Psychiatry Research 358 (2026) 116980

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Original article

Plasma proteome demonstrates sex-specific associations with mental health risks in adolescents

Alexey M. Afonin [a], Aino-Kaisa Piironen [a], Jordi Julvez [b,c,d], Irene van Kamp [e], Katja M. Kanninen [a,*]

[a] A.I. Virtanen Institute for Molecular Sciences University of Eastern Finland, Kuopio, Finland
[b] Clinical and Epidemiological Neuroscience (NeuroÈpia), Institut d'Investigació Sanitària Pere Virgili (IISPV), 43204 Reus, Spain
[c] ISGlobal, Barcelona, Spain
[d] CIBER Epidemiología y Salud Pública (CIBEREsp), Madrid, Spain
[e] Centre for Sustainability Environment and Health, National Institute for Public Health and the Environment, Bilthoven, the Netherlands

ARTICLE INFO

Keywords:
Adolescents
Mental health
LC-MS/MS
Plasma biomarkers
Strengths and difficulties questionnaire (SDQ)

ABSTRACT

Adolescence is a critical developmental period marked by significant physiological, psychological, and behavioural changes, many of which differ between the sexes. We aimed to investigate sex-specific associations between the plasma proteome and questionnaire-based mental health measures in adolescents. Liquid chromatography – tandem mass spectrometry proteomic analysis was used to measure the plasma proteome abundances in 197 adolescents (11-16 years old) from the WALNUTs cohort. Baseline analysis of sexual dimorphism revealed 76 proteins significantly differentially abundant between sexes, which were enriched in cell adhesion, collagen fibril organisation, and ossification pathways. Bioinformatic analysis revealed 37 proteins significantly associated with the total score of the Strengths and Difficulties Questionnaire (SDQ). Modelling the sex-specificity via interaction terms revealed 40 proteins with significant associations with SDQ in females and 1 protein in males. Plasma protein abundancies in males exhibited stronger correlations with SDQ externalizing subscale scores, while in females the associations with the internalizing score were more prominent, consistent with known behavioural sex differences. Female-associated proteins were enriched for haemostasis and complement pathways, while male-associated signals suggested distinct immune and cytoskeletal processes. These findings indicate that both shared and sex-specific plasma proteomic signatures are associated with SDQ scores in adolescents and that models adjusting only for sex may obscure sex-divergent biology. These exploratory results are hypothesis-generating and support the use of sex-aware proteomic analyses to refine biomarker discovery for adolescent mental health.

1. Introduction

Adolescence is a critical developmental period characterised by profound physiological, hormonal, and metabolic changes, many of which sex specific (Perng et al., 2019). These changes have long-term implications for health and disease trajectories, making it a crucial time to explore biological differences between females and males. As is the case with many diseases, sex also plays a role in mental health. For instance, depression and anxiety disorders are among the most prevalent mental health conditions worldwide, with significant sex differences observed in their prevalence, manifestation, and treatment responses (Steel et al., 2014). Epidemiological studies consistently report that women can be more than twice as likely as men to experience depression and anxiety disorders (Albert, 2015, Asher et al., 2017, Nolen-Hoeksema, 2001). Men, while exhibiting lower reported rates of these disorders, may underreport symptoms due to social stigma and are more likely to suffer from other problems, such as substance abuse, ADHD symptoms or externalizing problems (Steel et al., 2014).

The Strengths and Difficulties Questionnaire (SDQ) is a widely used screening tool assessing behavioural and emotional difficulties in children and adolescents across five domains: emotional symptoms, conduct problems, hyperactivity/inattention, peer relationship problems, and prosocial behaviour (Goodman, 2016, Goodman et al., 2003, Goodman and Goodman, 2011). The psychological measures such as the SDQ and

* Corresponding author at: Neulaniementie 2, Kuopio 70210, Finland.
E-mail address: katja.kanninen@uef.fi (K.M. Kanninen).

https://doi.org/10.1016/j.psychres.2026.116980
Received 22 April 2025; Received in revised form 26 January 2026; Accepted 27 January 2026
Available online 27 January 2026

the general psychopathology factor (p-factor) can both be influenced by the sex of the participant. Understanding these differences is crucial for accurate diagnosis, intervention, and support across developmental stages (NM et al., 2006). Boys tend to exhibit higher scores in hyperactivity/inattention and conduct problems, reflecting a greater prevalence of externalizing behaviours (van der Ende et al., 2012, Oltmanns et al., 2018). In contrast, girls often score higher in emotional symptoms and peer relationship problems, indicating a tendency toward internalizing issues (NM et al., 2006, Caspi et al., 2014). These differences may be attributed to biological factors, socialization processes, and gender norms influencing the expression and reporting of psychological symptoms (Eagly and Wood, 2013).

Significant sex-associated differences in different OMICs, ranging from whole-genome sequencing to metabolomics, were found in multiple studies (Chan et al., 2021, Jiang et al., 2022, Chen et al., 2024, Mittelstrass et al., 2011). The plasma proteome can provide important insights into health and disease, but also into differences between the sexes. Several studies have identified plasma proteins with significantly different levels between adult females and males (Bekaert et al., 2026, Curran et al., 2017, Miike et al., 2010). For instance, the apolipoproteins, integral to lipid transport and metabolism, often display sex-specific variations. Higher levels of apolipoprotein A-I have been observed in females, potentially offering protection against cardiovascular diseases (Bekaert et al., 2026). Conversely, males may exhibit higher levels of proteins associated with inflammatory responses, such as C-reactive protein (CRP), a known risk marker for cardiovascular events (The Emerging Risk Factors Collaboration, 2012). A study of circulating cardiometabolic-related proteins associated with the risk of incident myocardial infarction revealed significant sex-specific differences. Among over 11,000 Swedish adults, 45 proteins were linked to MI risk, with 13 showing sex-specific associations—most prominently among women (Titova et al., 2024). The immune system generally exhibits notable sex-specific characteristics: females generally mount stronger innate and adaptive immune responses than males, which is thought to contribute to the increased prevalence of autoimmune diseases in women (Wilkinson et al., 2022). Proteins such as interferons, interleukins, and immunoglobulins may be differentially expressed, influencing immune cell function and signalling pathways (Klein and Flanagan, 2016). Most studies thus far have concentrated on assessing sex-specific plasma protein levels in adults, and relatively little is known about the situation in adolescents. A longitudinal study of plasma proteome of children and adolescents showed multiple proteins to be significantly different between males and females, including anti-mullerian hormone and multiple insulin growth factors (Liu et al., 2017).

Sex-specific differences in mental health have been increasingly revealed through proteomics and other omics studies, highlighting distinct molecular mechanisms underlying psychiatric disorders between males and females. For instance, an untargeted proteomic analysis identified sex-specific biological abnormalities in late-life depression, finding 33 proteins uniquely associated with depression in females primarily related to immunoinflammatory control, and 6 unique proteins affecting a broader range of biological pathways in males (Xue et al., 2024). Similarly, a metabolomics study in children and adolescents with major depressive disorder identified sex-specific plasma biomarkers, with biliverdin as a male-specific biomarker and phosphatidylcholine as a female-specific biomarker (Jiang et al., 2022). Genomic studies further support these findings; for example, Labonté et al. discovered sex-specific transcriptional signatures in depression, demonstrating different gene expression patterns in males and females (Labonté et al., 2017). These insights underscore the importance of considering sex as a critical variable in mental health research, with significant implications for generating hypotheses for personalised diagnostic tools and therapeutic interventions tailored to sex-specific biological profiles.

We have previously shown that the total score of the SDQ is associated with changes in the plasma proteome in adolescents (de Sousa Maciel et al., 2023). In another study we also showed association of plasma proteins with the p-factor (Afonin et al., 2024). These papers show plasma proteins to be a legitimate way of investigating questionnaire-based mental wellness scores. Adjusting for sex in modelling is a routine method, based on the assumption of sex specific plasma differences. However, the SDQ measurements, specifically the sub-scales, are known to be dependent on sex, targeted investigation of these associations has not been previously directly addressed. Here we aimed to systematically investigate the sex-specific associations between plasma protein abundances and the SDQ scores in adolescents.

2. Methods

2.1. Participant recruitment and sample collection

The studies were reviewed and approved by CEIC Parc Salut Mar Clinical Research Ethics Committee (approval numbers: 2015/6026 Walnuts and 2020/9688–Equal-life). Written informed consent to participate in the original WALNUTs study was provided by the participants' legal guardian/next of kin. No additional consent was needed for this study, all the participants were offered free tickets to the science museum of Barcelona. The specifics of the Walnuts cohort formation were described in previous publications (Julvez et al., 2021, Pinar-Martí et al., 2022). The current manuscript used a subset of 372 baseline blood samples before any dietary intervention originally described in a previous work (Pinar-Martí et al., 2022). . Samples were drawn by a nurse using K2EDTA plus tubes, rested for one hour then centrifuged at 2500 x g for 20 min at 20°C, refrigerated at 4 °C, and frozen to -80 °C within 4 h after extraction (Pinar-Martí et al., 2022), stored at –80 °C, and were not thawed until the protein depletion was performed prior to the proteomics analysis. The biological sex information was obtained from school databases for the original WALNUTs manuscript (Julvez et al., 2021). The self-reported SDQ scores were used to assess the psychosocial status reflecting the risk of mental health issues (Table 1), the externalising and internalising sub-scores calculated as suggested in (Goodman et al., 2010). For this study, a cross-sectional sub-group of 197 samples was used to perform the proteomics analysis.

2.2. Proteome analysis

The specifics of the analysis of the first 91 samples are presented in (de Sousa Maciel et al., 2023). The analysis specifics for the second part of the cohort were as follows. The second analysis batch contained 120 samples, 106 of them new, and 14 previously analysed samples.

Albumin and IgG represent more than 70 % of total protein levels in human plasma samples (Ignjatovic et al., 2019). Therefore, the depletion of high-abundant proteins is essential to the identification and analysis of low-abundant proteins. The plasma samples were depleted from highly abundant plasma proteins with Thermo Scientific Top14 Abundant Protein Depletion Resin according to manufacturer instructions. The proteins of depleted samples were acetone precipitated. Precipitated proteins were dissolved in 8 M urea in 50 mM Tris-HCl, pH

Table 1
Demographic information of the subjects in the study.

	Males	Females	P-value
Sample size	111	86	-
SDQ total score (mean ± s.d.)	10 ± 5.7	10.19 ± 5.7	0.856
SDQ externalizing score (mean ± s.d.)	6.4 ± 3 .8	5.7 ± 3.9	0.194
SDQ internalising score (mean ± s.d.)	3.8 ±2.4	4.4 ± 3.0	0.148
Age (years) (mean ± s.d.)	13.8 ± 0.9	14.0 ± 0.9	0.252
BMI (kg/m^2) (mean ± s.d.)	20.5 ± 3.4	21.0 ± 3.2	0.263

n = number of samples; s.d. = standard deviation, SDQ = the Strength and Difficulties Questionnaire, BMI = body mass index (kg/m^2). Two-tailed Student's t-test for continuous variables

8. Samples were reduced with 10 mM D,L-dithiothreitol and alkylated with 40 mM iodoacetamide. Samples were digested overnight with sequencing grade modified trypsin (Promega). After digestion peptide samples were desalted with a Sep-Pak tC18 96-well plate (Waters) and evaporated to dryness. Samples were dissolved in 0.1 % formic acid and peptide concentration was determined with a NanoDrop device. For DIA analysis 800 ng of peptides were injected and analysed in random order. Wash runs were submitted between each sample to reduce potential carry-over of peptides The LC-ESI-MS/MS analysis was performed on a nanoflow HPLC system (Easy-nLC1200, Thermo Fisher Scientific) coupled to the Orbitrap Exploris 480 mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a nano-electrospray ionisation source and FAIMS interface. Compensation voltages of -40 V and -60 V were used. Peptides were first loaded on a trapping column and subsequently separated inline on a 15 cm C18 column (75 µm x 15 cm, ReproSil-Pur 3 µm 120 Å C18- AQ, Dr. Maisch HPLC GmbH, Ammerbuch-Entringen, Germany). The mobile phase consisted of water with 0.1 % formic acid (solvent A) or acetonitrile/water (80:20 (v/v)) with 0.1 % formic acid (solvent B). A 120 min gradient was used to elute peptides (62 min from 5 % to 21 % solvent B followed by 48 min from 21 % to 36 % solvent B and in 5 min from 36 % to 100 % of solvent B, followed by 5 min wash stage with solvent B). Samples were analysed by a data independent acquisition (DIA) LC-MS/MS method. MS data was acquired automatically by using Thermo Xcalibur 4.6 software (Thermo Fisher Scientific). In a DIA method a duty cycle contained one full scan (400 -1000 m/z) and 30 DIA MS/MS scans covering the mass range 400 -1000 with variable width isolation windows.

2.3. Protein identification and quantification analysis

Data analysis consisted of protein identifications and label free quantifications of protein abundances. Data was analysed by Spectronaut software (Biognosys; version 18.0.2). The direct DIA approach was used to identify proteins and label-free quantifications were performed with the MaxLFQ algorithm in Spectronaut. Main data analysis parameters in Spectronaut were: (i) Enzyme: Trypsin/P; (ii) up to 2 missed cleavages (iii) Fixed modifications: Carbamidomethyl (cysteine); (iv) Variable modifications: Acetyl (protein N-terminus) and oxidation (methionine); (v) Precursor FDR Cutoff: 0.01 (vi) Protein FDR Cutoff: 0.01 (vii) Quantification MS level: MS2 (viii) Quantification type: Area under the curve within integration boundaries for each targeted ion (iv) Protein database: Swiss-Prot 2022_05 Homo Sapiens (Bateman et al., 2023), Universal Protein Contaminant database (Frankenfield et al., 2022); and (v) Normalisation: Global median normalization. All the peptides were used for quantification.

2.4. Bioinformatic data analysis

The two cohorts were treated as two batches in ComBat batch correction algorithm. To simplify the necessary batch correction, fourteen samples were present in both batches. These samples were used to assess he batch correction effectiveness. The results of the batch correction are presented in Supplementary Fig. 1. Following batch correction, the mean Pearson correlation coefficient from 0.69 to 0.76, indicating a successful reduction in batch-specific variance. Data preprocessing and statistical analyses were performed using R (version 4.4.1.). Identified proteins with more than 20 % missing values were excluded from the analysis. Sample normalisation was performed using the medianCentering method from the proBatch package (Čuklina et al., 2021). Missing values remaining in the dataset were inputted using the Sample Minimum method, chosen based on reliability (Liu and Dongre, 2021, Peng et al., 2024). Proteins depleted prior to MS analysis that were considered a possible source of bias and thus were also excluded.

Multivariate statistical analysis was conducted to model the proteomic differences between males and females using Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) with the ropls package in R (1.40.0).

For the differential abundance analysis limma (v. 3.54.2) (Ritchie et al., 2015) package was used, with the SDQ score used as a continuous variable. The age, BMI and sex (where applicable) were included in the linear models to mitigate these confounding factors. Linear models to estimate associations between SDQ scores and protein abundance. In the primary analysis, we modelled SDQ as a continuous predictor, adjusting for sex, age and BMI (model 1: ~ SDQ + sex + age + BMI). This model estimates an overall SDQ–protein association assuming a common SDQ slope across sexes.

To examine whether SDQ-protein associations differed between males and females, we fitted an additional interaction model including (model 2: ~ SDQ + sex + SDQ:sex + age + BMI). Sex-specific SDQ effects were derived from this interaction model using appropriate contrasts. All models were fitted using limma's empirical Bayes procedure, and p-values were adjusted for multiple testing using the Benjamini–Hochberg method.As plasma proteomic datasets from adolescents represent a very low number of all the proteomic datasets (Ignjatovic et al., 2019), background gene list for the enrichment analyses contained only the proteins found in this study. To characterize the enriched pathways related to the identified proteins, significantly differently abundant proteins (adj. $p \leq 0.05$) were used in further bioinformatic data analyses. The STRINGdb package (v. 2.20.0) was used to retrieve the protein-protein interaction information for the significantly differentially abundant proteins from the STRING database (v. 12) (Szklarczyk et al., 2021).The enrichment analysis with Gene Ontology (Process, Function and Component), KEGG and Reactome pathways, PubMed publications, UniProt Keywords, and PFAM/INTERPRO/SMART domain databases was performed using the STRINGdb package (v. 2.20.0) and the ReactomePA package (v. 1.52.0) (Franceschini et al., 2012, Yu and He, 2016). The enrichment was considered significant if the BH adjusted p-values were < 0.05. The full list of found proteins was used as the background. Visualisations were performed using ggplot2 (v3.4.0) (Wickham, 2009) package.

3. Results

3.1. Analysis of sex differences in the plasma proteome

Mass spectrometry-based proteomics was used to successfully identify 2803 proteins in the WALNUTs plasma samples (mean 1414 proteins per sample, SD=176). The full list of the proteins is presented in Supplementary File 1. Out of these, 119 were identified as contaminants, and were removed. Only proteins detected in at least 80 % of the samples were used for all the subsequent analyses (N = 802).

To determine if the global plasma proteome contained enough information to distinguish between sexes, we employed Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA). Unlike standard clustering, this supervised model specifically isolates the variance correlated with sex from unrelated background noise (orthogonal variance).

For orthogonal partial least squares discriminate analysis (OPLS-DA), all proteins of the subjects were utilised. Male and female subjects with were distinctly separated in the OPLS-DA plot (Fig. 1). This OPLS-DA model explained 85 % of the variation in group membership and demonstrated acceptable predictive performance (Q2Y cumulative = 0.494). Sex therefore can serve as a reliable separating factor between the plasma proteomes of adolescents in the WALNUTs cohort.

To identify the proteins most robustly associated with sex we used linear modelling with limma. When correcting for age and BMI, linear modelling showed 76 proteins to be significantly different between sexes (Supplementary Table 1). Enrichment analysis for those proteins using Gene Ontology (GO) terms showed that the most significantly enriched categories were cell adhesion, collagen fibril organisation, extracellular matrix organisation, anatomical structure morphogenesis and ossification.

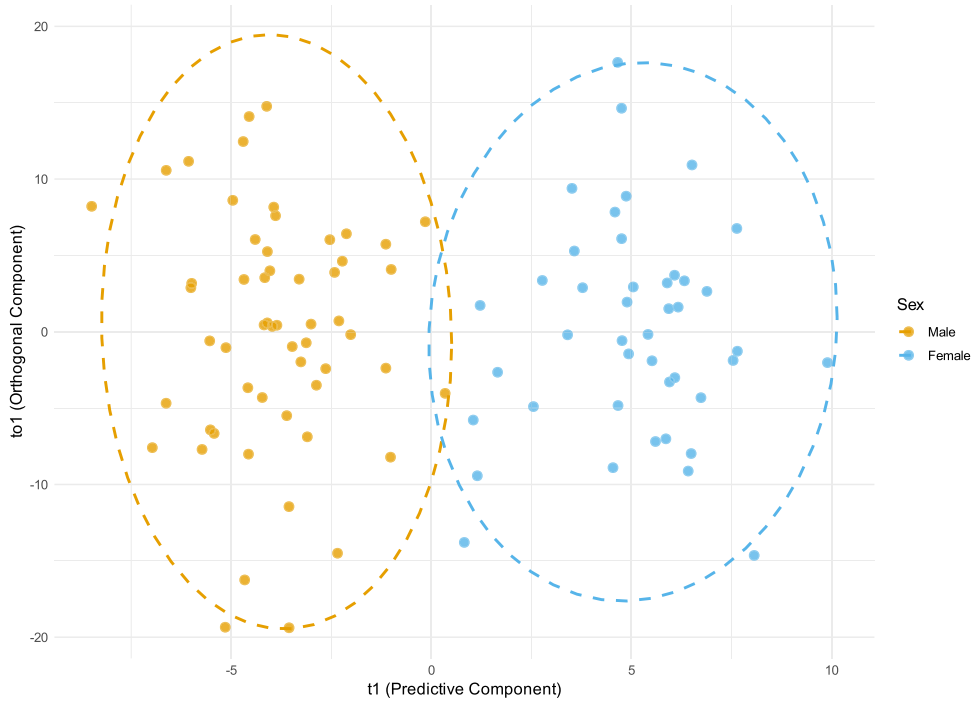

Fig. 1. OPLS-DA analysis shows a clear separation of the plasma proteome between the female and male plasma proteomes. Orange colour *in*dicates males and blue indicates females.

Out of the significant sex-specifically altered plasma proteins (Supplementary Table 1), Vitronectin, Prothrombin, Ficolin 2, and Alpha-1B-Glycoprotein were previously reported to be associated with the SDQ score in adolescents (de Sousa Maciel et al., 2023). We therefore fitted an interaction model to investigate sex-specific SDQ–protein associations, and report the estimated SDQ association for males and females separately.

3.2. *Proteins significantly associated with the full cohort*

The full WALNUTs cohort was used to identify the proteins significantly associated with SDQ. We identified 37 proteins to be significantly associated (adjusted p-value < 0.05) with the total SDQ score in this work. Sex-specific analysis showed that 40 proteins are associated with the SDQ in females and only 1 protein is associated with SDQ in males (adjusted p-value < 0.05) (Table 2).

Comparing the list of significant proteins to the sex-specific lists, 15 of the proteins were associated with SDQ in females and the full cohort, only one was associated with males and the full cohort. (Fig. 2).

To predict protein-protein interactions, we next constructed STRING protein interaction graph for the DEPs associated with the SDQ score. The interaction map for the entire cohort, females, and males separately are presented in Fig. 3.

We next performed pathway enrichment analysis on the lists of differentially expressed proteins (DEPs) to identify the biological processes involved. For the full cohort, analysis revealed significant Reactome enrichment in pathways related to the complement and coagulation cascades. Key Reactome pathways included the "Formation of Fibrin Clot (Clotting Cascade)," its "Intrinsic" and "Common" pathways, and the "Complement cascade" and its regulation.

A similar pattern was observed for the female-specific DEPs, which were also significantly enriched in pathways related to haemostasis, fibrin clot formation, and the complement cascade. Additionally, pathways such as "Defects of contact activation system (CAS) and kallikrein/kinin system (KKS)" and "Diseases of haemostasis" were enriched in this subgroup. Complete enrichment results are presented in Supplementary Table 2.

3.3. *Analysis of sex differences in the plasma proteome associated with the externalizing and internalizing subscales of SDQ*

An initial analysis of the association between the SDQ subscales and plasma protein abundances revealed that ten proteins were associated with the externalizing sub-score, whereas only two were associated with the internalizing sub-score (Table 3).

To further investigate these findings, we modelled sex-specific associations by including interaction terms and examining the sex-specific SDQ–protein slopes. Pearson correlation analysis showed that for males the correlation between the full SDQ score and the externalising score was higher than for females (0.89 in males and 0.86 in females), for the internalising score the opposite was true (0.72 in males and 0.76 for females). Linear modelling showed that for the internalizing subscale, Peptidyl-prolyl cis-trans isomerase (FKBP1A) was the only significantly associated protein in males, and the Polymeric immunoglobulin receptor (PIGR) was the only one in females. For the externalizing subscale, Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase (ST6GALNAC1) was significantly associated in males, while Serpin Family C Member 1 (SERPINC1) was significant in females. Of these, only the ST6GALNAC1 was not significant for the corresponding analysis for the whole cohort.

To better understand the differences between the protein plasma associations in males and females we opted to additionally investigate proteins with unadjusted p-values. The results of the analysis are presented in the Supplementary file 2. Fig. 4 shows the UpSet plot of all the analyses of associations between the total SDQ scores, the externalizing and internalizing subscales of the SDQ, and the protein abundances.

This analysis showed that in males, 38 proteins were in common between the total SDQ score and the SDQ externalising scores. On the contrary, in females 44 proteins were commonly associated with the full SDQ score and the SDQ internalizing scores, with. As the full SDQ is comprised of subscales, the clustering of significantly associated proteins was expected. The proteins common between males and females present in intersections with fewer than ten proteins and are shown in the Supplementary figure 2. The Reactome enrichment analysis showed that for the proteins associated with SDQ and both the subscales in

Table 2
Proteins associated with SDQ.

		Full cohort			Males			Females		
Protein ID	Gene name	Size. effect	P value	Adjusted p value	Size. effect	P value	Adjusted p value	Size. effect	P value	Adjusted p value
O15511	actin related protein 2/3 complex subunit 5	-0.135	0.001	0.036	-1.166	0.001	0.085	-0.489	0.176	0.511
O43895	X-prolyl aminopeptidase 2	-0.119	0.002	0.049	-1.210	0.003	0.106	-0.446	0.289	0.629
O75882	attractin	0.112	0.009	0.096	0.041	0.481	0.750	0.192	0.002	0.044
O95445	apolipoprotein M	0.020	0.001	0.031	0.069	0.116	0.407	0.153	0.001	0.032
P00450	ceruloplasmin	0.029	< 0.01	0.007	0.097	0.059	0.343	0.222	< 0.01	0.009
P00734	coagulation factor II, thrombin	0.024	0.001	0.032	0.025	0.637	0.842	0.252	< 0.01	0.004
P00736	complement C1r	0.104	0.010	0.100	0.040	0.463	0.740	0.176	0.002	0.048
P00740	coagulation factor IX	0.081	0.052	0.226	-0.011	0.846	0.957	0.183	0.002	0.045
P00742	coagulation factor X	0.100	0.005	0.065	0.042	0.374	0.687	0.165	0.001	0.036
P00747	plasminogen	0.099	0.004	0.057	0.030	0.514	0.770	0.176	< 0.01	0.021
P01008	serpin family C member 1	0.028	< 0.01	0.001	0.080	0.056	0.343	0.234	< 0.01	< 0.01
P01019	angiotensinogen	0.152	0.005	0.065	0.020	0.785	0.928	0.301	< 0.01	0.014
P01042	kininogen 1	0.102	0.003	0.047	0.028	0.535	0.786	0.185	< 0.01	0.016
P01833	polymeric immunoglobulin receptor	-0.071	< 0.01	0.003	-0.398	0.001	0.085	-0.377	0.003	0.056
P02652	apolipoprotein A2	0.114	0.002	0.086	0.056	0.266	0.597	0.180	0.001	0.029
P02746	complement C1q B chain	0.021	0.001	0.035	0.084	0.075	0.365	0.148	0.003	0.056
P02749	apolipoprotein H	0.092	0.018	0.141	0.009	0.867	0.965	0.186	0.001	0.031
P02763	orosomucoid 1	0.035	0.001	0.036	0.203	0.012	0.199	0.179	0.037	0.209
P02774	GC vitamin D binding protein	0.083	0.014	0.125	0.004	0.932	0.980	0.172	< 0.01	0.024
P02790	hemopexin	0.075	0.010	0.101	0.023	0.561	0.805	0.134	0.001	0.037
P03951	coagulation factor XI	0.029	< 0.01	0.012	0.098	0.083	0.369	0.226	< 0.01	0.016
P03952	kallikrein B1	0.094	0.006	0.072	0.031	0.491	0.758	0.165	0.001	0.028
P04003	complement component 4 binding protein alpha	0.028	< 0.01	0.012	0.110	0.042	0.321	0.199	0.001	0.027
P04004	vitronectin	0.099	0.003	0.147	0.048	0.278	0.608	0.156	0.001	0.031
P04180	lecithin-cholesterol acyltransferase	0.125	0.007	0.183	0.046	0.460	0.740	0.213	0.001	0.037
P04217	alpha-1-B glycoprotein	0.020	0.001	0.030	0.098	0.028	0.290	0.125	0.008	0.091
P05154	serpin family A member 5	0.027	0.002	0.047	0.069	0.289	0.611	0.241	0.001	0.026
P05155	serpin family G member 1	0.027	< 0.01	0.005	0.097	0.038	0.313	0.203	< 0.01	0.009
P05156	complement factor I	0.022	0.002	0.049	0.084	0.111	0.396	0.161	0.004	0.061
P05160	coagulation factor XIII B chain	0.023	0.002	0.049	0.056	0.312	0.628	0.207	0.001	0.026
P05543	serpin family A member 7	0.111	0.012	0.114	0.004	0.949	0.985	0.231	< 0.01	0.019
P06276	butyrylcholinesterase	0.139	0.003	0.148	0.040	0.517	0.770	0.250	< 0.01	0.016
P08603	complement factor H	0.025	< 0.01	0.011	0.070	0.131	0.427	0.210	< 0.01	0.008
P08697	serpin family F member 2	0.142	0.003	0.050	0.073	0.256	0.587	0.219	0.002	0.037
P09603	colony stimulating factor 1	-0.123	< 0.01	0.019	-0.987	0.007	0.168	-0.768	0.047	0.243
P10768	esterase D	-0.397	0.022	0.153	-0.043	0.853	0.961	-0.793	0.001	0.037
P10909	clusterin	0.098	0.003	0.155	0.041	0.367	0.676	0.162	0.001	0.028
P12259	coagulation factor V	0.086	0.022	0.155	0.007	0.897	0.967	0.176	0.001	0.035
P12955	peptidase D	0.029	0.001	0.023	0.083	0.183	0.506	0.245	< 0.01	0.017
P20851	complement component 4 binding protein beta	0.030	< 0.01	0.012	0.095	0.103	0.395	0.236	< 0.01	0.016
P20908	collagen type V alpha 1 chain	-0.107	< 0.01	0.011	-0.662	0.019	0.226	-0.850	0.005	0.068
P25311	alpha-2-glycoprotein 1, zinc-binding	0.077	0.037	0.184	0.002	0.974	0.994	0.162	0.002	0.048
P29622	serpin family A member 4	0.130	0.044	0.206	-0.012	0.888	0.966	0.290	0.002	0.042
P31150	GDP dissociation inhibitor 1	-0.097	0.002	0.042	-0.834	0.002	0.094	-0.362	0.195	0.530
P35542	serum amyloid A4, constitutive	0.022	0.001	0.023	0.076	0.109	0.396	0.172	0.001	0.028
P43251	biotinidase	0.030	< 0.01	0.011	0.125	0.030	0.295	0.207	0.001	0.028
P43652	afamin	0.092	0.006	0.074	0.039	0.386	0.692	0.151	0.002	0.039
P46531	notch receptor 1	-0.030	0.001	0.032	-0.215	0.002	0.102	-0.105	0.156	0.481
P51693	amyloid beta precursor like protein 1	-0.074	0.001	0.021	-0.489	0.005	0.153	-0.349	0.057	0.271
P55287	cadherin 11	-0.123	< 0.01	0.005	-0.726	0.005	0.153	-0.741	0.006	0.076
P55899	Fc gamma receptor and transporter	-0.134	0.001	0.036	-1.341	0.001	0.085	-0.469	0.274	0.620
P69891	hemoglobin subunit gamma 1	-0.156	0.001	0.035	-1.049	0.019	0.226	-0.766	0.106	0.383

(*continued on next page*)

Table 2 (*continued*)

Protein ID	Gene name	Full cohort			Males			Females		
		Size. effect	P value	Adjusted p value	Size. effect	P value	Adjusted p value	Size. effect	P value	Adjusted p value
Q04760	glyoxalase I	-0.142	< 0.01	0.012	-1.135	0.002	0.094	-1.139	0.003	0.056
Q14520	hyaluronan binding protein 2	0.095	0.007	0.083	0.027	0.576	0.809	0.173	0.001	0.028
Q15485	ficolin 2	0.035	< 0.01	0.013	0.216	0.003	0.106	0.165	0.028	0.186
Q16627	C-C motif chemokine ligand 14	-0.094	< 0.01	0.012	-0.550	0.004	0.147	-0.484	0.017	0.140
Q6ZP82	coiled-coil domain containing 141	-0.169	< 0.01	0.001	-0.969	0.001	0.085	-0.975	0.001	0.031
Q86SF2	polypeptide N-acetylgalactosaminyltransferase 7	-0.121	< 0.01	0.004	-1.065	< 0.01	0.027	-0.349	0.190	0.528
Q96HD1	cysteine rich with EGF like domains 1	-0.224	0.003	0.055	-0.119	0.248	0.574	-0.341	0.002	0.044
Q9H1U4	multiple EGF like domains 9	-0.041	0.001	0.021	-0.306	0.001	0.085	-0.136	0.153	0.476
Q9NSC7	ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 1	-0.135	< 0.01	0.015	-1.260	0.001	0.085	-0.536	0.162	0.486
Q9Y696	chloride intracellular channel 4	-0.135	0.002	0.042	-1.214	0.002	0.097	-0.642	0.118	0.409

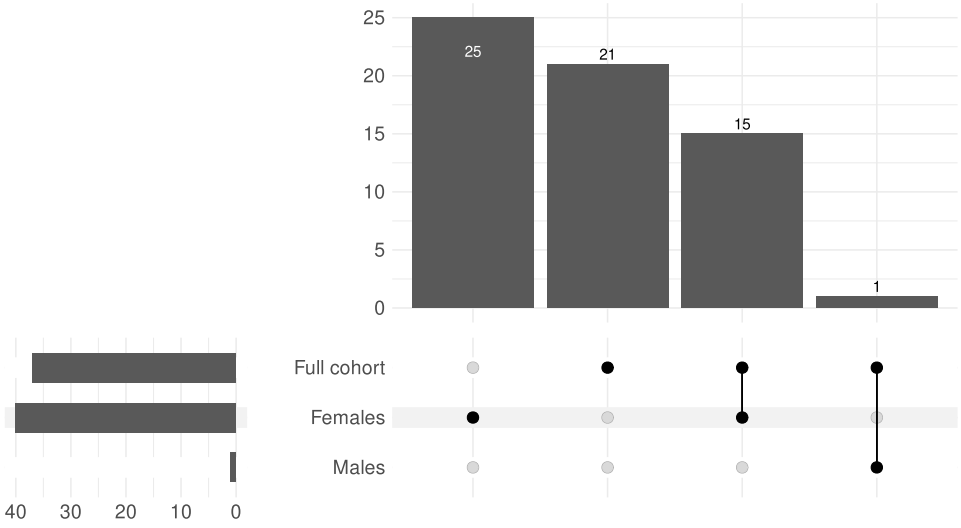

Fig. 2. UpSet plot showing the intersections between the set of protein associated with SDQ in the full cohort and in two subsets.

females were significantly enriched in blood coagulation and complement cascade proteins, whereas for the male subset, the proteins associated with the internalising score showed significant enrichment for three modules: Fcγ-receptor–dependent phagocytosis, EPHB/Ephrin signaling, and vesicle/mitochondrial trafficking (Miro GTPases and RHOBTB3). These categories indicate innate immune and cytoskeletal signalling responses associated with higher SDQ burden, unique to males. The results of the enrichment analysis are presented in supplementary file 2.

4. Discussion

In this study, we used untargeted LC-MS/MS plasma proteomics to investigate the sex-specific associations between plasma protein abundances and mental health indicator scores in adolescents. Investigation of these proteins showed sex-hormone-binding globulin to be associated with sex, possibly connected to different levels of sex hormones, which is to be expected (Hammond, 2016). Differences in proteins related to bone structure and ossification, such as osteopontin, which is consistent with the significant skeletal development occurring during puberty (van der Eerden et al., 2003) were identified, consistent with the literature (Nguyen et al., 2001). Previously it was shown that although males tend to display more inflammatory responses before puberty, inflammation is higher in females than in males after the puberty (Klein and Flanagan, 2016), which makes the investigation of inflammation-associated pathways complicated in mixed-sex cohorts. Given that puberty also occurs at different ages for males and females, developmental differences can additionally influence protein abundances (Blakemore et al., 2010, Mauvais-Jarvis, 2015), further emphasising the importance of analysing the sexes separately, even in an age-balanced cohorts like WALNUTs.

Investigation of SDQ-associate protein in the full cohort showed 37 proteins to be significantly associated with the total SDQ score using the 197 adolescents participating in the WALNUTs cohort. Of all the 76 plasma proteins found to be associated with sex, there were four proteins (Vitronectin, Thrombin, Ficolin 2, and Alpha-1B-Glycoprotein) that were previously shown by our group to be associated with the SDQ in the sub-cohort (de Sousa Maciel et al., 2023), and additionally Collagen alpha-1(V) found in this analysis. This coupled with the knowledge that SDQ is sex-specific, highlight the importance of investigating the associations of the plasma proteome with SDQ separately for females and males. This approach allowed us to test whether SDQ–protein associations differ between males and females rather than assuming a single common effect. Given the pronounced sex differences in adolescent mental health and immune biology, these sex-specific slopes provide additional insight that would be obscured in a purely pooled analysis.

Using linear modelling we found proteins, associated with the total SDQ score in either of the sexes; N-acetylgalactosaminyltransferase 7 in

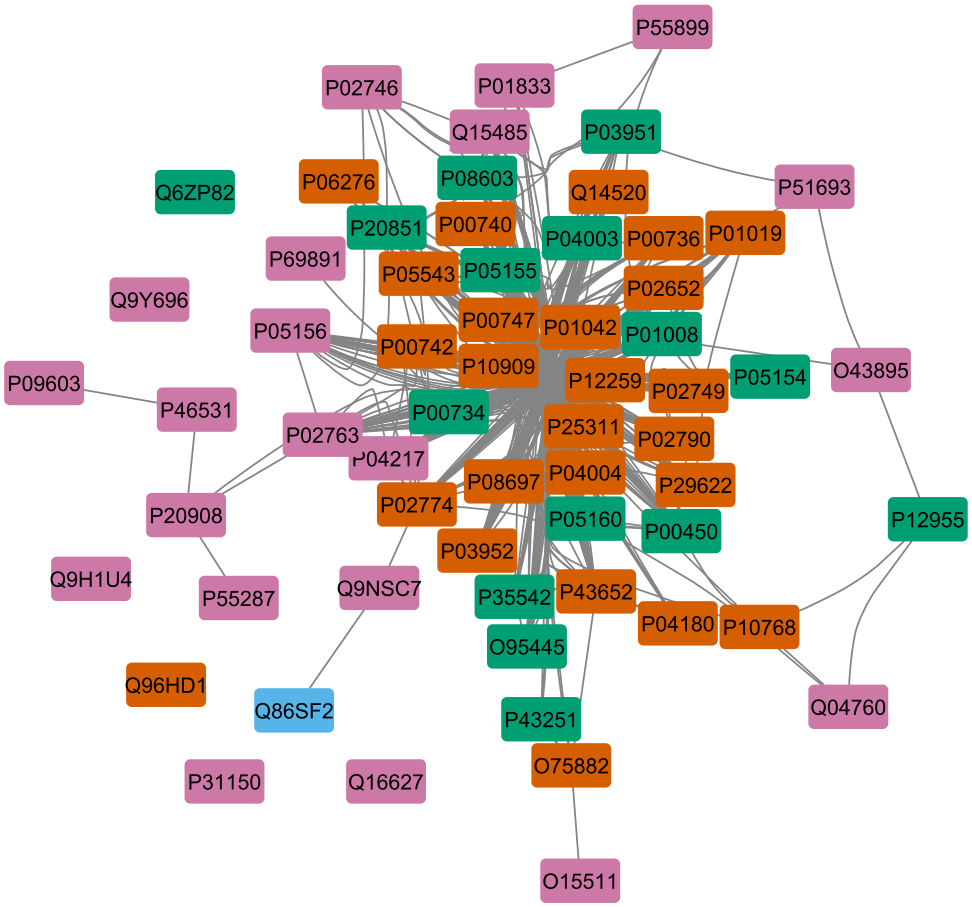

Fig. 3. This figure shows the STRING interaction network for proteins significantly associated with the total SDQ score in the full WALNUTs cohort. Node colors indicate the specific subgroups where the association was found: green for females and the full cohort; vermilion for females only; blue for males and the full cohort; and purple for the full cohort only. The thickness of the lines represents the confidence score of each interaction according to the STRING database. The proteins significantly changed in females both uniquely and shared with the full cohort form a tight interacting cluster. The proteins associated with the SDQ in the full cohort form the outer shell of the cluster, with only one protein associated in males.

males, forty proteins in females. In a previous study, the level of N-acetylgalactosaminyltransferase in the brain was connected to sex differences in depressive, anxious behaviours in a rat model (Mehta et al., 2013). The female-specific proteins both play a role in blood coagulation which has been previously linked to depression and anxiety (Dietrich-Muszalska and Wachowicz, 2017), and is in line with our previous study (de Sousa Maciel et al., 2023). The deregulation of blood coagulation proteins was previously reported to be associated with acute stress (Zgraggen et al., 2005) and depression (Yu et al., 2021) in adults.

Given the exploratory nature of this study and the reduced statistical power inherent in sex-stratified analyses, particularly within the male subset where only one protein remained significant after multiple testing correction, these findings should be interpreted as hypothesis-generating. Consequently, we decided to investigate nominal p-values to identify potential trends that warrant validation in larger cohorts. Using these subscales was previously recommended for the low-SDQ participants (Goodman et al., 2010). When looking at externalizing and internalizing subscales separately, we observed that in males the externalising SDQ score was more closely correlated with the full SDQ score, and the proteomic associations in males were also more similar between those, while in females the internalising score was more closely associated with the full SDQ, mirrored by the significant proteins for each score. This aligns with behavioural research suggesting that males often exhibit more outward-directed behavioural challenges, while females are more prone to internalizing conditions such as anxiety and depression (Caspi et al., 2014, Mehta et al., 2013), underscoring the need for a nuanced approach to studying adolescent psychological development. The involvement of immune response pathways and the enrichment of blood coagulation proteins in females points to potential sex-divergent molecular profiles associated with SDQ scores, warranting further investigation into whether these reflect true biological distinctiveness or differences in symptom severity. Males showed a completely different protein set associated with the internalising subscale, containing proteins connected to the actin skeleton in blood cells and phagocytosis. Multiple immunological markers were previously associated with depression and anxiety symptoms, suggesting a direct link between deregulation of immune response to depression (Milaneschi et al., 2021, Branchi et al., 2021).

The snapshot nature of this proteomics analysis makes it impossible to determine the causal relationship between the SDQ and the plasma levels of proteins. However it is important to remember, that the SDQ indicates the risk of developing a mental health condition later in life (Goodman et al., 2000), so the found associations may be connected to an underlying process which would result in a some form of mental disorder later in life. The associations of blood proteome signatures and mental health risk indicated by the p-factor were previously reported by our group (Afonin et al., 2024), and in the paper by English and colleagues, significantly different abundancies of levels of blood plasma proteins connected to the complement and coagulation cascade in children who would develop a psychotic disorder later in life (English et al., 2018). These findings provide a foundation for further investigations into the interplay between proteomic profiles, sex-specific development, and behavioural outcomes. The investigation of mixed-sex cohorts, even when the analyses include correction for the sex

Table 3
Proteins associated with SDQ subscales.

		Full cohort						Females						Males					
		Internalising SDQ			Externalising SDQ			Internalising SDQ			Externalising SDQ			Internalising SDQ			Externalising SDQ		
Protein ID	Gene name	Size effect	P value	Adjusted p value	Size effect	P value	Adjusted p value	Size effect	P value	Adjusted p value	Size effect	P value	Adjusted p value	Size effect	P value	Adjusted p value	Size effect	P value	Adjusted p value
P00450	ceruloplasmin	0.117	0.003	0.216	0.136	0.000	0.038	0.1763187	2.06E-03	0.126548538	0.181	0.001	0.092	0.103	0.042	0.422	0.041	0.464	0.904
P01008	serpin family C member 1	0.102	0.002	0.216	0.142	0.000	0.008	0.2235578	2.44E-06	0.001953571	0.146	0.001	0.099	0.076	0.064	0.462	0.050	0.294	0.814
P01833	polymeric immunoglobulin receptor	-0.383	0.000	0.017	-0.275	0.002	0.076	-0.1151941	3.85E-01	0.833630196	-0.524	0.000	0.012	-0.401	0.001	0.111	-0.218	0.090	0.632
P03951	coagulation factor XI	0.101	0.018	0.320	0.150	0.000	0.038	0.2044624	1.09E-03	0.126548538	0.155	0.008	0.159	0.106	0.055	0.453	0.039	0.534	0.917
P28300	lysyl oxidase	-0.227	0.298	0.691	-0.749	0.000	0.038	-0.5598886	7.49E-02	0.463395136	-0.221	0.454	0.759	-0.899	0.001	0.141	-0.235	0.463	0.904
P55287	cadherin 11	-0.341	0.078	0.523	-0.782	0.000	0.008	-0.7471305	7.07E-03	0.149028565	-0.412	0.115	0.424	-0.810	0.001	0.128	-0.257	0.364	0.858
P62942	FKBP prolyl isomerase 1A	-0.411	0.006	0.296	-0.262	0.080	0.338	-0.129118	5.63E-01	0.902346634	-0.015	0.940	0.978	-0.367	0.066	0.471	-0.876	0.000	0.048
Q04760	glyoxalase I	-0.653	0.017	0.320	-1.128	0.000	0.008	-1.3875915	4.47E-04	0.116873103	-0.356	0.332	0.690	-0.922	0.008	0.341	-1.002	0.012	0.345
Q15485	ficolin 2	0.124	0.020	0.320	0.181	0.001	0.044	0.1335773	8.46E-02	0.497273442	0.131	0.070	0.329	0.218	0.002	0.141	0.117	0.135	0.656
Q16627	C-C motif chemokine ligand 14	-0.235	0.104	0.556	-0.558	0.000	0.014	-0.5334673	1.03E-02	0.187670283	-0.214	0.272	0.633	-0.577	0.002	0.141	-0.259	0.221	0.753
Q6ZP82	coiled-coil domain containing 141	-0.835	0.000	0.029	-0.773	0.000	0.028	-0.8802512	4.57E-03	0.132517417	-0.670	0.017	0.210	-0.689	0.013	0.346	-1.028	0.001	0.151
Q9NSC7	ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 1	-0.336	0.222	0.662	-1.043	0.000	0.015	-0.5344903	1.71E-01	0.654073085	-0.297	0.425	0.738	-1.447	0.000	0.037	-0.382	0.343	0.858

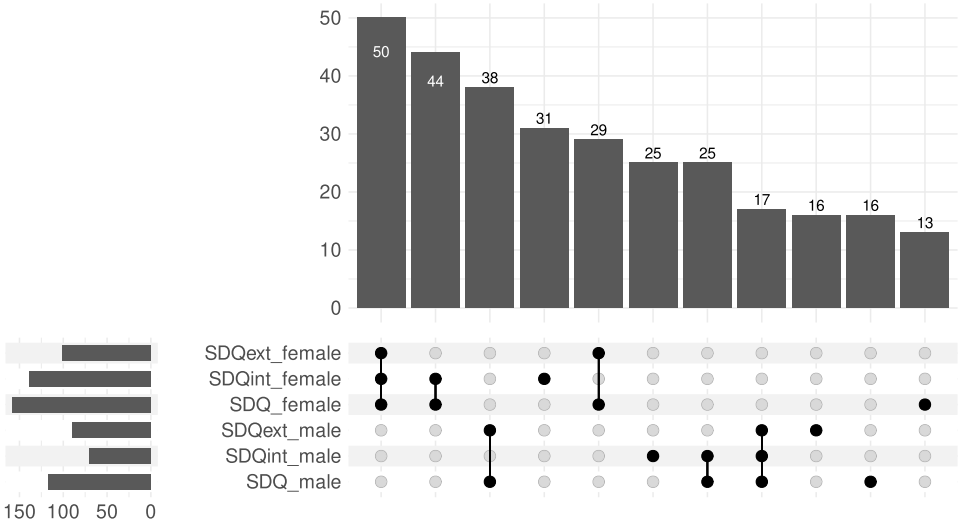

Fig. 4. UpSet plot showing the nominally significant proteome features associated with the SDQ score and its sub-scales in females and males. All the intersections with fewer than 10 proteins were removed for readability. The full plot is shown in Supplementary Figure. 2.

of the participants, is likely to show only those features universal for the whole cohort, while ignoring the specific associations making the results less nuanced. This limitation highlights the contribution of the present study compared to our previous work (de Sousa Maciel et al., 2023). While our earlier analysis, which treated sex as a covariate, successfully identified high-abundance markers shared across the population, it masked the sex-divergent signals. By employing a stratified approach here, we were able to unmask the male-specific associations enriched in cytoskeletal regulation, which were undetectable in the full cohort.

The study has several limitations. The cohort contains only adolescents from a specific region of Spain, making the results harder to generalise. The cross-sectional nature of this study does not allow us to suggest causal assumptions in the found associations. All the found differences may be due to three distinct, but not necessarily mutually exclusive causes: a) the observed differences in protein levels are the cause of the differences in the SDQ b) they are directly associated with the SDQ increase – such as the levels of peer pressure and stress can be associated with the plasma protein levels c) both the SDQ differences and the plasma proteome differences have the same underlying cause. The sex information was received from a questionnaire filled in by the parents of the participants, with the choice being binary, making the information possibly incomplete. The age range of the participants was more than two years, which can be critical when investigating adolescents. The information about the puberty stage markers is not available for the large part for the participants and could not be used for modelling. The puberty trajectories are known to influence both neurodevelopment and the onset of internalizing disorders, particularly in females (Juraska and Willing, 2017, Marceau et al., 2011), and the age correction is not a full substitute for this information. Sex-specific associations were estimated from interaction models, which increased the number of parameters and reduced statistical power for detecting sex-specific effects, particularly in males where the sample size was smaller. The second batch is homogenous with regards to SDQ, with most participants having low to moderate total SDQ scores, which hinders the analysis of associations with extreme SDQ values. The relative scarcity of significant proteins in males compared to females may reflect these limitations. Furthermore, the protein depletion method used in the plasma analyses affects the abundance estimation of multiple plasma proteins, including coagulation associated proteins. However these effects should affect all the sample in the same manner, not contributing to the observed SDQ associated difference. Other depletion methods can potentially yield more reliably detected proteins and improve repeatability (Ahsan et al., 2023). Despite the limitations of this study the results pave the way for future research. Our findings emphasise the importance of analysing male and female proteomes separately to uncover distinct biological pathways, particularly those related to coagulation, immune responses, and psychological health.

Future investigations should focus on validating the results in independent sets of samples using larger, multi-cohort datasets to improve the reproducibility and usefulness of the found associations. The statistical power of the OMICs studies should also be considered when planning future work with cohorts. To improve the results analysing each sex separately may be the better way forward for mental health investigations. Collection of pubertal staging data with circulating hormone levels would better model the effects of puberty on the proteomic signatures. Furthermore, integrating longitudinal data could reveal how proteomic profiles evolve during adolescence and their relationship with long-term psychological outcomes.

Data availability

The data analysed in this study is subject to the following licenses/restrictions: The Walnuts data is not publicly available due to the restrictions of informed consent. The data contains personal information of children and according to the ethical approval, they should be kept confidential. The studies were reviewed and approved by CEIC Parc Salut Mar Clinical Research Ethics Committee (approval numbers: 2015/6026 Walnuts and 2020/9688–Equal-life). Proteomics data is not publicly available due to the restrictions of informed consent. Reasonable requests to access these datasets should be directed to Katja Kanninen, University of Eastern Finland. To ensure the protection of privacy and compliance with national data protection legislation, a data use/transfer agreement is needed, the content and specific clauses of which will depend on the nature of the requested data.

Funding

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 874724. Equal-Life is part of the European Human Exposome Network. This study was also supported by "Instituto de Salud Carlos III" through the projects 'CP14/00108, PI16/00261, PI21/00266' (co-funded by European Regional Development Fund 'A way to make Europe'), and was funded by a Finnish Cultural Foundation grant 65242097.

CRediT authorship contribution statement

Alexey M. Afonin: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Aino-Kaisa Piironen:** Writing – review & editing, Formal analysis. **Jordi Julvez:** Writing – review & editing, Data curation. **Irene van Kamp:** Supervision, Project administration. **Katja M. Kanninen:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis.

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.

Acknowledgements

We gratefully acknowledge the contribution of the Early Environmental quality and life-course mental health effects (Equal-Life) project team, Ruth Koole and Rik Bogers (RIVM) in providing the selection protocol, the Turku Proteomics Facility team supported by Biocenter Finland for mass spectrometry, and the laboratory assistance of Ms. Mirka Tikkanen.

This WALNUTs project was supported by Instituto de Salud Carlos III through the projects 'CP14/00108, PI16/00261, and PI21/00266' (co-founded by the European Regional Development Fund 'A way to make Europe'). JJ holds a Miguel Servet-II contract (grant CPII19/00015) awarded by the Instituto de Salud Carlos III (confounded by the European Social Fund "Investing in your future"). The California Walnut Commission (CWC) gave support by supplying the walnuts for free for the Walnuts Smart Snack Dietary Intervention Trial. The funders had no role in the study design, collection, management, analysis, and interpretation of data, the writing of the report, or the decision to submit it for publication.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2026.116980.

References

Afonin, A.M., Piironen, A-K, de, Sousa, Maciel, I., Ivanova, M., Alatalo, A., Whipp, A.M., et al., 2024. Proteomic insights into mental health status: plasma markers in young adults. Transl. Psychiatry 14, 55.

Ahsan, N., Fornelli, L., Najar, F.Z., Gamagedara, S., Hossan, M.R., Rao, R.S.P., et al., 2023. Proteomics evaluation of five economical commercial abundant protein depletion kits for enrichment of diseases-specific biomarkers from blood serum. Proteomics 23.

Albert, PR., 2015. Why is depression more prevalent in women? J. Psychiatry Neurosci. 40, 219–221.

Asher, M., Asnaani, A., Aderka, IM., 2017. Gender differences in social anxiety disorder: a review. Clin. Psychol. Rev. 56, 1–12.

Bateman, A., Martin, M-J, Orchard, S., Magrane, M., Ahmad, S., Alpi, E., et al., 2023. UniProt: the universal protein knowledgebase in 2023. Nucleic Acids Res. 51, D523–D531.

E. D. Bekaert, P. Alaupovic, C. S. Knight-Gibson, G. Franceschini A, Sirtorit CR. 2026 Apol i poprotei n A-l sex-related differences in the concentration and composition of apoA-I- and apoB-containing lipoprotein particles.

Blakemore, S., Burnett, S., Dahl, R.E., 2010. The role of puberty in the developing adolescent brain. Hum. Brain Mapp. 31, 926–933.

Branchi, I., Poggini, S., Capuron, L., Benedetti, F., Poletti, S., Tamouza, R., et al., 2021. Brain-immune crosstalk in the treatment of major depressive disorder. Eur. Neuropsychopharmacol. 45, 89–107.

Caspi, A., Houts, R.M., Belsky, D.W., Goldman-Mellor, S.J., Harrington, H., Israel, S., et al., 2014. The p factor: one general psychopathology factor in the structure of psychiatric disorders? Clin. Psychol. Sci. 2, 119–137.

Chan, R.F., Copeland, W.E., Zhao, M., Xie, L.Y., Costello, J., Aberg, K.A., et al., 2021. A methylation study implicates the rewiring of brain neural circuits during puberty in the emergence of sex differences in depression symptoms. J. Child Psychol. Psychiatry 63, 802–809.

Chen, C.C., Howie, J., Ebrahimi, M., Teymouri, K., Woo, J.J., Tiwari, A.K., et al., 2024. Analysis of the complement component C4 gene with schizophrenia subphenotypes. Schizophr. Res. 271, 309–318.

Čuklina, J., Lee, C.H., Williams, E.G., Sajic, T., Collins, B.C., Rodríguez Martínez, M., et al., 2021. Diagnostics and correction of batch effects in large-scale proteomic studies: a tutorial. Mol. Syst. Biol. 17.

Curran, A.M., Fogarty Draper, C., Scott-Boyer, M-P, Valsesia, A., Roche, H.M., Ryan, M. F., et al., 2017. Sexual Dimorphism, Age, and Fat Mass Are Key Phenotypic Drivers of Proteomic Signatures. J. Proteome Res. 16, 4122–4133.

de Sousa Maciel, I., Piironen, A-K, Afonin, A.M., Ivanova, M., Alatalo, A., Jadhav, K.K., et al., 2023. Plasma proteomics discovery of mental health risk biomarkers in adolescents. Nat. Ment. Heal. 1, 596–605.

Dietrich-Muszalska, A., Wachowicz, B., 2017. Platelet haemostatic function in psychiatric disorders: effects of antidepressants and antipsychotic drugs. World J. Biol. Psychiatry 18, 564–574.

Eagly, A.H., Wood, W., 2013. The nature–nurture debates. Perspect. Psychol. Sci. 8, 340–357.

English, J.A., Lopez, L.M., O'Gorman, A., Föcking, M., Hryniewiecka, M., Scaife, C., et al., 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, 297.

Franceschini, A., Szklarczyk, D., Frankild, S., Kuhn, M., Simonovic, M., Roth, A., et al., 2012. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic. Acids. Res. 41, D808–D815.

Frankenfield, A.M., Ni, J., Ahmed, M., Hao, L., 2022. Protein contaminants matter: building universal protein contaminant libraries for DDA and DIA proteomics. J. Proteome Res. 21, 2104–2113.

Goodman, A., Goodman, R., 2011. Population mean scores predict child mental disorder rates: validating SDQ prevalence estimators in Britain. J. Child Psychol. Psychiatry 52, 100–108.

Goodman, R., Renfrew, D., Mullick, M., 2000. Predicting type of psychiatric disorder from Strengths and Difficulties Questionnaire (SDQ) scores in child mental health clinics in London and Dhaka. Eur. Child Adolesc. Psychiatry 9, 129–134.

Goodman, R., Meltzer, H., Bailey, V., 2003. The strengths and difficulties questionnaire: a pilot study on the validity of the self-report version. Int. Rev. Psychiatry 15, 173–177.

Goodman, A., Lamping, D.L., Ploubidis, GB., 2010. When to Use broader internalising and externalising subscales instead of the hypothesised five subscales on the strengths and difficulties questionnaire (SDQ): data from british parents, teachers and children. J. Abnorm. Child Psychol. 38, 1179–1191.

Goodman A. Scoring the strengths & difficulties questionnaire for age 4-17 or 18+. 2016.

Hammond, GL., 2016. Plasma steroid-binding proteins: primary gatekeepers of steroid hormone action. J. Endocrinol. 230, R13–R25.

Ignjatovic, V., Geyer, P.E., Palaniappan, K.K., Chaaban, J.E., Omenn, G.S., Baker, M.S., et al., 2019. Mass spectrometry-based plasma proteomics: considerations from sample collection to achieving translational data. J. Proteome Res. 18, 4085–4097.

Jiang, Y., Qin, M., Teng, T., Li, X., Yu, Y., Wang, J., et al., 2022. Identification of sex-specific plasma biomarkers using metabolomics for major depressive disorder in children and adolescents. Front. Psychiatry 13.

Julvez, J., Gignac, F., Fernández-Barrés, S., Romaguera, D., Sala-Vila, A., Ranzani, O.T., et al., 2021. Walnuts, long-chain polyunsaturated fatty acids, and adolescent brain development: protocol for the walnuts smart snack dietary intervention trial. Front. Pediatr. 9.

Julvez, J., Gignac, F., Fernández-Barrés, S., Romaguera, D., Sala-Vila, A., Ranzani, O.T., et al., 2021. Walnuts, long-chain polyunsaturated fatty acids, and adolescent brain development: protocol for the walnuts smart snack dietary intervention trial. Front. Pediatr. 9.

Juraska, J.M., Willing, J., 2017. Pubertal onset as a critical transition for neural development and cognition. Brain Res. 1654, 87–94.

Klein, S.L., Flanagan, KL., 2016. Sex differences in immune responses. Nat. Rev. Immunol. 16, 626–638.

Labonté, B., Engmann, O., Purushothaman, I., Menard, C., Wang, J., Tan, C., et al., 2017. Sex-specific transcriptional signatures in human depression. Nat. Med. 23, 1102–1111.

Liu, M., Dongre, A., 2021. Proper imputation of missing values in proteomics datasets for differential expression analysis. Brief. Bioinform. 22.

Liu, C-W, Bramer, L., Webb-Robertson, B-J, Waugh, K., Rewers, M.J., Zhang, Q., 2017. Temporal profiles of plasma proteome during childhood development. J. Proteomics. 152, 321–328.

Marceau, K., Ram, N., Houts, R.M., Grimm, K.J., Susman, EJ., 2011. Individual differences in boys' and girls' timing and tempo of puberty: modeling development with nonlinear growth models. Dev. Psychol. 47, 1389–1409.

Mauvais-Jarvis, F., 2015. Sex differences in metabolic homeostasis, diabetes, and obesity. Biol. Sex Differ. 6, 14.

Mehta, N.S., Wang, L., Redei, EE., 2013. Sex differences in depressive, anxious behaviors and hippocampal transcript levels in a genetic rat model. Genes Brain Behav. 12, 695–704.

Miike, K., Aoki, M., Yamashita, R., Takegawa, Y., Saya, H., Miike, T., et al., 2010. Proteome profiling reveals gender differences in the composition of human serum. Proteomics. 10, 2678–2691.

Milaneschi, Y., Kappelmann, N., Ye, Z., Lamers, F., Moser, S., Jones, P.B., et al., 2021. Association of inflammation with depression and anxiety: evidence for symptom-specificity and potential causality from UK Biobank and NESDA cohorts. Mol. Psychiatry 26, 7393–7402.

Mittelstrass, K., Ried, J.S., Yu, Z., Krumsiek, J., Gieger, C., Prehn, C., et al., 2011. Discovery of sexual dimorphisms in metabolic and genetic biomarkers. PLoS Genet. 7, e1002215.
Nguyen, TV., Maynard, L.M., Towne, B., Roche, A.F., Wisemandle, W., Li, J., et al., 2001. Sex differences in bone mass acquisition during growth. J. Clin. Densitom. 4, 147–157.
NM, Else-Quest, Hyde, J.S., Goldsmith, H.H., Van Hulle, CA., 2006. Gender differences in temperament: a meta-analysis. Psychol. Bull. 132, 33–72.
Nolen-Hoeksema, S., 2001. Gender differences in depression. Curr. Dir. Psychol. Sci. 10, 173–176.
Oltmanns, J.R., Smith, G.T., Oltmanns, T.F., Widiger, TA., 2018. General Factors of psychopathology, personality, and personality disorder: across domain comparisons. Clin. Psychol. Sci. 6, 581–589.
Peng, H., Wang, H., Kong, W., Li, J., Bin, Goh.WW, 2024. Optimizing differential expression analysis for proteomics data via high-performing rules and ensemble inference. Nat. Commun. 15, 3922.
Perng, W., Rifas-Shiman, S.L., Hivert, M-F, Chavarro, J.E., Sordillo, J., Oken, E., 2019. Metabolic trajectories across early adolescence: differences by sex, weight, pubertal status and race/ethnicity. Ann. Hum. Biol. 46, 205–214.
Pinar-Martí, A., Fernández-Barrés, S., Gignac, F., Persavento, C., Delgado, A., Romaguera, D., et al., 2022. Red blood cell omega-3 fatty acids and attention scores in healthy adolescents. Eur. Child Adolesc. Psychiatry. https://doi.org/10.1007/s00787-022-02064-w, 12 August 2022.
Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., et al., 2015. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 43 e47–e47.
Steel, Z., Marnane, C., Iranpour, C., Chey, T., Jackson, J.W., Patel, V., et al., 2014. The global prevalence of common mental disorders: a systematic review and meta-analysis 1980–2013. Int. J. Epidemiol. 43, 476–493.
Szklarczyk, D., Gable, A.L., Nastou, K.C., Lyon, D., Kirsch, R., Pyysalo, S., et al., 2021. The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 49, D605–D612.
The Emerging Risk Factors Collaboration, 2012. C-reactive protein, fibrinogen, and cardiovascular disease prediction. N. Engl. J. Med. 367, 1310–1320.
Titova, O.E., Yuan, S., Byberg, L., Baron, J.A., Lind, L., Michaëlsson, K., et al., 2024. Plasma proteome and incident myocardial infarction: sex-specific differences. Eur. Heart. J. 45, 4647–4657.
van der Eerden, B.C.J., Karperien, M., Wit, J.M., 2003. Systemic and local regulation of the growth plate. Endocr. Rev. 24, 782–801.
van der Ende, J., Verhulst, F.C., Tiemeier, H., 2012. Agreement of informants on emotional and behavioral problems from childhood to adulthood. Psychol. Assess. 24, 293–300.
Wickham, H., 2009. ggplot2. Springer New York, New York, NY.
Wilkinson, N.M., Chen, H-C, Lechner, M.G., Su, MA., 2022. Sex differences in immunity. Annu. Rev. Immunol. 40, 75–94.
Xue, X., Demirci, D., Lenze, E.J., Reynolds III, C.F., Mulsant, B.H., Wetherell, J.L., et al., 2024. Sex differences in plasma proteomic markers in late-life depression. Psychiatry Res. 334, 115773.
Yu, G., He, Q-Y., 2016. ReactomePA: an R/Bioconductor package for reactome pathway analysis and visualization. Mol. Biosyst. 12, 477–479.
Yu, C., Zhang, T., Shi, S., Wei, T., Wang, Q., 2021. Potential biomarkers: differentially expressed proteins of the extrinsic coagulation pathway in plasma samples from patients with depression. Bioengineered 12, 6318–6331.
Zgraggen, L., Fischer, J.E., Mischler, K., Preckel, D., Kudielka, B.M., von Känel, R., 2005. Relationship between hemoconcentration and blood coagulation responses to acute mental stress. Thromb. Res. 115, 175–183.