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Differential expression of haptoglobin in individuals at clinical high risk of psychosis and its association with global functioning and clinical symptoms

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A B S T R A C T

Background: Immune dysregulation has been observed in patients with schizophrenia or first-episode psychosis, but few have examined dysregulation in those at clinical high-risk (CHR) for psychosis. The aim of this study was to examine whether the peripheral blood-based proteome was dysregulated in those with CHR. Secondly, we examined whether baseline dysregulation was related to current and future functioning and clinical symptoms.

Methods: We used data from participants of the North American Prodromal Longitudinal Studies (NAPLS) 2 and 3 (n = 715) who provided blood samples (Unaffected Comparison subjects (UC) n = 223 and CHR n = 483). Baseline proteomic data was quantified from plasma samples using mass spectrometry. Differential expression was examined between CHR and UC using logistic regression. Psychosocial functioning was measured using the Global Assessment of Functioning scale (GAF). Symptoms were measured using the subscale scores from the Scale of Psychosis-risk Symptoms; positive, negative, general, and disorganised. Three measures of each outcome were included: baseline, longest available follow-up (last follow-up) and most severe follow-up (MSF). Associations between the proteomic data, GAF and symptoms were assessed using ordinal regression.

Results: Of the 99 proteins quantified, six were differentially expressed between UC and CHR. However, only haptoglobin (HP) survived FDR-correction (OR:1.45, 95 %CI:1.23–1.69, $p_{adj} = <0.001$). HP was cross-sectionally and longitudinally associated with functioning and symptoms such that higher HP values were associated with poorer functioning and more severe symptoms. Results were evident after stringent adjustment and poorer functioning was observed in both NAPLS cohort separately.

Conclusion: We demonstrate that elevated HP is robustly observed in those at CHR for psychosis, irrespective of transition to psychosis. HP is longitudinally associated with poorer functioning and greater symptom severity. These results agree with previous reports of increased HP gene expression in individuals at-risk for psychosis and with the dysfunction of the acute phase inflammatory response seen in psychotic disorders.

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1. Introduction

Psychotic disorders are among the most severe debilitating psychiatric disorders globally. Several studies have noted peripheral inflammatory dysregulation in individuals with both chronic and recent onset schizophrenia (Dickerson et al. (2016)), those with first episode psychosis (FEP) (Fraguas et al. (2019)) and those with FEP who were medication-naive (Upthegrove et al., 2014). A previous systematic review reported that the acute-phase response protein pathway was the most implicated in distinguishing psychiatric patients with psychosis from those without psychosis (Sabherwal et al., 2016). It has also been reported that dysregulation is evident in childhood prior to the onset of disorder (English et al. (2018)) and may be useful in predicting transition to psychosis from those at clinical high risk (CHR, Mongan et al. (2021)).

Beyond transition to psychosis, CHR status has been associated with a broad range of poorer outcomes (Addington et al., 2017; and Addington et al., 2011). Poorer functioning is evident in those with CHR who do not transition to psychosis and even in those whose psychotic symptoms subside (Addington et al., 2011). Few studies have examined inflammatory dysregulation in those with CHR relative to healthy individuals, often including short lists of well-known interleukins (Misiak et al., 2021; Stojanovic et al. (2014)). For example, results to date suggest IL6 may be elevated in those with CHR (Misiak et al., 2021). Little work has been done to examine dysregulation across the blood-based proteome (Karanikas et al., 2017; Kelsven et al., 2020, Perkins et al., 2015). Moreover, there is a dearth of studies that have examined whether inflammatory dysregulation is associated with functional and clinical outcomes in those with CHR beyond transition status, with the current evidence tentatively suggesting that these phenomena are linked (Zeni-Graiff et al.,2016; Susai et al., (2022a); Goldsmith et al. (2019); Perkins et al., 2015).

Utilising samples from two large cohort studies, the first aim of this investigation was to examine whether peripheral blood-based protein levels are differentially expressed between those with CHR relative to healthy individuals. Secondly, we examined whether the levels of differentially expressed proteins were related to concurrent and subsequent global functioning and clinical symptoms.

2. Methods

2.1. Participants

Participants involved in this investigation were part of two clinical high risk cohort studies, the North American Prodrome Longitudinal Study (NAPLS) 2 and the later phase of the study with a new, independent cohort, NAPLS 3. These studies have 8 and 9 sites across North America, respectively. Sites were located at Emory University, Harvard University, University of Calgary, University of California at Los Angeles, at San Diego, and at San Francisco, University of North Carolina Chapel Hill, Yale University, and Zucker Hillside Hospital. Individuals with CHR symptoms were referred to these sites from health care providers, educators, or social service agencies, or by self-referral as a result of community outreach. The recruitment procedures have been described by Addington et al. (2012) and Addington et al. (2022), for NAPLS2 and NAPLS3, respectively.

NAPLS2 consists of 720 CHR individuals while NAPLS3 consists of 560 CHR individuals (subjects' ages ranged from 12 to 35). Both studies also recruited participants who, along with first-degree relatives, were unaffected by psychiatric or neurologic disorders but who were demographically similar to those with CHR (UC, NAPLS2 n = 240; NAPLS3 n = 96). A subsample of UC and CHR participants in both studies provided blood samples which were collected at baseline. 715 of these samples were used in this investigation including 375 from NAPLS2 (CHR samples = 222; and UC samples = 153) and 340 from NAPLS3 (CHR samples = 261; and UC samples = 79). Thus, 483 CHR samples and 232 UC samples were included in this investigation.

2.1.2 Ethics. Appropriate ethical approval was obtained for the NAPLS2 and NAPLS3 studies at each of the study sites individually. Ethical approval for the plasma biomarker analysis in this study was obtained from the Royal College of Surgeons in Ireland research ethics committee (REC number: 202211009).

2.1.3 Defining Clinical High-Risk Status and Transition to Psychosis. Participants aged between 12 and 35 years undertook the Structured Interview for Psychosis-risk Syndromes (SIPS, McGlashan et al., 2010; Woods et al., 2019) to determine if they met Criteria of Prodromal Syndromes. Transition to psychosis was defined as meeting the Presence of Psychotic Syndrome (POPS) criteria (McGlashan et al., 2010): at least one of the five Scale of Prodromal Symptoms positive symptoms rated as level 6 in intensity, for a frequency of >1 h per day for 4 days per week during the past month; or that symptoms seriously impacted functioning to the level of severely disorganisation; or the participant presented a danger to themselves or others.

2.2.1. Mass Spectrometry. Detailed description of the sample preparation and mass spectrometry methods used in this investigation are described in a previous publication (Susai et al., (2022b)). Briefly, these samples were randomised using a block randomisation design (Burger et al., 2020). This allowed for the preservation of transition rate and the proportion of samples from each study across the randomisation sequence. Plasma samples were prepared for mass spectrometry (MS) using PreOmics kits (PreOmics GmbH). Samples were then transferred to EVOSEP tips and eluted. Samples were analysed in a Bruker tims TOF Pro mass spectrometer connected to an Evosep One liquid chromatography system which injected the samples.

2.2.2. Bioinformatics. MaxQuant was used to analyse the raw MS files and label-free quantification values were further processed with Perseus software. Proteins that were identified in more than 70 % of samples were brought forward for analysis. All proteins have been labelled in accordance with the National Centre for Biotechnology Information's Official Gene Symbol coding (https://www.ncbi.nlm.nih.gov/gene/). Missing LFQ values were treated as left-censored missing data (missing not-at-random due to quantity being below the limit of detection). That is, in our a left-censored imputation approach, missing values were replaced with values drawn from a normal distribution centred at -3 standard deviations below the mean for a protein. This distribution allowed for variation in a ratio of 0.3 times the standard deviation of values for that specific protein. Values for each protein were log2 transformed, standardised to z-scores, and winsorised at 4 standard deviations above and below the mean.

2.3. Outcome Measures. Our outcome measures included the Global Assessment of Functioning scale (GAF, American Psychiatric Association, 1987) and the subscales scores from the four sub- scales (positive, negative, disorganized and general) of the Scale of Psychosis-risk Symptoms (McGlashan al., 2010; Woods et al., 2019). Each outcome was measured at baseline, 6 months, 12 months, 18 months, and 24 months. In scenarios where the participant transitioned to psychosis, additional measurements were captured at the time of transition. Not all participants attended all follow-up time points. Thus, for the purpose of this investigation three different approaches to outcome measures were used; Baseline, Last Follow-Up, and Most Severe Follow-Up (MSF). Baseline levels were considered the first measurement for the outcome variables. Last Follow-Up is the last known outcome scores reported by the participant after baseline. MSF is the poorest known outcome score recorded for each participant in the study after baseline.

2.3.1 Global assessment of functioning. The global assessment of functioning scale (GAF, American Psychiatric Association, 1987) was used as a single measure assessing psychological, social, and occupational functioning in both cohorts.

2.3.2. Scale of Prodromal Symptoms. The Scale of Psychosis-risk symptoms (SOPS; McGlashan al., 2010) was used as to measure the dimensions of psychosis. The SOPS consists of four subsections enquiring about positive, negative, general, disorganised symptoms of psychosis.

Ratings of each symptom ranged 'Absent' (0) to 'Severe and Psychotic'/ 'Extreme' (6). For this investigation, symptom ratings in each of these sections were combined into a single section score. The sections of the SOPS are described below.

2.3.2.1 General Symptoms. General symptoms included rating on four symptoms including sleep disturbances, dysphoric mood, motor disturbances and impaired tolerance to normal stress.

2.3.2.2 Positive Symptoms. Positive symptoms included ratings on five symptoms including, unusual thought content, persecutory ideas, grandiosity, hallucinations, and disorganized communication.

2.3.2.3 Negative Symptoms. Negative symptoms include ratings on six items including social anhedonia, avolition, reduced expression of emotion, reduced interpretation of own expression of emotion, reduced ideation richness and occupational functioning.

2.3.2.4 Disorganised Symptoms. Disorganised symptoms include ratings on four symptoms including odd behaviour and appearance, trouble with focus and attention, bizarre thinking, and personal hygiene.

2.3.3 Confounders. Demographic characteristics such as age and sex of the participants were also collected. Lifestyle factors and clinical characteristics included current tobacco use (yes/no), cannabis use (yes/no), alcohol use (yes/no), baseline antidepressant or anti-psychotic medication use (yes/no). Sample preparation confounders included hours to freeze the sample after draw and the time from draw to mass spectroscopy quantification. In the NAPLS3 cohort, body mass index (BMI) was also recorded.

2.4 Statistical Analysis. Demographic and lifestyle characteristics of the samples were examined using t-tests and chi^2 analysis. Comparisons between UC and CHR participants on these characteristics were conducted in the overall sample and in each NAPLS cohort separately. Logistic regression was used to examine the differential expression of each of the identified proteins between UC and CHR participants. These analyses were adjusted for age, sex and cohort. False discovery rate correction was applied to the analysis using the Benjamini-Hochberg correction method. We also ran a bootstrapped rank choice analysis, using 500 iterations. This analysis examined the consistency of the rank of the proteins based on the magnitude of the differential expression between CHR participants and controls (larger effect sizes are ranked higher). We report the median rank of each protein and the corresponding 95th percentile confidence intervals (for further information see Harrell, 2015). Two sensitivity analyses were conducted to examine if 1) any effects were observed in both cohorts separately, and 2) were the effects observed in those who transition to psychosis and those who did not transition to psychosis.

Subsequently, we examined the association between proteins with FDR-corrected differential expression and our three measures (Baseline, Last Follow-Up, and MSF) of each outcome; general, positive, negative, and disorganised symptoms and GAF. These analyses were conducted using ordinal regression and we report the proportional odds ratios. These analyses were adjusted for all confounders and cohort in the overall analysis. Sensitivity analyses were additionally conducted to examine if effects were observed in both cohorts separately. Additional adjustment for BMI was only carried out for NAPLS3 participants, as this information was not available for the NAPLS2 cohort.

3. Results

3.1 Demographic Characteristics. Overall, the average age was 18.4 years (SD: 3.7, range 12–35) and 56 % (n = 400) of participants were male. Average body mass index for the participants (NAPLS3 only) was 24.2 (SD 5.8). Just over two thirds of these participants were at clinical high risk for psychosis (CHR: 67.6 %). There was no significant difference between the two cohorts (NAPLS2 and NAPLS3) in terms of age (t(713) = -0.95, p =.344, d = -0.07) or sex ($X^2(1) = 0.11$, p =.74). In Table 1 we give a breakdown of the demographic characteristics of the CHR group and UC (overall and when stratified by cohort). Overall, there were no significant differences between the UC and CHR in terms of age (t (713) = 1.95, p =.051, d = 0.16), sex ($X^2(1) = 0.08$, p =.77) and BMI (t (333) = -1.13, p =.26, d = -0.14). However, UC had significantly lower levels of tobacco use ($X^2(1) = 23.23$, p <.001), cannabis use ($X^2(1) = 22.19$, p <.001) and anti-depressant and/or antipsychotic medication use ($X^2(1) = 121.50$, p <.001). UC reported slightly higher alcohol intake ($X^2(1) = 8.27$, p =.004) relative to the CHR group. Within NAPLS2, there were significant differences between the UC and CHR in terms of age but with small effect sizes (t (373) = 2.91, p =.004, d = 0.30). There was no significant difference in the proportion of males and females ($X^2(1) = 0.28$, p =.60). Within NAPLS3, there were no significant differences between the UC and CHR in terms of age (t (338) = 0.04, p =.97, d = 0.005), sex ($X^2(1) = 0.007$, p =.94) and BMI (t (333) = -1.13, p =.26, d = -0.14). Similar to the overall sample, tobacco use, cannabis use and psychiatric medication use was higher in the CHR group in both NAPLS2 and NAPLS3 separately. 19.58 % (n = 148) of CHR participants went on to transition to psychosis (15.0 % of the overall sample including UC).

3.2 Between group Comparison. We examined the differential expression between UC with CHR individuals for each of these proteins. Six proteins had unadjusted differential expression between UC and individuals with CHR; HP (Haptoglobin), SERPIND1 (Serpin Family D Member 1), SERPINA6 (Serpin Family A Member 6), APCS (Antigen-presenting cells), ITIH2 (Inter-Alpha-Trypsin Inhibitor Heavy Chain 2) and ITIH1 (Inter-Alpha-Trypsin Inhibitor Heavy Chain 1). However, only HP survived FDR-correction (See Supplementary Table 1). The results indicated higher plasma HP levels in the CHR group relative to controls (AdjOR: 1.49, CI:1.26–1.78, FDR p <.001). In a bootstrapped comparison HP was the only protein consistently ranked among the most discriminating; Median Rank: 1st, Rank 95 %ile CI:1st−5th (see Supplementary Table 2). Sensitivity analyses reveal that the differential expression of HP was evident in both the NAPLS2 (AdjOR: 1.36, CI:1.10–1.67, p =.004) and NAPL3 (AdjOR: 1.71, CI:1.26–1.78, p =.001) cohort separately. Differential expression of HP was evident in both those who transitioned to psychosis (AdjIRR:1.36, CI:1.07–1.72, p =.01) and those who did not transition to psychosis (AdjIRR:1.54, CI:1.28–1.85, p <.001) relative to UC. HP was not differentially expressed between those who transition and those who did not transition (AdjIRR:0.88, CI:0.72–1.09,p =.260).

3.3. Protein expression and Clinical Outcome. Subsequent analyses investigated the relationship between HP with global functioning, general symptoms, positive symptoms, negative symptoms, and disorganised symptoms (see Table 2 for the proportional odds of the

Table 1
Demographic characteristics of UC and participants at CHR in the overall group and stratified by cohort.

	Overall		**NAPLS 2**		**NAPLS 3**	
	Controls(n = 232)	**CHR**n = 483)	**Controls**(n = 153)	**CHR**(n = 222)	**Controls**(n = 79)	**CHR**(n = 261)
Age x (SD)	18.8 (3.8)	18.3 (3.6)	19.0 (3.7)	17.9 (3.3)	18.6 (4.1)	18.5 (3.8)
Sex (% male)	55.2	56.3	54.9	57.7	55.7	55.2
BMI (NAPLS3 only) x (SD)	23.6 (4.9)	24.4 (6.0)	–	–	23.6 (4.9)	24.4 (6.0)
Smoking Status (% Yes)	6.5	20.7	5.9	24.5	7.6	17.4
Cannabis Use (% Yes)	10.0	25.1	10.5	26.4	8.9	24.0
Alcohol Use (% Yes)	50.7	39.3	51.3	39.6	49.4	39.0
Psychiatric Medication use (% Yes)	0.4	39.4	0.7	38.7	0.0	40.0

Table 2
Association between HP and the different outcome measures in the overall sample and when stratified by cohort.

Model	Overall Sample Unadjusted	Adjusted[a]	NAPLS2 only Unadjusted	Adjusted[a]	NAPLS3 only Unadjusted	Adjusted[b]
Global Assessment of Functioning (pOR, 95 %CI)						
Baseline (n = 715)	**0.77**(0.68 – 0.89)	**0.82**(0.71 – 0.94)	**0.77**(0.65 – 0.91)	0.86(0.72 – 1.04)	0.83(0.67 – 1.02)	**0.78**(0.62 – 0.97)
Last Follow-up (n = 651)	**0.74**(0.64 – 0.85)	**0.79**(0.69 – 0.91)	**0.72**(0.60 – 0.85)	**0.83**(0.69 – 0.99)	0.81(0.65 – 1.00)	**0.77**(0.61 – 0.99)
Most Severe Follow-up (n = 651)	**0.73** (0.63 – 0.83)	**0.79** (0.69 – 0.91)	**0.75** (0.63 – 0.89)	**0.77** (0.65 – 0.93)	**0.71** (0.57 – 0.89)	**0.70** (0.55 – 0.89)
SOPS Positive Symptoms (pOR, 95 %CI)						
Baseline (n = 715)	**1.27** (1.12–1.44)	**1.19** (1.04–1.36)	**1.21** (1.04–1.43)	**1.11** (0.94–1.33)	**1.28** (1.04–1.58)	1.25 (0.99–1.57)
Last Follow-up (n = 700)	**1.28** (1.12–1.46)	**1.23** (1.07–1.41)	**1.28** (1.08–1.52)	1.19 (0.99–1.43)	**1.23** (1.00–1.51)	1.23 (0.98–1.54)
Most Severe Follow-up (n = 700)	**1.35** (1.19–1.54)	**1.27** (1.12–1.46)	**1.33** (1.12–1.58)	**1.20** (1.01–1.44)	**1.29** (1.04–1.58)	**1.30** (1.04–1.63)
SOPS Negative Symptoms (pOR, 95 %CI)						
Baseline (n = 715)	**1.20** (1.06–1.37)	1.12 (0.98–1.29)	1.17 (0.99–1.38)	1.08 (0.90–1.30)	1.17 (0.95–1.43)	1.23 (0.98–1.54)
Last Follow-up (n = 664)	**1.26** (1.10–1.44)	**1.20** (1.04–1.38)	**1.29** (1.09–1.53)	**1.22** (1.01–1.47)	1.15 (0.93–1.44)	1.13 (0.87–1.46)
Most Severe Follow-up (n = 664)	**1.30** (1.14–1.49)	**1.21** (1.05–1.39)	**1.29** (1.08–1.53)	1.17 (0.97–1.40)	**1.24** (1.01–1.54)	**1.29** (1.01–1.64)
SOPS General Symptoms (pOR, 95 %CI)						
Baseline (n = 715)	**1.31** (1.16–1.50)	**1.23** (1.07–1.41)	**1.28** (1.09–1.51)	**1.19** (1.01–1.42)	**1.29** (1.05–1.58)	**1.32** (1.05–1.65)
Last Follow-up (n = 663)	**1.42** (1.24–1.63)	**1.33** (1.15–1.53)	**1.49** (1.25–1.78)	**1.38** (1.14–1.66)	**1.29** (1.04–1.61)	1.22 (0.96–1.56)
Most Severe Follow-up (n = 663)	**1.41** (1.23–1.62)	**1.30** (1.12–1.49)	**1.42** (1.20–1.70)	**1.30** (1.07–1.56)	**1.34** (1.08–1.66)	**1.32** (1.04–1.68)
SOPS Disorganised Symptoms (pOR, 95 %CI)						
Baseline (n = 715)	**1.25** (1.10–1.43)	**1.16** (1.01–1.33)	**1.27** (1.07–1.51)	1.15 (0.96–1.39)	1.15 (0.93–1.42)	1.16 (0.92–1.46)
Last Follow-up (n = 664)	**1.27** (1.11–1.46)	**1.21** (1.04–1.40)	**1.39** (1.16–1.66)	**1.35** (1.11–1.64)	1.11 (0.89–1.38)	1.07 (0.84–1.35)
Most Severe Follow-up (n = 664)	**1.31** (1.15–1.50)	**1.23** (1.07–1.42)	**1.40** (1.17–1.67)	**1.33** (1.10–1.60)	1.14 (0.92–1.41)	1.11 (0.88–1.41)

outcomes). Baseline, Last-Follow up and Most Severe Follow-up were investigated for each outcome. These analyses were conducted before and after adjustment for confounders age, sex, tobacco use, cannabis use, alcohol use, baseline psychiatric medication use, hours to freeze the sample, time between the sample freeze and mass-spectrometric protein quantification, cohort (in the overall analysis) and BMI (NAPLS3 only).

3.3.1. Global Functioning. In the overall sample, HP was significantly associated with global functioning before and after model adjustment (Baseline Adj-Model p =. 004; Last Follow-up: Adj-Model p =.001; and MSF: Adj-Model p =.001). Model adjustment had limited impact on effect size (range in change of pOR: 0.05–0.06). Overall, the results indicate that a one standard deviation higher HP value is associated with a ~20% lower proportional odds on all measures of functioning (see Table 2).

3.3.2. Positive Symptoms. HP was cross-sectionally (Adj-Model p =.009) and longitudinally (Last Follow-up: Adj-Model p =.004; and MSF Adj-Model p <.001) associated with positive symptoms, before and after model adjustment. Model adjustment had limited impact on effect size (range in change of pOR: 0.05–0.8). The results indicate that a one standard deviation higher HP value is associated with a 19–27% higher proportional odds in positive symptoms on all measures.

3.3.3. Negative Symptoms. HP was longitudinally, but not cross sectionally, associated with negative symptoms, before and after model adjustment (Baseline: Adj-Model p =.111; Last Follow-up: Adj-Model p =.013; and MSF Adj-Model p =.009). Model adjustment had limited impact on effect size (range in change of pOR: 0.06–0.09). The longitudinal results indicated that a one standard deviation higher HP value at baseline was associated with a ~20% higher proportional odds in subsequent negative symptoms.

3.3.4. General Symptoms. HP was significantly cross-sectionally associated with baseline general symptoms (Adj-Model p =.002). Model adjustment had limited impact on effect size (range in change of pOR: 0.08–0.11). HP was longitudinally associated with both Last Follow-Up general symptoms and MSF general symptoms before and after adjustment (Last Follow-up: Adj-Model p <.001; and MSF Adj-Model p <.001). The results indicate that increasing HP value by one standard deviation is associated with 23–33% higher proportional odds on all measures of general symptoms from the SOPS.

3.3.5. Disorganised Symptoms. HP was cross-sectionally (Adj-Model p =.045) and longitudinally (Last Follow-up: Adj-Model p =.009; and MSF Adj-Model p =.005) associated with disorganised symptoms before and after model adjustment. Model adjustment had limited impact on effect size (range in change of pOR: 0.06–0.09). The results indicate that a one standard deviation higher HP value is associated with a 16–23% higher proportional odds in disorganised symptoms on all measures.

3.4. Cohort Sensitivity analysis. We examined the association between HP and each outcome in NAPLS2 and NAPLS3 separately. Despite the reduction in power, many of the associations between HP with functioning and general symptoms were retained, with broadly similar effect sizes observed in both cohorts. The reduction in power was more consequential to an association between HP with specific symptom subscales; positive, negative, and disorganised symptom scales. In both cohorts, HP was significantly associated with most of the MSF outcomes on each of these symptom scales. Reduced associations were retained for the baseline and last follow up outcomes on these scales. The effect sizes in both cohorts were broadly similar to the overall effects, suggesting that loss of association is a result of the widening of the confidence interval due to a loss of power.

4. Discussion

Using the largest proteomic comparison between CHR and UC to date, our results indicate that HP levels are elevated in those at CHR compared to UC. Additionally, higher HP levels are associated with poorer global functioning and greater positive, negative, disorganised, and general symptoms. These findings were evident cross-sectionally and longitudinally. Furthermore, the findings relating to global functioning and general symptoms were evident in both cohorts separately, highlighting the robustness of the finding. HP levels were upregulated in those with CHR for psychosis, irrespective of transition status. Coupling this with the knowledge that the vast majority of those with CHR meet criteria for depression or anxiety, the findings suggest that HP may be a non-specific biological marker of poorer functioning and general symptoms. There is increased circulating HP level in individuals with depression (Zorrilla et al., 2001). Our results support the hypothesis that pro-inflammatory acute phase response processes are represented in the extended psychosis phenotype.

A previous review article has indicated that the acute phase immune response is the most dysregulated pathway in patients with schizophrenia (Sabherwal et al., 2016). HP is an acute phase protein which is elevated during inflammation response. It is a primary binding protein for haemoglobin in the blood, which has anti-inflammatory properties (Polticelli et al., 2008). Taken together, the results suggest an overactivation of the acute inflammatory response in those with CHR that is related to clinical presentation. Moreover, our results support a previous smaller study specifically demonstrating that CHR patients had

higher HP gene expression assay levels than healthy controls (Yee, et al., 2022). However, while observing a relationship between HP levels and cognition, Lee and colleagues' study did not find a significant association between HP levels with global functioning or symptoms. Thus, our investigations expand on this research by robustly indicating that HP is associated with current and subsequent global functioning and symptoms. It should be noted that the degree of dysregulation observed in the CHR samples was in line with low-level dysregulation, rather than severe inflammatory dysregulation as may occur in the context of acute infection or injury (Müller (2018)).

Other proteins (SERPIND1, SERPINA6, APCS, ITIH2 and ITIH1) involved in immune processes were differentially expressed. However, these did not survive correction for multiple comparisons nor were they consistently ranked highly within a bootstrapped rank order analysis. Few studies have investigated proteomic discrimination between participants with CHR and UC, with most of these concentrating on cytokines and C-reactive protein (Karanikas et al., 2017; Delaney et al., 2019). Meta-analytic evidence suggests that IL6 and IL1B are differentially expressed in those with CHR from healthy controls (Park & Miller, 2020; and Misiak et al., 2021). However due to the discovery nature of our mass spectrometry investigation and the low abundances of cytokines within peripheral blood samples, these proteins were not identified within the current samples, possibly as they were below the limit of detection. Thus, while we are confident in the findings regarding HP and the other measured proteins, our results do not preclude differential expression of other proteins which were not quantified in this investigation.

Apart from the increased HP levels in CHR of psychosis population, our study results showed that HP levels in the plasma could be a potential marker for poor functioning and higher symptomology. In humans, HP is coded by genes located on chromosome 16q22, and allelic combinations of these genetic information produces three major polymorphisms of HP protein (HP1-1, HP1-2 and HP2-2) (Bensi et al., 1985). In general HP is involved in various biological functions which include oxidative stress, immune reactions, angiogenesis and nitrous oxide metabolism (Polticelli et al., 2008). Previous papers provide preliminary evidence that micro and macro level biological properties of HP could be involved in the pathophysiology of psychosis. Particularly important evidence arises from the involvement of inflammatory pathways that link HP with psychosis (Wang et al., 2001; Yang et al., 2000; Arredouani et al., 2003; Guetta et al., 2007). Inflammatory cytokines such as IL6 and TNF are well known for their involvement in pathophysiology of psychosis as well as production of HP by liver (Wang et al., 2001; Yang et al., 2000). This immune relationship appears to be bidirectional as the HP also influences the production of IL6 by T-helper cells (Arredouani et al., 2003; Guetta et al., 2007).

In addition, HP related effects on nitrous oxide cause narrowing of blood vessels that can result in compromised circulation in internal organs including brain. Previous studies have reported higher levels of serum HP in diabetes and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease which share common neurovascular features such as brain atrophy, reduced cerebral glucose metabolism, and central nervous system insulin resistance (Verdile, Fuller & Martins, 2015; Rosales-Corral et al., 2015). From our results we speculate that HP-associated functional deterioration and clinical symptoms could be due to vascular and metabolic derangements in the brain, as observed in other neurodegenerative disorders. This opens up a future research option in exploring biological mechanisms underpinning the HP associated psychopathological alterations that predispose to psychosis. In support of this hypothesis, elevated HP has been reported in first-episode psychosis and schizophrenia, and was associated with depression and excitement symptoms on the Positive and Negative Syndrome Scale (PANSS) (Seal & Eist, 1966; Bock, Weeke & Rafaelsen, 1971; Maes et al., 1997; Yang et al., 2006; and Wan et al., 2007). Furthermore, a recent study by Yee et al (2022) found gene expression levels in CHR participants were elevated relative to healthy controls. Since the biological activity varies across different genotypes of HP, identification of specific genotypes in relation to psychosis could be therapeutically valuable for determining preventive measures in CHR psychosis.

5. Strengths and limitations.

This is a large investigation utilising biological data from two observational multi-site cohort studies both based in North America. Our results were robust to stringent adjustment for demographic, lifestyle, and sample preparation confounding. Our work adds to limited current knowledge on the biological discrepancies between those at CHR and UC. Such investigations of those meeting CHR criteria were requested in a recent review of the immunophenotype of schizophrenia (Miller et al. (2003) & Goldsmith, 2016). Our study has limitations. Given the large number of biological samples and the requirement of quantification in 70% of the observation, our proteomic profile for participants is relatively shallow ($k = 99$). This leaves open the possible differential expression of other proteins. Conversely, the number of proteins measured required quite a significant multiple comparison correction leaving open the risk of type II error. Not every participant from the studies will have given blood, suggesting room for representation bias. However, our comparison between those with CHR who did and did not provide a blood sample indicates little to no difference between these two groups (see Supplementary Table 3). Samples from NAPLS2 did not have BMI data, thus we are unable to adjust for it in the main analysis. Thus, we did use BMI as an additional confounder in the stratified analysis using the NAPLS3 cohort. Finally, while we observe an association between haptoglobin and functioning within the samples our result should not be interpreted that lower haptoglobin causes a reduction in functioning but more that lower haptoglobin appears to co-occur and precede lower functioning and higher symptom scores.

6. Conclusion

We found robust evidence that HP is elevated in those with CHR relative to UC. HP levels were associated with current and future global functioning and clinical symptoms. In line with previous research, our results suggest that acute phase inflammatory response dysfunction, seen in psychotic disorders, is also observable in those at risk for psychosis, irrespective of transition status. This suggests that such dysfunction may be a transdiagnostic marker of distress and poorer functioning.

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Declaration of competing interest

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

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2023.12.018.

References

Addington, J., Cornblatt, B.A., Cadenhead, K.S., Cannon, T.D., McGlashan, T.H., Perkins, D.O., Heinssen, R., 2011. At clinical high risk for psychosis: outcome for nonconverters. Am. J. Psychiatry 168 (8), 800–805.

Addington, J., Cadenhead, K.S., Cornblatt, B.A., Mathalon, D.H., McGlashan, T.H., Perkins, D.O., Cannon, T.D., 2012. North American prodrome longitudinal study (NAPLS 2): overview and recruitment. Schizophr. Res. 142 (1–3), 77–82.

Addington, J., Piskulic, D., Liu, L., Lockwood, J., Cadenhead, K.S., Cannon, T.D., Woods, S.W., 2017. Comorbid diagnoses for youth at clinical high risk of psychosis. Schizophr. Res. 190, 90–95.

Addington, J., Liu, L., Brummitt, K., Bearden, C.E., Cadenhead, K.S., Cornblatt, B.A., Cannon, T.D., 2022. North American prodrome longitudinal study (NAPLS 3): methods and baseline description. Schizophr. Res. 243, 262–267.

American Psychiatric Association (1987). Diagnostic and statistical manual of mental disorders (3rd. Ed. rev.) Washington, D.C.

Arredouani, M., Matthijs, P., Van Hoeyveld, E., Kasran, A., Baumann, H., Ceuppens, J.L., Stevens, E., 2003. Haptoglobin directly affects T cells and suppresses T helper cell type 2 cytokine release. Immunology 108 (2), 144–151.

Bensi, G., Raugei, G., Klefenz, H., Cortese, R., 1985. Structure and expression of the human haptoglobin locus. EMBO J. 4 (1), 119–126.

Bock, E., Weeke, B., Rafaelsen, O.J., 1971. Serum proteins in acutely psychotic patients. J. Psychiatr. Res.

Burger, B., Vaudel, M., Barsnes, H., 2020. Importance of block randomization when designing proteomics experiments. J. Proteome Res. 20 (1), 122–128.

Delaney, S., Fallon, B., Alaedini, A., Yolken, R., Indart, A., Feng, T., Javitt, D., 2019. Inflammatory biomarkers in psychosis and clinical high-risk populations. Schizophr. Res. 206, 440–443.

Dickerson, F., Stallings, C., Origoni, A., Schroeder, J., Katsafanas, E., Schweinfurth, L., Yolken, R., 2016. Inflammatory markers in recent onset psychosis and chronic schizophrenia. Schizophr. Bull. 42 (1), 134–141.

English, J.A., Lopez, L.M., O'Gorman, A., Föcking, M., Hryniewiecka, M., Scaife, C., Cotter, D.R., 2018. Blood-Based Protein Changes in Childhood Are Associated With increased risk for later psychotic disorder: evidence from a nested case–control study of the ALSPAC Longitudinal Birth Cohort. Schizophr. Bull. 44 (2), 297–306.

Fraguas, D., Díaz-Caneja, C.M., Ayora, M., Hernández-Álvarez, F., Rodríguez-Quiroga, A., Recio, S., Arango, C., 2019. Oxidative stress and inflammation in first-episode psychosis: a systematic review and meta-analysis. Schizophr. Bull. 45 (4), 742–751.

Goldsmith, D.R., Haroon, E., Miller, A.H., Addington, J., Bearden, C., Cadenhead, K., Perkins, D.O., 2019. Association of baseline inflammatory markers and the development of negative symptoms in individuals at clinical high risk for psychosis. Brain Behav. Immun. 76, 268–274.

Guetta, J., Strauss, M., Levy, N.S., Fahoum, L., Levy, A.P., 2007. Haptoglobin genotype modulates the balance of Th1/Th2 cytokines produced by macrophages exposed to free hemoglobin. Atherosclerosis 191 (1), 48–53.

Harrell, F.E., 2015. Regression Modelling Strategies. Springer, New York.

Karanikas, E., Manganaris, S., Ntouros, E., Floros, G., Antoniadis, D., Garyfallos, G., 2017. Cytokines, cortisol and IGF-1 in first episode psychosis and ultra high risk males. Evidence for TNF-α, IFN-γ, TNF-β, IL-4 deviation. Asian J. Psychiatr. 26, 99–103.

Kelsven, S., de la Fuente-Sandoval, C., Achim, C.L., Reyes-Madrigal, F., Mirzakhanian, H., Domingues, I., Cadenhead, K., 2020. Immuno-inflammatory changes across phases of early psychosis: The impact of antipsychotic medication and stage of illness. Schizophr. Res. 226, 13–23.

Maes, M., Delange, J., Ranjan, R., Meltzer, H.Y., Desnyder, R., Cooremans, W., Scharpé, S., 1997. Acute phase proteins in schizophrenia, mania and major depression: modulation by psychotropic drugs. Psychiatry Res. 66 (1), 1–11.

McGlashan, T., Walsh, B., Woods, S., 2010. The psychosis-risk syndrome: handbook for diagnosis and follow-up. Oxford University Press.

Miller, T.J., McGlashan, T.H., Rosen, J.L., Cadenhead, K., Ventura, J., McFarlane, W., Woods, S.W., 2003. Prodromal assessment with the structured interview for prodromal syndromes and the scale of prodromal symptoms: predictive validity, interrater reliability, and training to reliability. Schizophr. Bull. 29 (4), 703–715.

Misiak, B., Bartoli, F., Carra, G., Stańczykiewicz, B., Gładka, A., Frydecka, D., Miller, B.J., 2021. Immune-inflammatory markers and psychosis risk: A systematic review and meta-analysis. Psychoneuroendocrinology 127, 105200.

Mongan, D., Föcking, M., Healy, C., Susai, S.R., Heurich, M., Wynne, K., McGuire, P., 2021. Development of proteomic prediction models for transition to psychotic disorder in the clinical high-risk state and psychotic experiences in adolescence. JAMA Psychiat. 78 (1), 77–90.

Müller, N., 2018. Inflammation in schizophrenia: pathogenetic aspects and therapeutic considerations. Schizophr. Bull. 44 (5), 973–982.

Park, S., Miller, B.J., 2020. Meta-analysis of cytokine and C-reactive protein levels in high-risk psychosis. Schizophr. Res. 226, 5–12.

Perkins, D.O., Jeffries, C.D., Addington, J., Bearden, C.E., Cadenhead, K.S., Cannon, T.D., Heinssen, R., 2015. Towards a psychosis risk blood diagnostic for persons experiencing high-risk symptoms: preliminary results from the NAPLS project. Schizophr. Bull. 41 (2), 419–428.

Polticelli, F., Bocedi, A., Minervini, G., Ascenzi, P., 2008. Human haptoglobin structure and function–a molecular modelling study. FEBS J. 275 (22), 5648–5656.

Rosales-Corral, S., Tan, D. X., Manchester, L., & Reiter, R. J. (2015). Diabetes and Alzheimer disease, two overlapping pathologies with the same background: oxidative stress. Oxidative Medicine and Cellular Longevity, 2015.

Sabherwal, S., English, J.A., Föcking, M., Cagney, G., Cotter, D.R., 2016. Blood biomarker discovery in drug-free schizophrenia: the contribution of proteomics and multiplex immunoassays. Expert Rev. Proteomics 13 (12), 1141–1155.

Seal, U.S., Eist, H., 1966. Elevation of serum protein-bound carbohydrates and haptoglobin in schizophrenia. Clin. Chem. 12 (10), 709–716.

Stojanovic, A., Martorell, L., Montalvo, I., Ortega, L., Monseny, R., Vilella, E., Labad, J., 2014. Increased serum interleukin-6 levels in early stages of psychosis: associations with at-risk mental states and the severity of psychotic symptoms. Psychoneuroendocrinology 41, 23–32.

Susai, S.R., Healy, C., Mongan, D., Heurich, M., Byrne, J.F., Cannon, M., Cotter, D., 2022a. Evidence that complement and coagulation proteins are mediating the clinical response to omega-3 fatty acids: A mass spectrometry-based investigation in subjects at clinical high-risk for psychosis. Transl. Psychiatry 12 (1), 1–10.

Susai, S.R., Mongan, D., Healy, C., Cannon, M., Cagney, G., Wynne, K., Cotter, D., 2022b. Machine learning based prediction and the influence of complement–Coagulation pathway proteins on clinical outcome: Results from the NEURAPRO trial. Brain Behav. Immun. 103, 50–60.

Upthegrove, R., Manzanares-Teson, N., Barnes, N.M., 2014. Cytokine function in medication-naive first episode psychosis: a systematic review and meta-analysis. Schizophr. Res. 155 (1–3), 101–108.

Verdile, G., Fuller, S.J., Martins, R.N., 2015. The role of type 2 diabetes in neurodegeneration. Neurobiol. Dis. 84, 22–38.

Wan, C., La, Y., Zhu, H., Yang, Y., Jiang, L., Chen, Y., He, L., 2007. Abnormal changes of plasma acute phase proteins in schizophrenia and the relation between schizophrenia and haptoglobin (Hp) gene. Amino Acids 32 (1), 101–108.

Wang, Y., Kinzie, E., Berger, F.G., Lim, S.K., Baumann, H., 2001. Haptoglobin, an inflammation-inducible plasma protein. Redox Rep. 6 (6), 379–385.

Woods, S.W., Walsh, B.C., Powers, A.R., McGlashan, T.H., 2019. Reliability, validity, epidemiology, and cultural variation of the Structured Interview for Psychosis-risk Syndromes (SIPS) and the Scale Of Psychosis-risk Symptoms (SOPS). International perspectives on early identification and intervention, Handbook of attenuated psychosis syndrome across cultures, pp. 85–113.

Yang, F., Ghio, A.J., Herbert, D.C., Weaker, F.J., Walter, C.A., Coalson, J.J., 2000. Pulmonary expression of the human haptoglobin gene. Am. J. Respir. Cell Mol. Biol. 23 (3), 277–282.

Yang, Y., Wan, C., Li, H., Zhu, H., La, Y., Xi, Z., He, L., 2006. Altered levels of acute phase proteins in the plasma of patients with schizophrenia. Anal. Chem. 78 (11), 3571–3576.

Yee, J.Y., Chow, S.Q., Lim, K., Goh, W., Sng, J., Lee, T.S., Lee, J., 2022. Haptoglobin in ultra-high risk of psychosis–Findings from the longitudinal youth at risk study (LYRIKS). Brain. Behavior, & Immunity-Health, 23.

Zeni-Graiff, M., Rizzo, L.B., Mansur, R.B., Maurya, P.K., Sethi, S., Cunha, G.R., Brietzke, E., 2016. Peripheral immuno-inflammatory abnormalities in ultra-high risk of developing psychosis. Schizophr. Res. 176 (2–3), 191–195.

Zorrilla, E.P., Luborsky, L., McKay, J.R., Rosenthal, R., Houldin, A., Tax, A., Schmidt, K., 2001. The relationship of depression and stressors to immunological assays: a meta-analytic review. Brain Behav. Immun. 15 (3), 199–226.