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Association of Complement and Coagulation Pathway Proteins With Treatment Response in First-Episode Psychosis: A Longitudinal Analysis of the OPTiMiSE Clinical Trial

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***Background and Hypothesis*: Treatment response to specific antipsychotic medications is difficult to predict on clinical grounds alone. The current study hypothesizes that the baseline complement pathway activity predicts the treatment response and investigates the relationship between baseline plasma biomarkers with treatment response to antipsychotic medications. *Study Design*: Baseline plasma samples were collected from first episode of psychosis patients (n = 243) from a multi-center clinical trial. The participants were treated with amisulpride for 4 weeks. Levels of complement and coagulation proteins at baseline were measured using both data-dependent and data-independent mass spectrometry approaches. The primary outcome was remission status at 4 weeks and the secondary outcomes included change in psychotic and functional symptoms over the period of treatment. In addition, immunoassays were performed at baseline for complement C1R, as well as for activation markers C4a and sC5b-9. *Study Results*: The plasma level of complement variant C4A was significantly associated with remission at 4 weeks. Moreover, higher levels of several complement and coagulation pathway proteins were associated with a reduction in psychotic symptoms and an improvement in functioning. Immunoassays showed an association of baseline levels of C1R and C4a as well as complement activation marker sC5b-9 levels with treatment response. *Conclusion*: The results demonstrated that the response to antipsychotic treatment might be related to pretreatment levels of plasma complement and coagulation pathway proteins. This is consistent with independent evidence associating immune dysfunction with the pathophysiology of psychosis. Moreover, these results inform the development of novel therapeutic approaches that target the complement system for psychosis.**

Key words: Schizophrenia/psychotic disorder/immune markers/biomarkers/complement system proteins/antipsychotic agents

Introduction

Around a third of patients with psychosis do not respond to initial treatment with antipsychotic medication.[1–3] Because the response to treatment is difficult to predict based on the clinical presentation, the effectiveness of antipsychotic medication in an individual patient has to be determined empirically, through the careful evaluation of a course of treatment. However, recent research suggests that it may be possible to predict the therapeutic response by assessing biomarkers, measured using neuroimaging[4–7] or in peripheral blood.[8–10]

The role of immune system dysfunction in psychosis is increasingly acknowledged.[11–17] More specifically, there is some evidence that response to antipsychotic medication may be related to the plasma levels of inflammatory markers[8,10,18] and auto-antibodies[19] prior to treatment. Immune dysfunction may also be manifested in changes in the concentration of proteins of the complement system.[20,21] Complement activity in both the peripheral and the central nervous system is crucial to the overall immune response. Complement proteins are mostly present in the circulation as inactive zymogens, which are converted into active enzymes and proteolytic fragments acting as opsonins or chemotactic molecules. This is triggered through the activation of 1 of the 3 complement pathways: the classical, lectin, and alternative pathways (figure 1). These pathways converge in a terminal common pathway that results in formation of the membrane attack complex (MAC or C5b-9). Complement C1R is one of the initiating serine proteases of the C1 complex, crucial for propagation of the classical complement pathway leading to C4 cleavage into proteolytic fragments C4b (opsonin) and C4a. Sequential proteolytic cleavage of C4 and C2 allows the formation of the C3 convertase (C4b2a), which cleaves C3, and if left unregulated, C5.[20] This generates quantifiable activation products, including C4a, the anaphylatoxins C3a and C5a, and terminal pathway activation marker sC5b-9.[22] C4 exists as 2 isotypes, C4A and C4B. A Genome-wide association study reported that the C4 alleles of the major histocompatibility complex were linked to an increased risk of psychosis, and that this risk was proportionate to the expression of C4A.[23–28] We recently found that altered plasma levels of complement and coagulation pathway proteins, which are closely connected to each other, were associated with the onset of psychosis in people at clinical high-risk.[29–31]

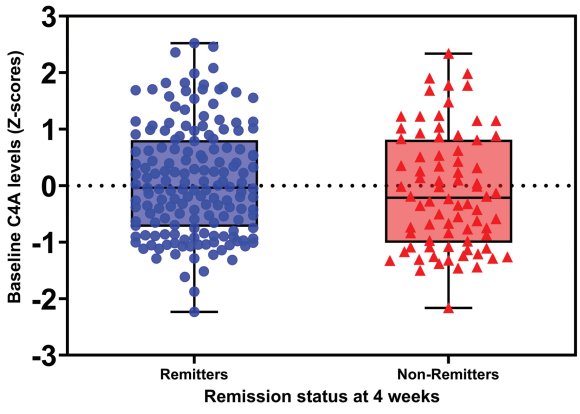

Fig. 1. Group difference in pre-treatment complement C4A levels between patients who were in remission, or not in remission after 4 weeks of treatment with amisulpride.

The aim of the present study was to investigate the relationship between levels of complement and coagulation proteins and the response to treatment with antipsychotic medication. By conducting the study in patients with first-episode psychosis who were either medication-naïve or had minimal previous exposure to antipsychotic medications, the potentially confounding effects of previous treatment were minimized. Moreover, because all the patients were treated with the same antipsychotic medication (amisulpride) in a clinical trial, differences in response were unlikely to be due to variation in the type or dose of treatment. We previously performed a pilot study based on a subset of participants in the OPTiMiSE trial (optimization of treatment and management of schizophrenia in Europe) using data-dependent acquisition (DDA) discovery-based proteomic analysis of plasma samples.[32] Comparing responders to non-responders, there was preliminary evidence for dysregulation of several complement and coagulation proteins in relation to responders.

In the current study, we tested the hypothesis that the levels of complement and coagulation proteins prior to treatment would be associated with the subsequent response to treatment, as indexed by remission status and the change in symptom severity. To test our hypothesis, we undertook 2 complementary mass spectrometry approaches. First, we undertook a discovery-based approach called "data-dependent acquisition" (DDA), to extend our earlier pilot study[32] to the whole OPTiMiSE population and to confirm the findings using the same mass spectrometric methods as previously. Second, to investigate associations between proteins and symptomatic and treatment response, we used a data-independent acquisition (DIA) mass spectrometry approach to allow us to extract data on complement and coagulation pathway proteins in a more targeted manner.

Materials and Methods

Participants

The patients were participants in the OPTiMiSE study, a multi-center trial of amisulpride conducted at 20 institutions and 15 countries (Clinicaltrials.gov identifier NCT01248195). The trial protocol has been described in detail elsewhere.[33] Five-hundred patients with the

first episode of schizophrenia, schizophreniform disorder, or schizoaffective disorder as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) were verified with the Mini International Neuropsychiatric Interview Plus.[34] Participants were aged between 18 and 40 years, had a maximum interval of 2 years between the onset of psychosis and study entry, and had a history of antipsychotic medication of no longer than 2 weeks in the previous year or 6 weeks lifetime.[33] Ethical approval for the proteomic analyses was granted by the Research Ethics Committee of the Royal College of Surgeons Ireland [REC No: 1699].

Outcomes

We examined 3 outcomes. The primary outcome was symptomatic remission as defined by Andreasen et al.[35] Participants were assessed for remission after treatment for 4 weeks. In secondary analyses, we examined 2 further outcomes: a change in Positive and Negative Symptom scale (PANSS)[36] score from baseline to 4 weeks and a change in Personal and Social Performance Scale (PSP)[37] score from baseline to 4 weeks.

Mass Spectrometry Based DDA and DIA Proteomics

Two different mass spectrometry (MS) based proteomic methods were used to relatively quantify the levels of plasma proteins.

First, data-dependent acquisition (DDA) was performed to measure the relative abundance of complement and coagulation proteins in the immunodepleted plasma. Second, the proteins were quantified using targeted extraction from data-independent acquisition (DIA), which was based on sequential window acquisition of all theoretical mass spectra (SWATH) analysis in which all peptide precursor ions in the scanning interval are scanned at ultra-high speed and subjected to secondary fragmentation.[38,39]

The protocols for these 2 methods are described in detail in the supplementary methods.

Enzyme-Linked Immunoassay (ELISA) Quantifying Complement Protein C1R, and Activation Markers C4a and sC5b-9

We used standardized enzyme-linked immunosorbent assays (ELISA) to validate key mass spectrometry findings and measure the impact on complement activation by quantifying plasma concentrations of C1R (ABCAM Ltd, USA), and downstream activation markers C4a (Abbexa Ltd, UK) and sC5b-9 (Pathway Diagnostics Ltd, UK). C4a is proportional to C4 activation and sC5b-9 measures complement activation of the common terminal pathway (see supplementary methods).

Statistical Analyses

Statistical analyses were conducted using IBM SPSS statistics version 26 and STATA IC version 16. The set of complement and coagulation proteins that were analyzed were selected based on the UNIPROT functional annotation of pathway proteins. The LFQ values of plasma proteins that were expressed in at least 70% of the samples were considered for the statistical analysis. The mass spectrometry proteomic data were $\log_2$ transformed and standardised to *z*-scores for further analysis.

Associations Between Protein Levels and Remission Status We first tested whether the levels of complement and coagulation proteins at baseline were associated with remission status (remission/non-remission) after 4 weeks of treatment. For this analysis, baseline standardized plasma protein levels were measured using both DDA and DIA proteomic approaches. The proteomic data were used as the independent variable. For both DDA and DIA data, associations with remission status at 4 weeks were evaluated for each protein using logistic regression models adjusted for age, sex, BMI, smoking status (current smoker/past smoker/no smoker), and study site. *P*-values were corrected for multiple comparisons using the Benjamini-Hochberg method with a false discovery rate (FDR) of 5%.[40] A receiver operator curve (ROC) analysis was performed for the significant protein C4A to assess its diagnostic value to predict remission status in FEP participants.

Association Between Plasma Protein Levels and Changes in Symptoms and Functioning To evaluate associations between individual protein levels at baseline and changes in psychotic symptoms and functioning, we used the absolute protein levels from the DIA-SWATH proteomics as exposures. Linear regression analyses were performed using changes in PANSS total score and PSP score between baseline and 4 weeks as outcomes and each protein as the exposure. Models were adjusted for age, sex and BMI, smoking status, study site, and baseline PANSS/PSP score. *P*-values were corrected for multiple comparisons using the Benjamini-Hochberg method with a false discovery rate (FDR) of 5%.[40]

Enzyme-Linked Immunoassay (ELISA) Quantifying Complement Protein C1R, and Activation Markers C4a and sC5b-9

Finally, we examined associations between log-transformed standardized concentrations of the complement component C1R, and activation fragments C4a and sC5b-9, as measured by ELISA, and the outcomes measures (see supplementary methods).

Results

Complement and Coagulation Proteins From DDA and DIA Proteomic Analysis

Of the 534 participants initially recruited, a total of 294 participants had provided baseline samples and had also completed Phase I of the clinical trial. This subgroup was considered for the present study and the proteomic analysis was performed in baseline plasma samples from 294 participants. After the exclusion of participants who already met remission criteria at baseline, 243 participants with complete baseline and follow-up clinical data comprised the analytical sample. The baseline characteristics of the sample are given in table 1.

Data-dependent acquisition (DDA) mass spectrometry identified a total of 455 plasma proteins in all samples. Of these, 111 proteins passed the quality control (identified in more than 70% of plasma samples) and the missing values were imputed using minimum LFQ values of corresponding protein. Out of 111 proteins, 34 proteins belonged to the complement and coagulation cascade activity (proteins of interest) and these proteins were considered for statistical analysis (supplementary table 1). Using DIA proteomics (SWATH), a total of 648 proteins were qualified (based on the coefficient of variation filtering). Since the DIA quantification is reproducible, the samples with poor co-efficient of variation (CVs) were rerun and the raw data were normalized in MSStats data analysis pipeline (see supplementary methods). Out of 648 proteins, 48 proteins belonged to the complement and coagulation cascade (proteins of interest). The baseline plasma levels of these proteins were further used for statistical analysis (supplementary table 2).

Associations With Remission Status After 4 Weeks of Treatment

Data-Dependent Acquisition (DDA) In the discovery proteomic approach (DDA data), complement components C1R, C2, and coagulation factor XII (F12) were associated with remission status at 4 weeks with odds ratios (95% CI) of 1.42 (1.08, 1.88), 1.47 (1.04–1.88), and 1.40 (1.04, 1.93), respectively. However, these associations were not significant after adjusting for multiple corrections (supplementary table 1).

Data-Independent Acquisition (DIA) In logistic regression models of the DIA proteomic data, baseline plasma levels of complement protein C4A associated with the primary outcome (remission status) after 4 weeks of treatment, with an odds ratio of 1.60 [CI = 1.20, 2.14] (FDR adjusted *p*-value .046) (supplementary table 2). The group difference between remitters and non-remitters for C4A is shown in figure 1. In the ROC analysis, baseline levels of C4A indicated a prediction of remission status with an area under the curve (AUC) of 63% (p = .001) (supplementary figure 1). Other complement and coagulation proteins at baseline did not show any association with remission status (supplementary table 2).

Associations With Changes in Psychotic Symptoms

In linear regression models using DIA data, the levels of 16 proteins at baseline were significantly associated (after FDR correction) with a reduction in total PANSS score over the first 4 weeks of treatment (table 2). These comprised 11 complement proteins (complement C1R, C1S, C2, C5, C6, C7, C8A, C8B, CFI, FCN3, and MASP1),

Table 1. Baseline Characteristics of a Sub-group of Participants Who Showed Remission (Remitters) and Did Not Show Remission (Non-Remitters) to Amisulpride From the OPTiMiSE Trial

Baseline Characteristics		Non-Remitters (n = 78)	Remitters (n = 165)
Age in years, mean ± SD		24 ± 5	27 ± 7
Sex	Female, *n* (%)	58 (74.4%)	110 (66.7%)
	Male, *n* (%)	20 (25.6%)	55 (33.3%)
BMI in kg/m^2, mean ± SD		23.76 ± 4.62	23.16 ± 3.95
Smoking status	Never smoked, *n* (%)	27 (34.6%)	67 (40.6%)
	Current smoker, *n* (%)	45 (57.7%)	75 (45.5%)
	Past smoker, *n* (%)	6 (7.7%)	22 (13.3%)
PANSS, mean ± SD	Total	85.65 ± 16.45	78.98 ± 17.66
	Positive	21.33 ± 5.32	20.45 ± 5.28
	Negative	23.03 ± 7.27	19.3 ± 6.87
	General	41.29 ± 8.71	39.22 ± 9.35
Personal and Social Performance Scale, mean ± SD		45 ± 15	47 ± 14
Calgary Depression Scale, mean ± SD		13.84 ± 4.56	13.58 ± 4.68

Note: SD, Standard deviation; BMI, body mass index; PANSS, positive and negative symptom score.

Table 2. Results of Linear Regression Model Showing Association of Complement and Coagulation Proteins With Change in Psychopathology Scores After 4 Weeks of Treatment

Outcome	Change in Total PANSS					Change in PSP Scores				
Protein Names	Coef.	*P* Value	[95% Conf. Interval]		FDR *p* value	Coef.	*P* value	[95% Conf. Interval]		FDR *p* value
Mannan-binding lectin serine protease	1.36	.18	−0.63	3.35	.26	−0.35	.78	−2.32	1.62	.84
Ficolin-3	***−4.11***	***.00****	***−6.20***	***−2.02***	***.00****	2.82	.01*	0.73	4.91	.06
Coagulation factor XIII A chain	1.56	.12	−0.41	3.54	.20	0.04	.96	−1.88	1.97	.96
Prothrombin	−2.21	.03*	−4.20	−0.22	.07	1.68	.09	−0.29	3.65	.22
Complement C1R subcomponent	***−4.39***	***.00****	***−6.35***	***−2.44***	***.00****	***3.31***	***.00****	***1.37***	***5.25***	***.02****
Coagulation factor IX	***−3.73***	***.00****	***−5.69***	***−1.77***	***.00****	2.36	.02*	0.41	4.32	.09
Coagulation factor X	***−3.73***	***.00****	***−5.69***	***−1.76***	***.00****	***2.98***	***.00****	***1.09***	***4.88***	***.02****
Complement factor D	−1.50	.14	−3.48	0.47	.21	−0.14	.89	−2.06	1.79	.93
Coagulation factor XII	−0.68	.51	−2.71	1.34	.63	1.00	.32	−0.97	2.98	.48
Complement factor B	−1.90	.07	−3.94	0.15	.15	2.06	.04*	0.06	4.06	.12
Complement C3	−1.02	.33	−3.07	1.04	.43	0.45	.66	−1.54	2.43	.84
Complement C5	***−2.61***	***.01****	***−4.60***	***−0.62***	***.03****	1.79	.07	−0.18	3.76	.18
Fibrinogen alpha chain	−0.45	.66	−2.48	1.58	.73	0.30	.76	−1.64	2.23	.84
Fibrinogen beta chain	−0.38	.72	−2.40	1.65	.75	0.12	.91	−1.83	2.06	.93
Fibrinogen gamma chain	−0.48	.64	−2.52	1.56	.73	0.46	.64	−1.49	2.41	.84
Complement C1q subcomponent subunit A	−0.55	.59	−2.56	1.46	.70	−0.34	.74	−2.32	1.64	.84
Complement C1q subcomponent subunit B	0.41	.69	−1.60	2.42	.74	−1.17	.23	−3.08	0.74	.44
Complement C1q subcomponent subunit C	−1.70	.09	−3.69	0.29	.17	0.98	.32	−0.97	2.92	.48
Complement component C9	−1.76	.08	−3.75	0.24	.17	1.07	.28	−0.89	3.03	.47
Coagulation factor XI	−1.70	.10	−3.71	0.30	.17	0.68	.49	−1.27	2.62	.67
Vitronectin	−1.29	.20	−3.29	0.71	.28	2.16	.03*	0.20	4.11	.11
Complement factor I	***−3.62***	***.00****	***−5.62***	***−1.62***	***.00****	***3.45***	***.00****	***1.52***	***5.39***	***.02****
Coagulation factor XIII B chain	1.05	.31	−0.96	3.06	.41	−0.37	.71	−2.33	1.59	.84
Heparin cofactor 2	***−3.46***	***.00****	***−5.45***	***−1.47***	***.00****	***3.70***	***.00****	***1.77***	***5.63***	***.00****
Complement C2	***−2.98***	***.00****	***−4.94***	***−1.02***	***.01****	1.60	.10	−0.33	3.52	.23
Complement component C8 alpha chain	***−4.05***	***.00****	***−6.01***	***−2.10***	***.00****	2.44	.02*	0.48	4.40	.09
Complement component C8 beta chain	***−3.02***	***.00****	***−4.98***	***−1.06***	***.01****	1.33	.18	−0.62	3.28	.37
Complement component C8 gamma chain	0.09	.93	−1.90	2.07	0.93	1.19	.22	−0.70	3.08	.43
Complement factor H	−1.83	.09	−3.92	0.27	0.17	1.19	.25	−0.83	3.20	.45
Complement C1s subcomponent	***−3.29***	***.00****	***−5.28***	***−1.31***	***0.00****	2.11	.04*	0.15	4.08	.12
Complement C4-A	−2.34	.02*	−4.31	−0.37	0.05	1.97	.04*	0.07	3.87	.12
Complement C4-B	−1.62	.12	−3.66	0.41	0.20	−0.73	.46	−2.67	1.20	.65
Complement component C7	**−2.48**	**.01***	**−4.44**	**−0.52**	**0.04***	1.05	.29	−0.89	2.99	.47
Clusterin	−2.35	.02*	−4.35	−0.35	0.06	***2.82***	***.01****	***0.87***	***4.77***	***.05****
Mannose-binding protein C	1.37	.18	−0.62	3.37	0.26	−1.00	.31	−2.94	0.93	.48
Coagulation factor V	1.69	.10	−0.31	3.68	0.17	−1.96	.04*	−3.83	−0.09	.12
Complement component C6	***−3.98***	***.00****	***−5.89***	***−2.06***	***0.00****	2.18	.03*	0.25	4.12	.10
Fibulin-1	***−2.79***	***.01****	***−4.76***	***−0.82***	***0.02****	1.85	.06	−0.06	3.76	.15
Complement factor H-related protein 2	−0.13	.90	−2.13	1.87	0.92	0.30	.77	−1.66	2.26	.84
Mannan-binding lectin serine protease 1	***−2.93***	***.00****	***−4.92***	***−0.93***	***0.01****	2.33	.02*	0.40	4.25	.09
Complement factor H-related protein 1	−0.81	.42	−2.79	1.16	0.53	0.32	.74	−1.60	2.24	.84

Table 2. Continued

Outcome	Change in Total PANSS					Change in PSP Scores				
Protein Names	Coef.	*P* Value	[95% Conf. Interval]		FDR *p* value	Coef.	*P* value	[95% Conf. Interval]		FDR *p* value
Multimerin-1	−0.58	.57	−2.59	1.42	0.69	0.25	.80	−1.68	2.19	.85
Ficolin-2	0.43	.67	−1.54	2.40	0.73	0.70	.46	−1.19	2.59	.65
Complement factor H-related protein 5	1.98	.05	−0.02	3.97	0.12	−2.31	.02*	−4.23	−0.39	.09
Complement C1R subcomponent-like protein	−1.46	.15	−3.43	0.52	0.23	1.06	.27	−0.83	2.95	.47
Protein Z-dependent protease inhibitor	***−3.29***	***.00****	***−5.23***	***−1.35***	***0.00****	2.23	.02*	0.33	4.14	.09

Note: The results of significant proteins from the complement and coagulation pathway were marked bold and italic. FDR-False discovery rate, *significant, PANSS, Positive and Negative Symptom Scale; PSP, Personal and Social Performance scale.

and 5 coagulation pathway proteins (coagulation factor F9, F10, FBLN1, Serine protease inhibitor SERPINA10, and SERPIND1).

Associations With Change in Functional Status

In linear regression models using DIA data, increased expression of baseline levels of 5 proteins were associated with increased PSP score from baseline to 4 weeks of treatment (table 2): C1R (β 3.3, 95% CI 1.37–5.35, p = .015); CFI (β 3.5, 95% CI 1.52–5.39, p = .015); Clusterin (β 2.8, 95% CI 0.87–4.77, p = .05); Coagulation factor F10 (β 2.9, 95% CI 1.09–4.88); and SERPIND1 (β 3.7, 95% CI 1.77–5.63, p < .001).

Enzyme-Linked Immunoassay (ELISA) Results

In a logistic regression model, the baseline C4a activation marker concentration was significantly associated with remission status [Odds ratio (CI) = 1.48 (1.08–2.04); p-value = .02] (table 3). This is in alignment with the proteomics C4A findings, which also detected an increased expression of C4A associated with remission status. In the proteomic analysis, the high levels of protein C1R at baseline showed a significant association with a reduction in PANSS score. Using ELISA quantification, we observed a similar trend of association between baseline C1R concentration and a reduction in PANSS score (coef = −0.27, 95% CI = −0.54, 0.001, p = .05) (supplementary figure 2). The sC5b-9 concentration was inversely associated with an increase in PANSS score (coef = 0.28, 95% CI = 0.03, 0.53, p = .03) (table 3).

Discussion

The present study examined the relationship between complement and coagulation proteins and the response to treatment in psychosis. We tested the hypothesis that the levels of these proteins at baseline would be associated with the response to subsequent treatment with antipsychotic medication. Our results from both DDA and DIA approaches provided results pointing to a similar trend indicating an association between increased baseline levels of complement and coagulation proteins with treatment response to antipsychotics. The DDA proteomic results of complement and coagulation proteins indicated that higher levels of C1R, C2, and F12 were associated with remission (supplementary table 1), but these findings did not survive adjustment for multiple comparisons. Qualitatively similar findings were evident with a DIA proteomic analysis (supplementary table 2), which identified a strong association between higher levels of C4A with remission following amisulpride treatment in FEP. The association between C4A variant levels and remission was further evaluated by quantifying C4 activation fragment C4a, which was similarly associated with remission, indicating an association between elevated C4 activation with treatment response in FEP. In a ROC analysis, C4A indicated a diagnostic property with an AUC value of 63% for predicting remission status in FEP. Collectively, these results suggest that elevated baseline levels of several complement and coagulation proteins are associated with a positive early clinical response to amisulpride.

The DIA proteomic investigation focused on the complement and coagulation pathways and showed an association for 19 complement and coagulation proteins with the change in PANSS-rated symptom score over 4 weeks of treatment. Out of these, 16 proteins (C1R, C1S, C2, C5, C6, C7, C8A, C8B, CFI, FCN3, MASP1, F9, F10, FBLN1, SERPINA10, and SERPIND1) demonstrated a significant association after FDR correction. Similarly, increased expression of 17 complement and coagulation proteins was associated with improvement in PSP-rated functioning over the same period. Of these proteins, the association of 5 proteins (C1R, CFI, CLU, F10, and SERPIND1) remained significant after adjusting for multiple comparisons. Among the proteins that were associated with treatment response, the classical complement protein C1R demonstrated a strong association

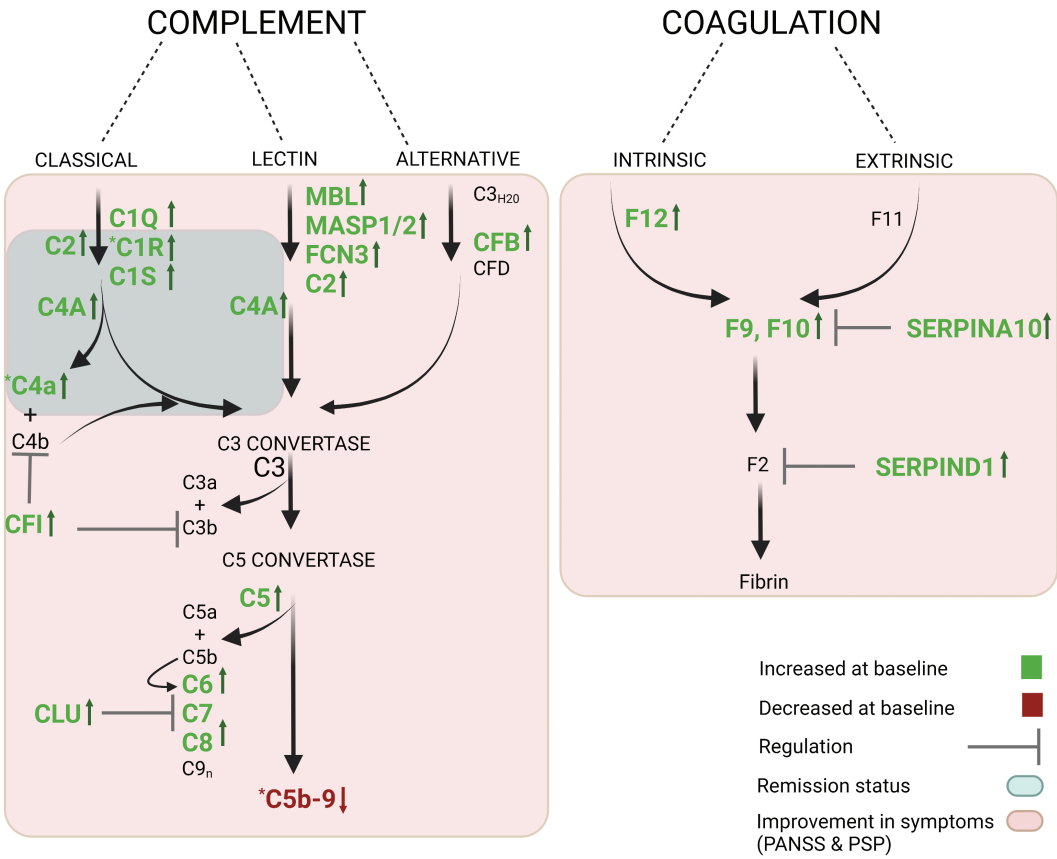

Fig. 2. Schematic representation of complement cascade proteins dysregulated at baseline in participants with a poor symptomatic response to treatment Created with BioRender.com.

with both, a decrease in psychotic symptoms and an improvement in functioning. This was confirmed in the C1R ELISA (supplementary figure 2).

Our findings showed that increased levels of both the activators and some inhibitors of the complement cascade are associated with symptomatic improvement in FEP patients (Figure 2). For instance, C1R contributes to the activation of the classical complement pathway, while complement factor I (CFI) is an integral complement inhibitor acting across all pathways.[41,42] Clusterin is a crucial terminal pathway inhibitor of the terminal product of the complement pathway called C5b-9. The fluid phase sC5b-9 which was quantified in our study is a biomarker of overall complement activity.[42] In contrast to other complement pathway proteins, the terminal activator complex sC5b-9 levels were significantly lower at baseline among subjects who responded well to amisulpride. Although we observe comparatively lower sC5b-9 levels, the absence of healthy controls limits us from understanding whether the changes in complement activation markers in relation to treatment response is a return to, or a further deviation from, normal physiological limits. Further mechanistic studies are required to elucidate the precise sequence of these changes.

Other than the biological significance, the results carry a potential clinical relevance in the prediction of remission after antipsychotic medication. The ROC analysis of baseline levels of complement protein C4A indicated a weak prediction of remission status (63% AUC) at follow-up after amisulpride treatment. However, the association of several complement cascade proteins with symptomatic response suggests that future studies with a combined complement score calculated from multiple complement proteins could have more predictive value than single biomarkers for the prediction of treatment response in FEP. Several studies have investigated the role of peripheral complement pathway proteins in the pathophysiology of psychosis[43,44] including the involvement of C4A, which has been associated with schizophrenia risk in genome-wide association studies.[23,24] To the best of our knowledge, the present study is the first to examine the relationship of several complement cascade proteins with symptomatic response to antipsychotic medication. A recent study by Mondelli et al. in patients with psychosis found that a higher baseline C4 level was associated with the number of relapses of psychosis in FEP at one-year follow-up.[9] Other studies have investigated associations

Table 3. Results of Logistic Regression Models for Immune-Assays of C5b-9, C1R, and C4a (Immuno-Assay Data) With the Treatment Response

Proteins at Baseline	Outcome	Odds Ratio	*p*-value	[95% Conf. Interval]	
C4a	Remission status	***1.48***	***.016****	***1.08***	***2.04***
C1R		1.11	.496	0.83	1.48
C5b-9		1.02	.896	0.76	1.36
Proteins at Baseline	**Outcome**	**Coefficient**	***p*-value**	**[95% Conf. Interval]**	
C4a	Change in PANSS total score	−0.051	.69	−0.30	0.20
C1R		−0.268	.05	−0.54	0.001
C5b-9		***0.279***	***.03****	***0.026***	***0.532***
Proteins at Baseline	**Outcome**	**Coefficient**	***p*-value**	**[95% conf. Interval]**	
C4a	Change in PSP total score	−0.089	.47	−0.327	0.150
C1R		0.053	.69	−0.211	0.317
C5b-9		0.060	.64	−0.188	0.308

Note: The results of significant proteins from the complement and coagulation pathway were marked bold and italic. For C5b-9 and C1R the participants were grouped based on the change PANSS score from baseline to 4 weeks follow-up. The odds ratio was obtained based on the association of baseline proteins predicting the group with higher reduction in PANSS score. For C4a the association of baseline protein levels were analyzed against the remission status based on the Andreasen criteria. C, complement; PANSS, Positive and Negative Symptom Scale.

between other peripheral immune markers and the response to treatment in psychosis. In the OPTiMiSE cohort, Martinuzzi et al. found an association between non-remission after 4 weeks of treatment and lower levels of the cytokine interleukin (IL)-15, but higher levels of the C-X-C motif chemokine 12 (CXCL12).[45] They also reported that in participants who were medication-naïve, plasma neurotrophin levels were associated with poor functional status after follow-up at 1-year, suggesting the existence of different pathological mechanisms in relation to antipsychotic medication.[46] Similarly, lower cortisol-awakening response and higher cytokine levels (IL-6 and TNF-α) have been linked with a poor response to antipsychotic treatment after 12 weeks,[8] whereas serum levels of NMDA receptor auto-antibodies do not appear to predict treatment response.[47]

Our study has several strengths. Investigating first-episode patients with minimal previous exposure to treatment with antipsychotic medication reduced the risk that the response to the trial medication was confounded by the effects of previous treatment or chronic illness. Moreover, all patients were treated with the same antipsychotic medication, following the same dose regimen, so differences in response are unlikely to have been related to variations in the type or dose of medication. We used 2 independent, state-of-the-art mass spectrometry-based approaches (DDA-based discovery and DIA-SWATH semi-targeted analysis) that provided complementary results. In the current study, we restricted the MS-based proteomic analysis to samples that passed stringent quality criteria filters, excluding 16% of the original plasma samples. Several findings were validated using ELISA-based methods.

The study has several limitations. The 2 proteomic methods used slightly different methods of plasma protein depletion, and the influence of such differences in methodology on relative abundance of valuable proteins is not addressed.[48–50] In addition, the large-scale discovery proteomic analysis did not find an association of complement protein C4A variant levels and treatment response, despite there being a strong association found in the DIA analysis. C4A, which was identified in the discovery of MS data in more than 40% of the samples, did not pass the stringent quality criteria applied, and therefore the label-free quantification intensities (normalized measures) of C4A were not considered for the DDA data analysis. Finally, the present study examined group-level differences in biomarkers and is not able to address whether proteomic measures can be used to make predictions about treatment response at the individual level. We plan to investigate this in subsequent work.

Conclusion

We report evidence for an association of increased levels of several complement and coagulation proteins in plasma with early clinical response to amisulpride in first-episode psychosis. These results are consistent with evidence that immune dysfunction contributes to the pathophysiology of psychosis and may inform the development of novel therapeutic approaches for psychosis that target the complement system.

Supplementary Material

Supplementary material is available at https://academic.oup.com/schizophreniabulletin/.

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