative symptoms, information which could have utility in informing treatment decisions, such as recommending specific interventions which aim to reduce negative symptoms to individuals with elevated complement and coagulation regulator levels. Further prognostic factor research will form the basis for the development of a prognostic model for negative symptoms and aid the prevention of severe negative symptoms in individuals at risk.

CRediT authorship contribution statement

Jonah F. Byrne: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Colm Healy:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Melanie Föcking:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Meike Heurich:** Writing – review & editing, Conceptualization. **Subash Raj Susai:** Writing – review & editing, Methodology, Investigation. **David Mongan:** Writing – review & editing, Methodology, Investigation. **Kieran Wynne:** Writing – review & editing, Methodology, Investigation. **Eleftheria Kodosaki:** Writing – review & editing, Conceptualization. **Scott W. Woods:** Writing – review & editing, Investigation. **Barbara A. Cornblatt:** Writing – review & editing, Investigation. **William S. Stone:** Investigation, Writing – review & editing. **Daniel H. Mathalon:** Investigation, Writing – review & editing. **Carrie E. Bearden:** Investigation, Writing – review & editing. **Kristin S. Cadenhead:** Investigation, Writing – review & editing. **Jean Addington:** Investigation, Writing – review & editing. **Elaine F. Walker:** Investigation, Writing – review & editing. **Tyrone D. Cannon:** Investigation, Writing – review & editing. **Mary Cannon:** Conceptualization, Writing – review & editing. **Clark Jeffries:** Conceptualization, Writing – review & editing. **Diana Perkins:** Conceptualization, Investigation, Writing – review & editing. **David R. Cotter:** Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Requests for access to study data can be made to the NAPLS coordinators (napls.ucsf.edu). The code used to generate the results in this study are available upon request from the corresponding author.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2024.03.049.

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