BRAIN
ORIGINAL ARTICLE

Connecting dementia risk loci to the CSF proteome identifies pathophysiological leads for dementia

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Genome-wide association studies have successfully identified many genetic risk loci for dementia, but exact biological mechanisms through which genetic risk factors contribute to dementia remains unclear. Integrating CSF proteomic data with dementia risk loci could reveal intermediate molecular pathways connecting genetic variance to the development of dementia.

We tested to what extent effects of known dementia risk loci can be observed in CSF levels of 665 proteins [proximity extension-based (PEA) immunoassays] in a deeply-phenotyped mixed memory clinic cohort [$n = 502$, mean age (standard deviation, SD) = 64.1 (8.7) years, 181 female (35.4%)], including patients with Alzheimer's disease (AD, $n = 213$), dementia with Lewy bodies (DLB, $n = 50$) and frontotemporal dementia (FTD, $n = 93$), and controls ($n = 146$). Validation was assessed in independent cohorts ($n = 99$ PEA platform, $n = 198$, mass reaction monitoring-targeted mass spectroscopy and multiplex assay). We performed additional analyses stratified according to diagnostic status (AD, DLB, FTD and controls separately), to explore whether associations between CSF proteins and genetic variants were specific to disease or not.

We identified four AD risk loci as protein quantitative trait loci (pQTL): *CR1*-CR2 (rs3818361, $P = 1.65 \times 10^{-8}$), *ZCWPW1*-PILRB (rs1476679, $P = 2.73 \times 10^{-32}$), *CTSH*-CTSH (rs3784539, $P = 2.88 \times 10^{-24}$) and *HESX1*-RETN (rs186108507, $P = 8.39 \times 10^{-8}$), of which the first three pQTLs showed direct replication in the independent cohorts. We identified one AD-specific association between a rare genetic variant of *TREM2* and CSF IL6 levels (rs75932628, $P = 3.90 \times 10^{-7}$). DLB risk locus *GBA* showed positive *trans* effects on seven inter-related CSF levels in DLB patients only. No pQTLs were identified for FTD loci, either for the total sample as for analyses performed within FTD only.

Protein QTL variants were involved in the immune system, highlighting the importance of this system in the pathophysiology of dementia. We further identified pQTLs in stratified analyses for AD and DLB, hinting at disease-specific pQTLs in dementia. Dissecting the contribution of risk loci to neurobiological processes aids in understanding disease mechanisms underlying dementia.

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Received September 04, 2023. Revised January 29, 2024. Accepted February 23, 2024. Advance access publication March 25, 2024

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Keywords: protein quantitative trait loci (pQTL); CSF; Alzheimer's disease (AD); dementia

Introduction

Genetic factors play a pivotal role in the development of dementia, as supported by high heritability estimates for Alzheimer's disease (AD), dementia with Lewy bodies (DLB) and frontotemporal dementia (FTD).[1-3] Large-scale genome-wide association studies (GWAS) have identified many common single nucleotide polymorphisms (SNPs) as risk or protective variants for AD-type dementia,[4-12] DLB[13,14] and FTD.[15-18] Most of these genetic risk loci have small effect sizes and are often located within non-coding regions rather than in a specific gene. Hence, the functional interpretation that link these genetic risk factors to biological mechanisms of disease has been extremely challenging. One of the proposed mechanisms is that genetic variation in coding and non-coding variants affects gene expression and consequently contributes to disease via alterations in protein abundances.[19,20] As proteins have a close relationship with pathophysiological processes, genetic studies on protein levels [i.e. protein quantitative trait loci (pQTL)] can provide a biological understanding on how genetic variation influences the risk for dementia.[21] Unravelling the biological processes and specific proteins that are affected by genetic risk variants could thus provide biomarkers, but also novel targets and therapeutic strategies to prevent or counter the development of different types of dementia.[22] Of note, quantitative trait loci (QTL) effects can be either tissue-shared or tissue-specific, and therefore QTL studies in specific tissue types or biofluids relevant to neurodegenerative disease are essential.[23-25]

CSF reflects several pathophysiological processes that take place *in vivo* in the human brain, and thus serves as an important source to identify potential biomarkers and drug targets for neurodegenerative disease.[26] The integration of genetic data with the CSF proteome has improved our understanding of the gene regulatory landscape underlying dementia,[27-29] as illustrated by the *cis* and *trans* effects of AD risk factors *APOE-ε4*[29] and *MS4A*[30] and *trans* effects of FTD risk factor *TMEM106B* on neurofilament light.[31] However, protein QTL studies using CSF proteomic information related to a wider range of biological mechanisms are limited[24] or have only been performed in cognitively healthy subjects.[28] Including patients in pQTL studies is relevant as it will naturally increase the frequency of rare risk alleles, which (often) have stronger associations with disease risk compared to common risk alleles.[32] The inclusion of patients further allows us to detect potential QTL effects specific to neurodegenerative disease, which has not been examined before in the field of dementia.

In this study, we aimed to identify CSF proteins associated with genetic risk factors for three major dementia types, AD, DLB and FTD, to better understand their underlying pathophysiological processes. We report results on the association between known genetic risk loci for AD, DLB and FTD, and 665 CSF protein levels (measured using proximity extension-based immunoassays) in a well characterized dementia cohort ($n = 502$) (Amsterdam Dementia Cohort, ADC).[33] Independent data from two other CSF substudies [ADC: $n = 99$; Alzheimer's disease Neuroimaging Initiative (ADNI) cohort: $n = 198$][34] were used to validate our findings. We identified several pQTLs for AD and DLB, but not for FTD. Identified CSF proteins were related to immune system and glucose metabolism.

Materials and methods

Study sample

Supplementary Fig. 1 provides an overview of cohorts included in discovery and replication analyses. All participating studies were approved by their respective Medical Ethics Committee.

Discovery cohort

For the discovery phase, we selected 502 subjects from the ADC[33] who had both genetic[35,36] and CSF proteome data available.[37]

Diagnostic groups included control subjects ($n = 146$), and patients with mild cognitive impairment (MCI; $n = 37$), AD ($n = 176$), DLB ($n = 50$) or FTD ($n = 93$). All patients with MCI had abnormal CSF amyloid-β (Aβ) and most ($n = 28/37$, 75.7%) had a positive AD CSF biomarker status (defined by an abnormal ratio of CSF t-tau/Aβ[38]). As these biomarker statuses have been associated with an intermediate-to-high likelihood to progress to AD-type dementia, we grouped MCI and AD patients together.[39]

All patients from the ADC underwent a standardized multidisciplinary assessment, consisting of medical history, informant-based history, neurological and medical examination, neuropsychological investigation, EEG, brain MRI, standard laboratory work-up and lumbar puncture.[33] Global cognition was estimated using the Mini-Mental State Examination (MMSE).[40] Diagnoses were assigned according to diagnostic criteria for psychiatric and neurodegenerative disease guidelines.[41-44] All AD diagnoses in the ADC cohort were supported by a positive AD CSF biomarker status, as assessed using the ratio CSF total tau/Aβ.[38] Controls had subjective cognitive decline (SCD). SCD was assigned to subjects that did not meet clinical criteria for psychiatric or neurodegenerative disorders and had no objective cognitive abnormalities on neuropsychological testing. This study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants.

Extended CSF proteome cohort

To analyse whether the levels of pQTL-related proteins identified in this study differed across diagnostic groups, we used the corresponding proteomic data from the extended CSF proteome cohort used in this study.[37] The larger CSF proteome study included a total of 747 CSF samples from patients with AD ($n = 230$), DLB ($n = 123$), FTD ($n = 199$) and 195 control subjects.

Replication cohorts

Replication analyses were performed in independent cohorts from ADC ($n = 99$ controls only) and ADNI ($n = 198$; $n = 67$ controls, $n = 88$ patients with MCI, $n = 43$ AD patients)[34] (Supplementary material). All AD diagnoses within the ADNI replication cohort were supported by a positive AD CSF biomarker status.

CSF proteome measurements

CSF samples were collected via lumbar puncture, using a 25-gauge needle and syringe. CSF levels of amyloid-β_{42} ($A\beta_{42}$), total tau (t-tau) and hyperphosphorylated 181 tau (p-tau) were determined as part of the diagnostic work-up, using ELISA (Innotest, Fujirebio).[33] For the ADC cohort, CSF $A\beta_{42}$ values were adjusted for drift over time, as previously described.[45] Biomarker abnormality cut-offs for the ADC cohort are CSF $A\beta_{42} < 813$ pg/ml, CSF t-tau > 375 pg/ml, ratio t-tau/$A\beta_{42} > 0.52$.[38] For the ADNI cohort, cut-off points for biomarker abnormality are $A\beta_{42}$ levels < 192 pg/ml and t-tau levels > 93 pg/ml, ratio t-tau/$A\beta_{42} > 0.39$.[46]

In total, 979 CSF proteins were measured using 11 multiplex antibody-based protein panels based on the proximity extension assay (PEA) (i.e. cardiometabolic, cardiovascular II and III, cell regulation, development, immune response, inflammation, metabolism, neurology, oncology II and organ damage) (Olink proteomics).[47] Each panel can measure up to 92 proteins, and 30 proteins were measured in two different panels (replicates). All characteristics and validation data for each assay are available on the manufacturer's webpage (www.olink.com). Protein assessment and quality control has been described in detail previously.[37] To control for potential interplate variation, samples were randomized across plates and intra- and interplate quality controls. To control for batch effects, 16 bridging samples were included. Proteins with values below the lower limit of detection (LOD) in >15% of the total samples were removed, leading ultimately to 665 proteins (642 unique proteins, some proteins were measured multiple times) included for further analysis (see Supplementary Tables 1 and 2 for a list of included and excluded proteins, respectively). Remaining raw values under LOD (2.7% of all measurements that were similarly distributed across diagnostic groups: 3.4% for controls, 1.6% for MCI, 2.6% for AD, 2.4% for DLB, 2.5% for FTD) were kept as provided by the manufacturer. Protein levels are reported in log2-scale as Normalized Protein eXpression (NPX).

CSF proteome data from the ADNI cohort were measured with mass reaction monitoring (MRM)-targeted mass spectroscopy (for ADNI methods, see Supplementary material and Spellman *et al.*[29]). We combined MRM protein fragments into a single protein score when these were correlated with $r > 0.5$, as previously described.[48] CSF IL6 was measured as part of another study using multiplex assays and normalized using inverse-rank normalization.[49]

Genotyping and imputation

All genetic samples from the ADC were genotyped on the Illumina Global Screening Array (GSA) v1, human genome build 37. ADNI samples were genotyped using the Illumina OmniQuad array[34] and were retrieved online from https://adni.loni.usc.edu/. Standard quality control methods were performed, as described in depth elsewhere.[35,36] Briefly, SNPs with a significant deviation from Hardy-Weinberg equilibrium ($P < 1 \times 10^{-6}$), or a variant call rate < 98% were excluded from the total sample. Individuals with sex mismatches or an individual call rate <98% were excluded from analyses. SNPs were imputed to the Haplotype Reference Consortium (HRC) reference panel, with the use of the Sanger imputation server (https://imputationserver.sph.umich.edu).[50,51]

To identify genetic ethnic outliers, a principal component (PC) analysis of ancestry was performed (based on 1000Genomes clustering) using EIGENSOFT. Individuals of non-European ancestry were excluded from analysis ($n = 25$). Relatedness was assessed through identity by descent and family relations up to second degree (i.e. identity-by-descent ≥ 0.3) were excluded. To account for population structure, PCs were calculated on the whole sample and subsamples of diagnostic groups for stratified analyses.

Genetic risk loci were selected based on their previous genome-wide association with AD ($n = 98$),[4-12,52,53] DLB ($n = 9$)[13,14] or FTD ($n = 9$).[15-17] All SNPs with the strongest signal within the risk loci and SNPs in linkage disequilibrium (LD) with these ($r^2 > 0.8$) were extracted from genetic data based on rsID and/or base pair location in the genome. For SNPs that were not available in our data, we selected SNPs in LD ($r^2 > 0.6$) with the risk SNP of interest. The final SNP selection included 2244 SNPs for AD, 198 SNPs for DLB and 328 SNPs for FTD (Supplementary Table 3).

Statistical analysis

Association signals between genetic risk variants and CSF protein levels were performed using a linear regression model in PLINK (version 2.0).[54,55] Additional statistical analyses and data visualization were performed using R (version 4.0.3, Bunny-Wunnies freak

out, R Development Core team 2010). Analyses were performed in the total sample ($n = 502$) and corrected for age, sex and population structure (PC1–4). SNPs with a minor allele frequency (MAF) < 0.001 were excluded from the final results. The pQTLs were defined as *cis* when the risk SNP was within ±1 Mb of the transcriptional start site (TSS) of the gene of the corresponding CSF protein, and as *trans* if the SNP was outside this ±1 Mb region. SNPs were assigned to gene names using positional mapping, and for intergenic genes this was done by selecting the nearest located gene. To examine whether pQTL associations were only detectable in specific dementia subtypes, additional analyses were performed stratified according to diagnostic status only (AD, DLB, FTD and controls separately).

Results based on the total and stratified samples were corrected for multiple testing using a 5% Bonferroni significance threshold [i.e. $P < 0.05$/number of genetic risk loci (98 risk loci for AD, nine for DLB, nine for FTD) × number of unique CSF proteins]. Nominal significance was defined as $P < 0.05$/number of genetic risk loci (Supplementary Table 3). As correcting for the total number of 2244 SNPs would be overly stringent, we used an LD-based significance threshold by dividing $\alpha = 0.05$ by the number of independent genetic markers (116 genetic risk loci for dementia) multiplied by the number of CSF proteins. Replication analyses were performed for significant pQTL associations. For replication analyses, the significance threshold was $P < 0.05$ (unadjusted). To examine whether pQTLs in the discovery and replication data were driven by the same genetic signal, LD patterns were examined using LDpair (1000 Genomes, European ancestry) and zoomplot.[56,57]

To analyse the pathophysiological and clinical relevance of the proteins associated to the pQTL, CSF protein levels were compared between pairs of diagnostic groups (i.e. diagnosis associated with the genetic risk loci and controls) within the extended CSF proteome cohort ($n = 747$), using nested linear models, corrected for age and sex, as previously described.[37] Multiplicity (per group comparison) was taken into account by controlling the false discovery rate (FDR) at $q \leq 0.05$.[58] Spearman rank-order correlations were used to examine the correlation between CSF proteins identified by the pQTL analysis and classical AD CSF biomarkers ($A\beta_{42}$, t-tau, p-tau and t-tau/$A\beta_{42}$ ratio) and MMSE scores.

Co-localization analyses

To examine whether dementia and CSF protein levels share similar causal genetic variants, we performed Bayesian co-localization analyses using COLOC package[59] (https://chr1swallace.github.io/coloc/articles/a03_enumeration.html). CSF proteins for which we identified a significant pQTL in the total sample were selected for this analysis, including CR2, PILRB, CTSH and RETN. For each CSF protein, we selected a genetic region using a 50 kb window surrounding the most significant SNP in the pQTL analysis. COLOC uses a Bayesian framework to generate posterior probability (PP) for five mutually exclusive hypotheses regarding the sharing of causal genetic variants between two traits (i.e. dementia and CSF protein levels), including: 0, no genetic associations with either dementia or the CSF protein (PP0); 1, association with dementia only (PP1); 2, association with the CSF protein level only (PP2); 3, association with dementia and CSF protein levels from distinct causal variants (PP3); and 4, shared causal variant between dementia and CSF protein levels (PP4). We defined co-localization evidence as supportive for a shared causal variant (PP4) or distinct causal variant (PP3) between dementia and CSF protein levels, when the PP was higher than the other posterior probabilities.

Enrichment analysis for Gene Ontology biological processes

To gain more insight into the biological properties of the pQTL associations, enrichment analyses on CSF proteins nominally (suggestive $\alpha = 0.05$/number of genetic risk loci, see Supplementary Table 3) associated with risk loci for dementia were performed using g:Profiler (https://biit.cs.ut.ee/gprofiler/gost).[60,61] G:Profiler is a public web server designed for characterizing gene lists resulting from mining high-throughput genomic data, such as enrichment analysis.

Proteins were annotated using the Gene Ontology (GO) biological processes database. Enrichment analyses were performed including those CSF proteins (with protein code availability in g:Profiler) with nominal associations in the complete ($n = 44$ proteins) or disease-specific stratified analysis (AD: 29 proteins, DLB: 13 proteins, FTD: six proteins).

Enrichment results were corrected for multiple comparisons using the g:SCS algorithm, which is specially developed to estimate thresholds in complex and structured functional profiling data such as the GO database. Enrichment analyses were repeated, including the 642 unique proteins of OLINK as a background list as sensitivity analysis.

Results

Demographic and clinical characteristics of the discovery sample ($n = 502$) are presented in Table 1. Control subjects were younger than all dementia patient groups. The percentage of females was lower in DLB compared to all other diagnostic groups. MMSE scores were lowest in AD, followed by DLB, FTD, MCI and controls.

Protein quantitative trait loci mapping of dementia risk loci

A total of 2244 genetic risk variants for AD, DLB and FTD were tested for their association with 665 CSF protein levels. In the total sample ($n = 502$ including controls, AD, DLB and FTD), we identified 32 significant pQTL SNP-protein pairs between four AD genetic risk loci (*CR1*, *HESX1*, *ZCWPW1* and *CTSH*) and CSF levels of four proteins (CR2, resistin, PILRB, CTSH, respectively) (Fig. 1, Table 2 and Supplementary Fig. 2). A full overview of all 672 nominally significant ($P < 4.95 \times 10^{-4}$) pQTL-protein pairs between 30 genetic loci and 90 CSF proteins is presented in Supplementary Table 4. The first pQTL locus, *CR1* on chromosome 1, significantly associated with CSF levels of protein CR2. The most significant SNP is the intronic variant rs3818361 (effect allele = A, $P = 1.65 \times 10^{-8}$), of which the effect allele related to higher CR2 CSF protein levels (Fig. 2A and Supplementary Fig. 3). The second pQTL locus *HESX1* on chromosome 3 was based on one rare intergenic variant (rs186108507-T, MAF = 0.002, $P = 8.30 \times 10^{-8}$), which associated with higher levels of CSF resistin (Fig. 2B and Supplementary Fig. 4). The third locus, *ZCWPW1*, significantly associated with PILRB CSF levels (Fig. 2C and Supplementary Fig. 5). The most significant SNP is the intronic variant rs1476679 (effect allele = C, $P = 2.73 \times 10^{-32}$), of which the protective, effect allele associated with lower PILRB CSF levels. Explorative analysis on the same genetic variant of gene *PILRA* (effect allele = C, SNP rs1476679) with CSF PILRA protein levels showed similar results in direction of effect but did not reach genome-wide significance ($P = 1.80 \times 10^{-3}$). The last pQTL was the previously reported *cis* association between *CTSH* and CSF CTSH,

Table 1 Demographic and clinical characteristics from discovery data ($n = 502$), Amsterdam Dementia Cohort

	All $n = 502$	Controls $n = 146$	Prodromal AD/MCI $n = 37$	AD-type dementia $n = 176$	DLB $n = 50$	FTD $n = 93$	P
Age, mean (SD)	64.1 (8.7)	57.7 (7.7)	68.0 (7.0)	66.0 (7.7)	69.0 (7.6)	66.5 (7.7)	$<2.0 \times 10^{-16}$[a]
Female, *n* (%)	181 (35.4%)	46 (31.5%)	17 (45.9%)	70 (39.8%)	3 (6.0%)	45 (48.4%)	5.3×10^{-6}[b]
MMSE, median (IQR)	25 (21.75–28)	29 (27–29)	27 (25–28)	21 (17–24)	23 (21–26)	25 (23–28)	$<2.0 \times 10^{-16}$[c]
APOE-e4, at least one allele, *n* (%)	223 (45.8%)	36 (25.0%)	27 (73.0%)	104 (60.5%)	29 (60.4%)	27 (31.4%)	7.0×10^{-13}[d]
Abnormal CSF t-tau/Aβ ratio, *n* (%)	232 (46.8%)	1 (0.69%)	28 (75.7%)	175 (100%)	12 (24.5%)	16 (18.0%)	$<2.2 \times 10^{-16}$[e]

Data are presented as mean (standard deviation, SD) for normally distributed continuous variables, as median (first quartile–third quartile) for non-normally distributed continuous variables and as *n* (%) for categorical variables. *Post hoc* comparisons were performed using χ^2 tests or *t*-tests where appropriate. Missing values: two for MMSE, 15 for *APOE*-status, six for ratio total tau/Aβ.

Aβ = amyloid-β; AD = Alzheimer's disease; DLB = dementia with Lewy bodies; FTD = frontotemporal dementia; IQR = interquartile range; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination (range 0–30).

[a]*Post hoc* comparisons with $P < 0.05$ age: controls < prodromal AD, AD, DLB, FTD.

[b]*Post hoc* comparisons with $P < 0.05$ sex (% female): DLB < controls, prodromal AD, AD, FTD and controls < FTD.

[c]*Post hoc* comparisons with $P < 0.05$ MMSE: controls > prodromal AD > FTD > DLB > AD.

[d]*Post hoc* comparisons with $P < 0.05$ *APOE*-e4 carriership: controls < prodromal AD, AD, DLB and FTD < prodromal AD, AD, DLB.

[e]Abnormal CSF t-tau/Aβ ratio: controls < FTD, DLB < prodromal AD < AD.

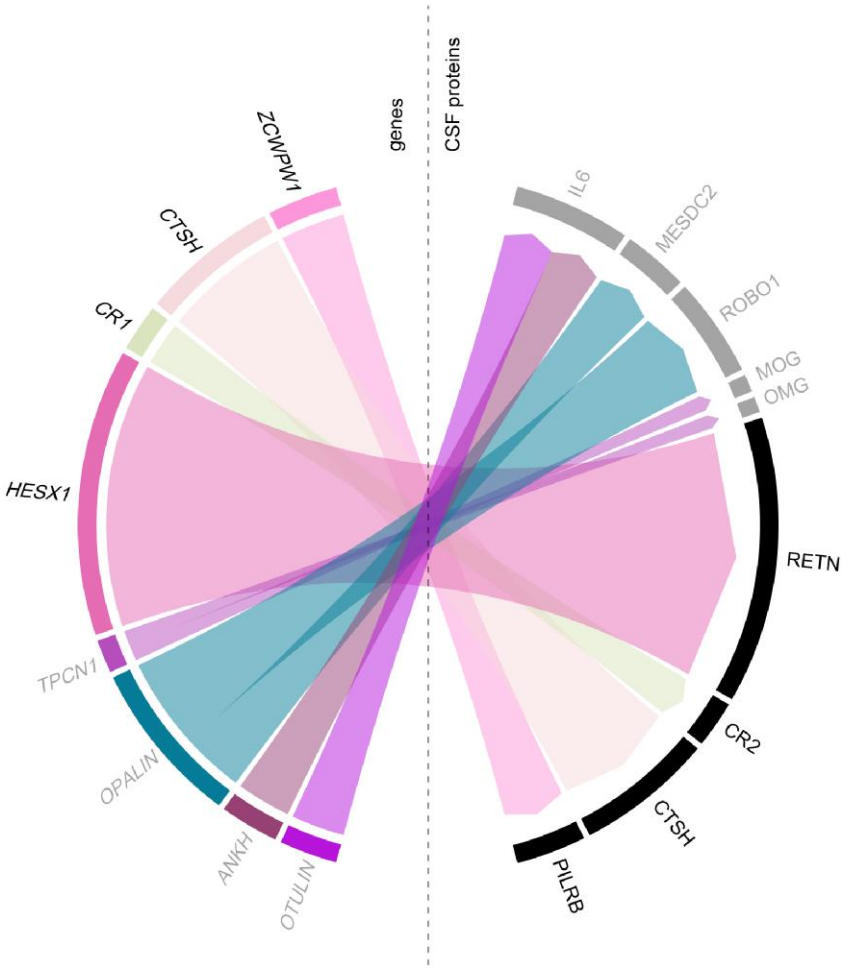

Figure 1 Relation between Alzheimer's disease genetic risk variants (*left*) and CSF protein concentrations (*right*) in the total sample. In this figure, we selected the top 10 protein quantitative trait loci (pQTL) associations between Alzheimer's disease (AD) genetic risk variants (*left*) and CSF protein levels (*right*) in the total sample ($n = 502$). Significant associations ($P < 7.71 \times 10^{-7}$) replicated in the independent cohorts are depicted in black text. Suggestive associations ($P < 4.95 \times 10^{-4}$) are depicted in grey. The size of the arrows indicates the strength of the association (beta estimate) between genetic variants and CSF protein levels. Supporting data are presented in Supplementary Table 4.

Table 2 Significant associations between genetic risk loci for Alzheimer's disease and CSF protein levels in the total sample ($n = 502$)

SNP	Genetic locus	CSF protein	Chr	Bp				Discovery, $n = 502$			Replication, $n = 99$ ADC		replication, $n = 198$ ADNI	
					Ref	Alt	% alt	n	Beta	P	Beta	P	Beta	P
rs3818361	*CR1*	CR2	1	207 784 968	G	A	20	502	0.25	1.65×10^{-8}	0.25	1.66×10^{-3}	NA	NA
rs1476679	*ZCWPW1*	PILRB	7	100 004 446	T	C	30	502	–0.37	2.73×10^{-32}	–0.38	7.82×10^{-8}	NA	NA
rs3784539	*CTSH*	CTSH	15	79 219 188	C	T	14	502	–0.70	2.88×10^{-24}	–0.70	2.40×10^{-7}	NA	NA
rs186108507[a]	*HESX1*	RETN	3	57 228 084	C	T	0.2	502	3.04	8.39×10^{-8}	–0.33	0.01	0.75	1.17×10^{-3}

The Beta values represent the single nucleotide polymorphism (SNP) effect (of the alternative allele) on the corresponding CSF protein. For proteins with multiple SNPs associated with CSF levels, the bi-allelic genetic variant with the lowest P-value for the association with the CSF protein level is shown. CSF protein levels are measured using proximity extension assay (PEA), unless stated otherwise. In the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort, CSF RETN levels were measured using proteomics mass reaction monitoring MRM targeted mass spectroscopy. Genetic Alzheimer's disease (AD) risk loci were selected based on association with AD risk in previous genome-wide association studies (GWAS): *CR1* (Beta = 0.02, $P = 2.01 \times 10^{-18}$), *ZCWPW1* (Beta = –0.02, $P = 6.04 \times 10^{-14}$), *HESX1* (Beta = 0.17 $P = 4.17 \times 10^{-7}$)[4] and *CTSH* (Beta = –0.72, $P = 4.2 \times 10^{-9}$).[11] alt = alternative allele/effect allele; Bp = base pair location; Chr = chromosome; *CR1* = complement receptor 1; CR2 = complement receptor 2; CTSH = Cathepsin H; *HESX1* = HESX homeobox 1; NA = not applicable; PILRB = paired immunoglobulin-like type 2 receptor beta; ref = reference allele; RETN = resistin; *ZCWPW1* = zinc finger CW-type and PWWP domain containing 1.

[a]The SNP rs186108507 was not used in replication analyses because it was not measured (ADNI) or had a too low minor allele frequency (MAF) [Amsterdam Dementia Cohort (ADC) replication cohort]. Both replication SNPs were in linkage equilibrium with discovery variant rs186108507 (rs9860863 for ADC D' = 1, R2 = 0.002; rs4637258 for ADNI D' = 1, R2 = 0.005) and direction of effects (Beta values) can therefore not be compared between discovery and replication stages. Discovery and replication results may reflect independent effects within the same genetic region.

of which rs3784539-T associated with lower CSF CTSH protein levels ($P = 2.88 \times 10^{-24}$) (Fig. 2D and Supplementary Fig. 6).[21,24,28]

Disease-specific protein quantitative trait loci

We next examined the presence of pQTL associations specific to dementia types. The identified CSF protein associations with *CR1*, *ZCWPW1* and *CTSH* remained significant after stratification for AD and controls, indicating that these pQTLs are not specific to AD-type dementia (Supplementary Fig. 2 and see Supplementary Table 5 for a complete overview of suggestive results). The *HESX1*-RETN pQTL could be tested in controls only ($P = 6.58 \times 10^{-5}$), as the AD patient group did not include rs186108507-T carriers.

Within AD patients ($n = 212$), we identified an AD-specific association between a rare genetic variant on *TREM2* (rs75932628-T) and CSF protein levels of IL6 ($P = 3.90 \times 10^{-7}$, $P > 0.5$ in the total sample). Within DLB patients ($n = 50$), we observed that a rare SNP-allele on *GBA* (rs12742181-T) showed positive pleiotropic *trans* associations with CSF protein levels of FCRL1 ($P = 1.94 \times 10^{-6}$) and nominal significant associations with CD79B ($P = 5.47 \times 10^{-5}$), SELL ($P = 9.73 \times 10^{-5}$), TNFRS13B ($P = 1.07 \times 10^{-4}$), IGLC2 ($P = 2.36 \times 10^{-4}$), CD1C ($P = 4.47 \times 10^{-4}$) and CXCL13 ($P = 6.86 \times 10^{-4}$) ($P > 0.5$ in the total sample, $n = 502$) (Fig. 3). None of the *GBA* associations were significant after excluding the $n = 1$ homozygous risk allele carrier (rs12742181-T/rs12742181-T) (Supplementary Fig. 7). FTD risk loci did not significantly associate with any of the CSF proteins analysed in this study, either for the total sample as for analyses performed within FTD patients only.

Replication results

Supplementary Table 6 describes characteristics from the replication datasets, including ADC ($n = 99$, measured with PEA platform) and ADNI ($n = 198$, RETN measured with MRM-targeted mass spectroscopy, IL6 measured with a multiplex assay) cohorts. Three pQTL-protein pairs were directly replicated, including risk locus *CR1* with CSF CR2 (ADC cohort: rs3818361, $P = 1.66 \times 10^{-3}$, ADNI: not measured), risk locus *ZCWPW1* with CSF PILRB levels (ADC cohort: rs1476679, $P = 7.82 \times 10^{-8}$, ADNI: not measured) and risk locus *CTSH* with CSF CTSH (ADC cohort: rs3784539, $P = 2.40 \times 10^{-7}$, ADNI: not measured). The association between AD risk locus *HESX1* and CSF resistin was indirectly replicated, since the discovery SNP (rs186108507) or SNPs in LD were not available in our validation cohorts (ADC cohort: rs4637258, $P = 0.01$; ADNI cohort rs9860863: $P = 1.17 \times 10^{-3}$) (Table 2 and Supplementary Table 7). Similarly, we observed an indirect replication between genetic risk locus *TREM2* (discovery SNP: rs75932628) and CSF IL6 (rs6937336: $P = 0.02$) (Supplementary Table 7). As the discovery SNPs for both *HESX1* and *TREM2* were not in LD with their replication SNPs, respectively, discovery and replication results may reflect independent effects within the same genetic region. DLB-specific associations for *GBA* could not be tested as replication cohorts did not include DLB patients and had a low frequency of the rare *GBA* SNP allele ($n = 7/99$ heterozygous carriers of rs12742181-T, MAF = 0.04%).

Protein quantitative trait loci proteins associate with clinical and pathophysiological phenotypes

To assess the clinical and pathophysiological relevance of the pQTL-associated proteins to specific dementias, we compared levels of these CSF proteins across diagnostic groups and analysed their associations to AD pathological proxies (i.e. CSF Aβ, t-tau,

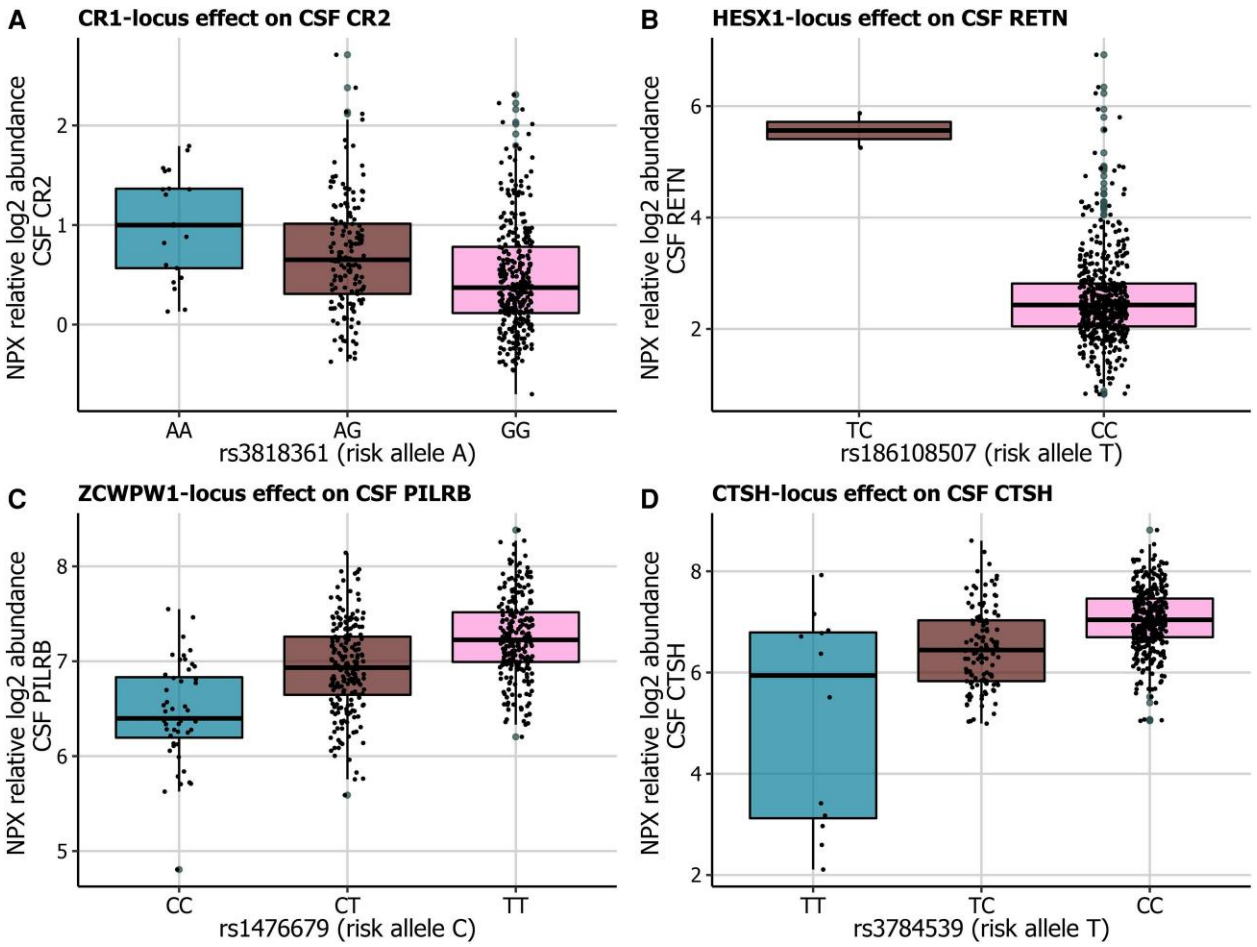

Figure 2 Significant pQTL associations in the total sample ($n = 502$). (**A**) Genetic risk locus *CR1* on CSF CR2. (**B**) Genetic risk locus *HESX1* on CSF RETN. (**C**) Genetic risk locus *ZCWPW1* on CSF PILRB. (**D**) Genetic risk locus *CTSH* on CSF CTSH. In this visualization, groups are based on the carriership of the risk allele. For example, in (**A**), 'AA' corresponds to subjects homozygous for the rs3818361-A allele, 'AG' to heterozygous subjects and 'GG' to subjects homozygous for the rs3818361-G allele. The discovery and replication cohorts did not include homozygous rs186108507-T carriers (*HESX1*). CR1 = complement receptor 1; CR2 = complement receptor 2; CTSH = Cathepsin H; *HESX1* = HESX homeobox 1; PILRB = paired immunoglobulin-like type 2 receptor beta; pQTL = protein quantitative trait loci; RETN = resistin; *ZCWPW1* = zinc finger CW-type and PWWP domain containing 1.

p-tau, ratio t-tau/Aβ) and cognitive function (MMSE), using the data from the extended cohort employed in this study.[37]

Of the four CSF proteins associated with AD risk loci (i.e. CR2, resistin, PILRB and CTSH), resistin and PILRB were increased in AD compared to controls and the group of non-AD dementias (FTD and DLB, $q < 0.05$, Supplementary Fig. 8A). CTSH CSF protein levels were decreased in the non-AD dementia group compared to AD. None of these proteins correlated with CSF Aβ protein levels. CSF levels of PILRB, CR2, resistin and CTSH were all associated with t-tau (PILRB: $r > 0.2$, $P < 0.0001$, CR2 and resistin: $r < 0.2$ $P < 0.05$, CTSH: $r > 0.15$, $P < 0.001$) and p-tau (PILRB, CR2, restitin: $r > 0.1$, $P < 0.001$, and CTSH: $r < 0.1$, $P < 0.05$). CSF PILRB and resistin were also associated with t-tau/Aβ (r: 0.15–0.30, $P < 0.0001$). All three proteins, except for CTSH, correlated with MMSE scores, albeit weakly ($r < 0.2$, $P < 0.05$, Supplementary Fig. 8C).

Of all pQTL proteins that associated with the DLB risk loci *GBA* (FCRL1, CD79B, SELL, TNFRS13B, IGLC2, CD1C and CXCL13), only CXCL13 showed a nominal increase in DLB compared to controls ($P < 0.05$, Supplementary Fig. 8B). CXCL13 was also increased in the AD group. None of the proteins correlated with CSF Aβ. Significant correlations of CD79B, SELL, CD1C and CXCL13 with t-tau, t-tau/Aβ ratio or MMSE scores were detected, albeit weakly ($r < 0.2$). A strong in-between correlation ($r > 0.35$) was observed for pQTL proteins, suggesting that these proteins might be involved in similar biological pathways (Supplementary Fig. 8C).

Co-localization analysis

We performed co-localization analyses to assess whether identical or different genetic variants underlie AD and CSF proteins for which we identified a significant pQTL in the total sample, including CR2, PILRB, CTSH and RETN. For CR2, PILRB and CTSH the co-localization analysis indicated that genetic variants at the same locus associate with both AD and the CSF protein levels (model PP4). For RETN we found that distinct genetic variants (at the same genetic locus) are associated with AD and CSF protein levels of RETN (model PP3) (Supplementary Tables 8 and 9).

Enrichment analysis

No enrichment in any specific biological process was observed when all proteins analysed within this study were included as

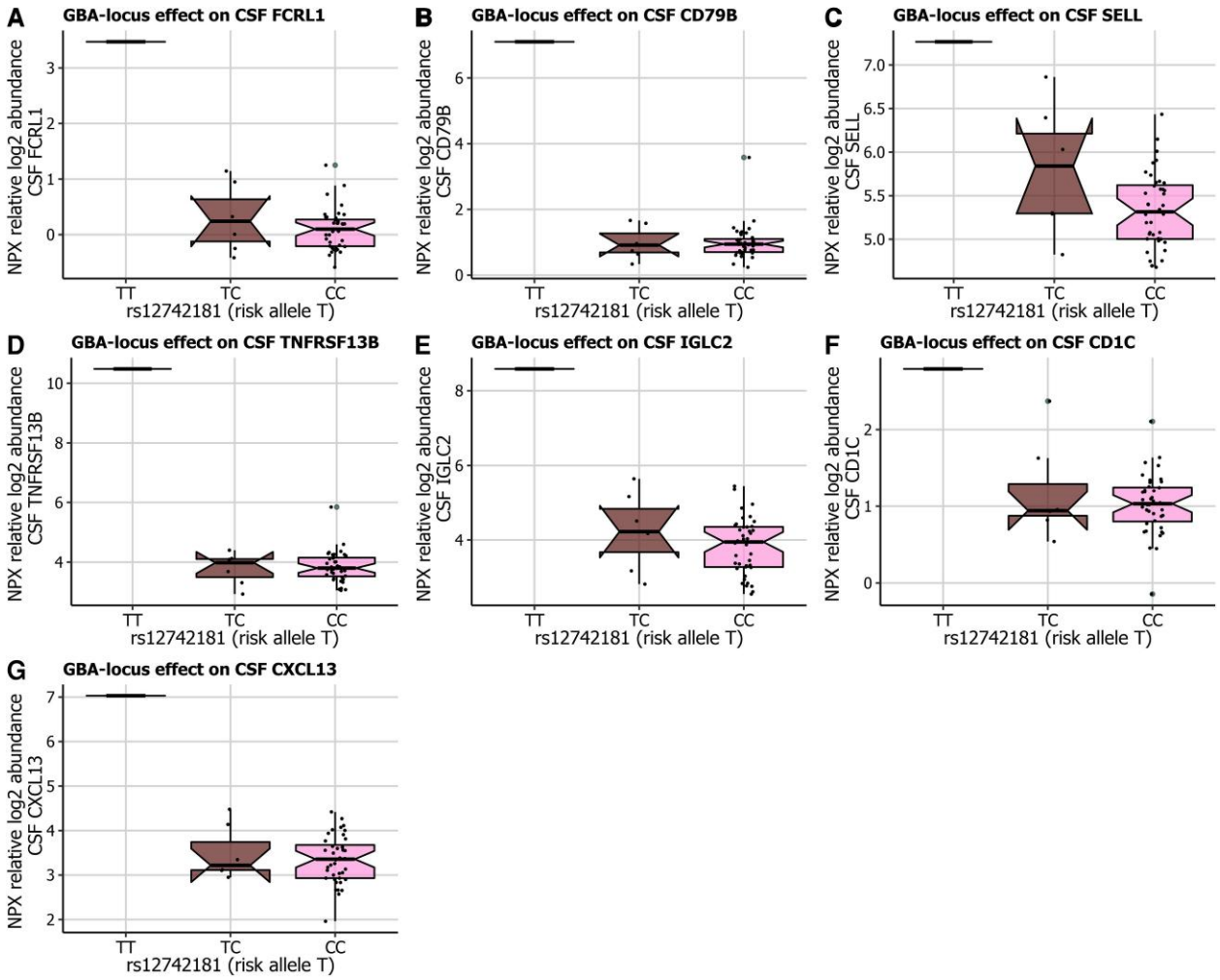

Figure 3 pQTL associations for *GBA* locus in patients with dementia with Lewy bodies ($n = 50$). (**A**) CSF FCRL1, (**B**) CSF CD79B, (**C**) CSF SELL, (**D**) CSF TNFRS13B, (**E**) CSF IGLC2, (**F**) CD1C and (**G**) CSF CXCL13. Protein levels are reported in log2-scale as normalized protein expression (NPX). CD1C = CD1c molecule; CD79B = CD79b molecule; CXCL13 = C-X-C motif chemokine ligand 13; FCRL1 = Fc receptor like 1; *GBA* = glucocerebrosidase; IGLC2 = immunoglobulin lambda constant 2; pQTL = protein quantitative trait loci; SELL = selectin L; TNFRSF13B = TNF receptor superfamily member 13B.

background list. Without correcting for this background list, we observed an enrichment of biological processes related to the immune function in those proteins showing nominal associations with dementia risk loci (e.g. GO:0002376 immune system process, GO:0002687 positive regulation of leucocyte migration, range $P_{adj} = 8.12 \times 10^{-8}$ to 4.55×10^{-2}, Supplementary Table 10).

Discussion

Here, we integrated genetic and CSF proteomic data to better understand the biological context underlying the genetic architecture of dementia. We identified and replicated pQTL associations between three AD risk loci and three CSF proteins (*CR1*-CR2, *ZCWPW1*-PILRB, *CTSH*-CTSH). Disease-specific analyses revealed a pQTL effect of *TREM2* on CSF IL6 in AD patients only, which warrants replication in another study. Within DLB patients, the DLB risk locus *GBA* showed pleiotropic positive *trans*-effects on FCRL1, CD79B, SELL, TNFRSF13B, IGLC2, CD1C and CXCL13 CSF levels. FTD risk loci did not significantly associate with any of the CSF proteins analysed in this study.

This study provides novel additional information, such as the identification of novel CSF pQTL loci and dementia-specific insights, to the few CSF pQTL studies performed so far.[24,27-29] Together with a recent multi-tissue study on 971 CSF samples (of which 249 had an AD diagnosis and 717 were controls),[24] our study is unique by the inclusion of a large set of samples from patients with different dementia types, which allowed us to identify disease-specific CSF proteome-genome associations. The proteins identified through our pQTL analysis provide new insights into biological processes by which the genetic risk loci may contribute to the pathogenesis of the different dementia types (described in detail below). These types of analyses are relevant as treatment targets with genetic support are more likely to succeed and to be approved compared to those without.[62]

Our study revealed an association between the *CR1* loci and the levels of CSF CR2, which could be explained by the close interplay of CR1 and CR2.[63] CSF CR1 protein levels were not measured within our proteomic array and thus we could not test the association between *CR1* and CSF CR1. Both CR1 and CR2 are part of the complement system, which is involved in immune regulation[63] and can play a relevant role in AD pathophysiology.[52,64]

The strongest association detected in this study was between *ZCWPW1* risk variants and CSF PILRB protein levels. A previous study has shown that these loci also influence gene expression of PILRB.[65-67] The identified protective variant associated with lower levels of PILRB in CSF. In line with such findings, we also observed higher CSF PILRB levels in AD compared to controls or other non-AD dementias, underpinning the role that this protein may play in AD pathophysiology. PILRB is a cellular signalling activating receptor expressed in myeloid cells and activates the immune response by binding the tyrosine kinase-binding protein (TYROBP/DAP12), the strongest main microglia network regulator associated with AD pathophysiology.[68,69] TYROBP is directly involved in Aβ turn-over and neuronal damage and has been shown to modulate tau pathology as well as cognitive deficits in AD mice models.[70-73] Accordingly, TYROBP or its adaptor proteins, such as PILRB, have been proposed as attractive immune-related targets for therapy development.[74]

We also detected an association between the AD risk gene *HESX1* and CSF resistin. The discovery analysis included only two heterozygous rs186108507-T carriers and thus, results should be interpreted with caution and need replication in samples including more carriers of the rare allelic variant of rs186108507. In line with previous CSF findings, we observed that CSF resistin levels were higher in AD compared to controls and patients with non-AD dementias.[75] Resistin is an adipokine that regulates glucose metabolism and can exert pro-inflammatory effects via the secretion of tumour necrosis factor-α and various interleukins.[76] Multiple studies have shown that this protein contributes to the pathogenesis of different age-related disorders through changes in metabolism and inflammatory processes.[76]

Emerging studies suggest that QTL effects are dynamic, meaning that they can depend on physiological context, such as sex,[77] environment[78] and disease.[79] The discovery of these context-dependent QTL effects does not only provide insight into the dynamic, genetic regulation of disorders, but it may reveal relevant molecular players that cannot otherwise be detected through population-based studies. Indeed, the targets identified through context-dependent QTL have been shown to play a major role in the pathophysiology of the disease.[79] Within AD patients only, we observed a *trans*-pQTL between AD risk locus *TREM2* and IL6 CSF protein levels in two independent datasets, which is supported by experimental cell models of microglia activation.[80] This AD-specific result suggests that this QTL effect might be a relevant contributor to AD pathogenesis. Within DLB patients only, we observed a *trans*-pQTL of the *GBA* gene with CSF protein levels of seven intercorrelated proteins associated with the immune response. It should be further noted that all associations with *GBA* were driven by a homozygous risk allele carrier of the *GBA* risk variant. While this may hint toward a *GBA*-induced dysregulation of closely interacting proteins, current results should be interpreted with caution and need replication in independent cohorts.

Several limitations should be considered. The coverage of all genetic variants within known risk loci was not high, and additional pQTL associations relevant to dementia have likely been missed. In addition, proteome and genetic studies with samples from non-AD dementias are limited, and thus, it is essential to extend such studies to identify additional pQTL effects related to non-AD dementias. Furthermore, the frequency of rare risk alleles (e.g. rs186108507 on *HESX1* and rs6937336 on *TREM2* for AD) was low. As such rare variants often have stronger associations with disease risk compared to common risk alleles,[32] future studies should aim to increase sample sizes in order to enlarge such groups. As only a few genetic risk loci for DLB ($n = 7$) and FTD ($n = 9$) have been identified compared to AD ($n = 98$), larger collaborative GWAS efforts on DLB and FTD are essential to enable further gene discovery and discovery of disease-specific effects in these dementias. FTD particularly requires studies of larger sample sizes, as the high clinicopathological heterogeneity of FTD likely hampers the identification of common biological pathways, as also seen in previous biomarker discovery studies.[81] Furthermore, our study consisted solely of European-ancestry individuals. Future studies must include diverse ancestral samples to better grasp the relationship between genetic variation and CSF proteomics. Finally, it should be noted that pQTL studies cannot be used for causal inference. Therefore, experimental validation is essential to further understanding of the relationship between genetic variance to protein levels and disease pathogenesis, to eventually identify which biological mechanisms are suitable targets for treatment strategies.

In conclusion, the results of this study reveal that genetic risk loci for AD and DLB serve as pQTLs, regulating the levels of specific proteins in CSF (i.e. *CR1*-CR2, *ZCWPW1*-PILRB, *CTSH*-CTSH). While preliminary, our targeted approach of including well phenotyped patients with specific causes of dementia, further allowed us to identify disease-specific pQTLs of *TREM2*-IL6 in AD and *GBA*-interconnected inflammatory proteins in DLB. Considering that the CSF biochemical profile may reflect brain pathological changes, these results pinpoint the specific proteins and pathways by which genetic variants associated to AD and DLB contribute or prevent the development of these dementia types. Most pQTLs associated with CSF proteins were involved in the immune function, supporting previous data and highlighting the importance of this system in the pathophysiology of dementia.

Data availability

CSF proteomic data can be made available upon request. Data sharing of subject-level genetic data may be restricted by consent given by research participants within each contributing cohort and by European GDPR regulations, which currently excludes data sharing with a number of non-European countries. ADNI data can be downloaded from adni.loni.usc.edu.

Acknowledgements

Research of the Alzheimer Center Amsterdam is part of the neurodegeneration research program of Amsterdam Neuroscience.

Funding

Genotyping of the Dutch case-control samples was performed in the context of EADB (European Alzheimer DNA biobank) funded by the JPco-fuND FP-829-029 (ZonMW projectnumber 733051061). L.M.R. was funded by the ZonMW Memorabel fellowship 'Identifying biological and clinical relevance of (epi)genetic risk factors in sporadic FTD' (ZonMW projectnumber: 10510022110012). The computational work in this manuscript was carried out on the Dutch national e-infrastructure with the support of SURF Cooperative. Computing hours were granted in 2016, 2017, 2018 and 2019 to Henne Holstege by the Dutch Research Council (project name: '100plus'; project numbers 15318 and 17232). The Alzheimer Center Amsterdam is supported by Stichting Alzheimer Nederland and Stichting VUmc fonds. The clinical database structure was developed with funding from Stichting Dioraphte.

Competing interests

M.d.C. has been an invited speaker at Eisai. L.V. received a grant for the CORAL consortium by Olink. B.M.T. and P.J.V. are inventors on a patent (WO2020197399A1; owned by Stichting VUmc). D.J.I. is a Scientific Advisory Board Member for Denali Therapeutics. W.M.v.d.F. has performed contract research for Biogen MA and Boehringer Ingelheim. W.M.v.d.F. has been an invited speaker at Boehringer Ingelheim, Biogen MA, Danone, Eisai, WebMD Neurology (Medscape) and Springer Healthcare. W.M.v.d.F. is consultant to Oxford Health Policy Forum CIC, Roche and Biogen MA. W.M.v.d.F. participated in advisory boards of Biogen MA and Roche. All funding is paid to her institution. W.M.v.d.F. is member of the steering committee of PAVE and Think Brain Health. W.M.v.d.F. was associate editor of Alzheimer's Research & Therapy in 2020/2021. W.M.v.d.F. is associate editor at Brain. C.E.T. has a collaboration contract with ADx Neurosciences, Quanterix and Eli Lilly and performed contract research or received grants from AC-Immune, Axon Neurosciences, Bioconnect, Bioorchestra, Brainstorm Therapeutics, Celgene, EIP Pharma, Eisai, Grifols, Novo Nordisk, PeopleBio, Roche, Toyama and Vivoryon. She serves on editorial boards of Medidact Neurologie/Springer, Alzheimer's Research & Therapy and Neurology: Neuroimmunology & Neuroinflammation and is editor of a Neuromethods book (Springer). She had speaker contracts for Roche, Grifols and Novo Nordisk.

Supplementary material

Supplementary material is available at *Brain* online.

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