Neurology-related protein biomarkers are associated with general fluid cognitive ability and brain volume in older age

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Abstract

Identifying the biological correlates of late life cognitive function is important if we are to ascertain biomarkers for, and develop treatments to help reduce, age-related cognitive decline. This study investigated the associations between plasma levels of 91 neurology-related proteins (Olink® Proteomics) and general fluid cognitive ability in the Lothian Birth Cohort 1936 (LBC1936, N=798), the Lothian Birth Cohort 1921 (LBC1921, N=165), and the INTERVAL BioResource, (N=4,451). In LBC1936, we also examined mediation of protein-cognitive ability associations by MRI-derived indices of brain structure. In the LBC1936, 22 of the proteins and the first principal component (PC) created from a PC analysis of the 91 proteins, were associated with general fluid cognitive ability (β between -0.11 and -0.17, $p<0.0029$). Total brain volume partially mediated the association between 10 of these proteins and general fluid cognitive ability. Effect sizes for the 22 proteins, although smaller, were all in the same direction as in LBC1936 in an age-matched subsample of INTERVAL. Similar effect sizes were found for the majority of these 22 proteins in the older LBC1921. The associations were not replicated in a younger subset of INTERVAL. In conclusion, we identified plasma levels of a number of neurology-related proteins that were associated with general fluid cognitive ability in later life, some of which were mediated by brain volume.

Introduction

As populations in developed countries continue to age, there is a growing need to understand the biological correlates of individual differences in cognitive ability in later life. Aging-related cognitive changes are thought to be driven – at least in part - by structural changes in the brain [1]. For example, global atrophy, grey matter and white matter volumes, white matter microstructure, and measures such as white matter hyperintensities (WMH) and perivascular spaces (PVS)—which are markers of cerebral small vessel disease (SVD)—have been associated with reduced cognitive ability and risk of dementia in both cross-sectional and longitudinal studies [2–7].

Large scale genome-wide association studies have shown that cognitive ability in later life is highly heritable and polygenic [8–12]. Due to the highly polygenic nature of this trait, it is challenging to identify relevant biological pathways from the genetic variants associated with it. However, gene expression is itself determined by a combination of genetic, ontogenetic, and environmental factors. Because proteins are the proximal products of transcribed and expressed genetic code, directly measuring protein levels can increase power to identify biological pathways in later life cognitive function. Protein levels are more directly linked than genetic variants to individual variation in cognitive function and structural brain phenotypes, with post-translational buffering as a potential mechanism for mitigating many environmental factors [13]. Until recently, it has been relatively difficult and cost-prohibitive to measure multiple proteins in large numbers of plasma samples [14]. Technological advances have enabled high-throughput and cost-effective measurement of plasma proteins, enabling us to link plasma proteomics to cognitive function and brain structure in three large population samples for the first time.

In this study we measured 91 neurology-related protein biomarkers using the Proseek Multiplex Neurology I 96 × 96 reagents kit produced by Olink® Proteomics (Uppsala, Sweden) [15,16]. These proteins have been implicated in neurological processes and/or diseases, cellular regulation, immunology, development or metabolism [17]. The participants were ~800 members of the Lothian Birth Cohort 1936 (LBC1936) [18], ~170 members of the Lothian Birth Cohort 1921 (LBC1921) [18] and ~4,500 members of

INTERVAL [19]. We investigated the association of 91 plasma proteins with general fluid cognitive ability in 5,414 samples. In the LBC1936 cohort we tested for association with brain volumes (total brain, grey matter, normal appearing white matter, WMH), PVS, and white matter tract measures derived from quantitative tractography (fractional anisotropy [FA], mean diffusivity [MD]). We investigated whether any associations between the neurology-related plasma protein levels and general fluid cognitive ability were mediated by structural brain variables.

Results

Descriptive statistics for general fluid cognitive ability in the LBC1936, LBC1921, INTERVAL-Old and INTERVAL-Young samples and for the brain magnetic resonance imaging (MRI) variables (LBC1936 only) are shown in Tables 1 and 2.

Principal Component Analysis of the 91 neurology-related protein biomarkers

Principal component analysis (PCA) indicated that, for all four cohorts, the majority of the variance in the biomarker data was explained by the first 17 components (64%-74%), with greater than 30% explained by principal component (PC) 1 (Supplementary Table 1, Figure 1). The component loadings for PC1-PC5 are shown in Supplementary Tables 2-5. The coefficient of factor congruence between the four cohorts ranged between |0.70 to 1.00| for the first three principal components (Supplementary Table 1). Therefore, protein-PC1-PC3 were selected for further analyses. INTERVAL-Old protein-PC3 components were multiplied by -1 so that the components were scaled in the same direction in all cohorts.

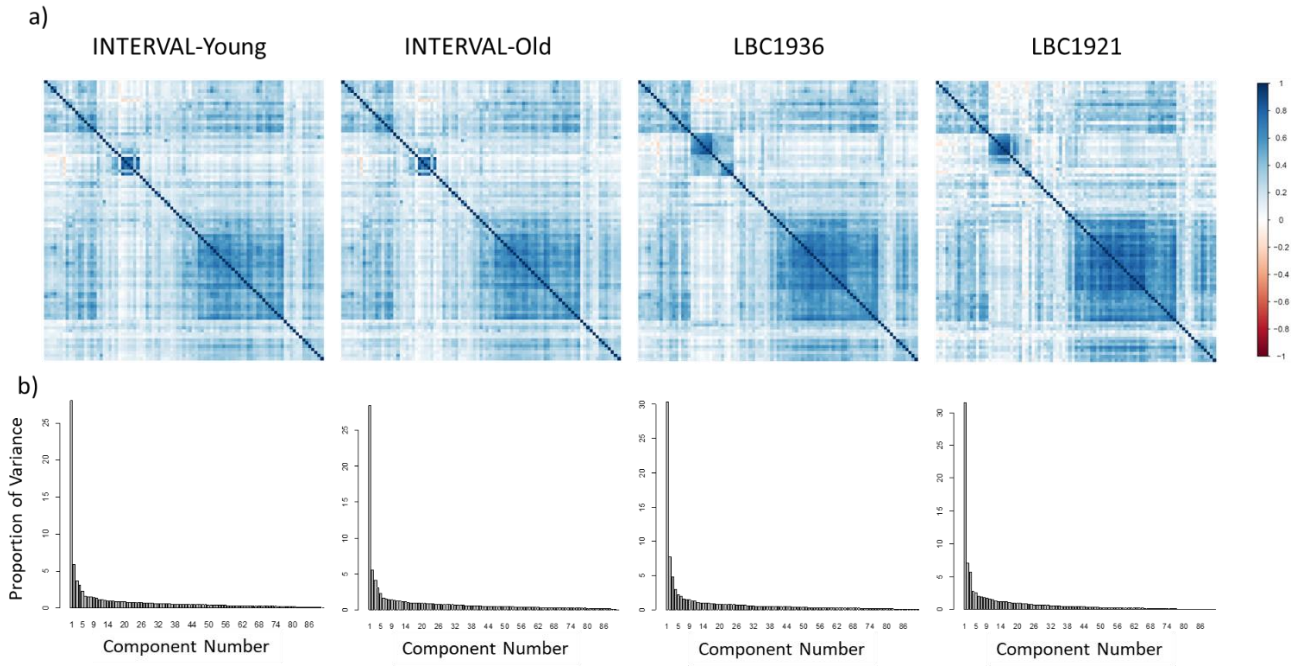

Fig. 1. a) heatmaps and b) screeplots illustrating the principal component analysis on the 91 neurology-related proteins in INTERVAL-Young, INTERVAL-Old, LBC1936 and LBC1921.

Association of 91 neurology-related protein biomarkers and protein-PC1-PC3 with general fluid cognitive ability

Twenty-two proteins and protein-PC1 were associated with general fluid cognitive ability in the LBC1936 (N=798, age ~73 years) (β between -0.11 and -0.17, $p<0.0029$) (Supplementary Table 6 and Table 3). In all instances lower protein levels were associated with higher cognitive ability. Of these 23 associations, two [carboxypeptidase M (CPM) and sialic acid binding Ig like lectin 1 (SIGLEC1)] were nominally associated with general fluid cognitive ability in the age-matched INTERVAL-Old cohort (N=975, age ≥67 years) (β = -0.07 and -0.08 respectively, $p<0.05$). Sixteen associations were significant in a meta-analysis of the LBC1936 and INTERVAL-Old groups (β between -0.07 and -0.10, $p<0.0029$) (Supplementary Table 6). The remaining seven associations were nominally significant in the meta-analysis (β between -0.05 and -0.07, $p<0.05$) (Supplementary Table 6). Direction of effect was consistent

in both cohorts, but effect sizes were smaller in INTERVAL-Old, for all 22 proteins and for protein-PC1. Fourteen of the 23 associations showed evidence of heterogeneity in the meta-analysis (ChiSq between 4.1 and 8.9, $p<0.05$), indicating that the effect sizes were significantly different between the two cohorts. The most significant association in both the LBC1936 and the meta-analysis was with ectodysplasin A2 receptor (EDA2R).

In the older and smaller LBC1921 (N=165, age ~87 years), eight of the 23 proteins/protein-PC1 (including EDA2R) were nominally significantly associated with general fluid cognitive ability (β between -0.16 and -0.20, $p<0.05$), and the direction of effect was the same for all 23. The effect sizes were similar to the LBC1936 results for most of them (Supplementary Table 6). In the larger INTERVAL-Young (N=3476, age ≤66 years), there was no replication ($p>0.05$) of the LBC1936 associations and direction of effect was consistent for half (12/23) of LBC1936 associations (Supplementary Table 6). Supplementary Figure 1 shows scattergraphs indicating effect sizes for 91 proteins and protein-PC1-PC3 for all four cohorts. The LBC1936 is positively correlated with INTERVAL-Old, LBC1921, and INTERVAL-Young (Pearson correlation coefficients=0.21, 0.38 and 0.41 respectively, $p<0.05$), indicating that, in general, associations with general cognitive function across all 91 proteins were similar in effect between the LBC1936 and the other three cohorts. A negative correlation was identified between the LBC1921 and INTERVAL-Young cohorts (Pearson correlation coefficients=-0.21, $p<0.05$).

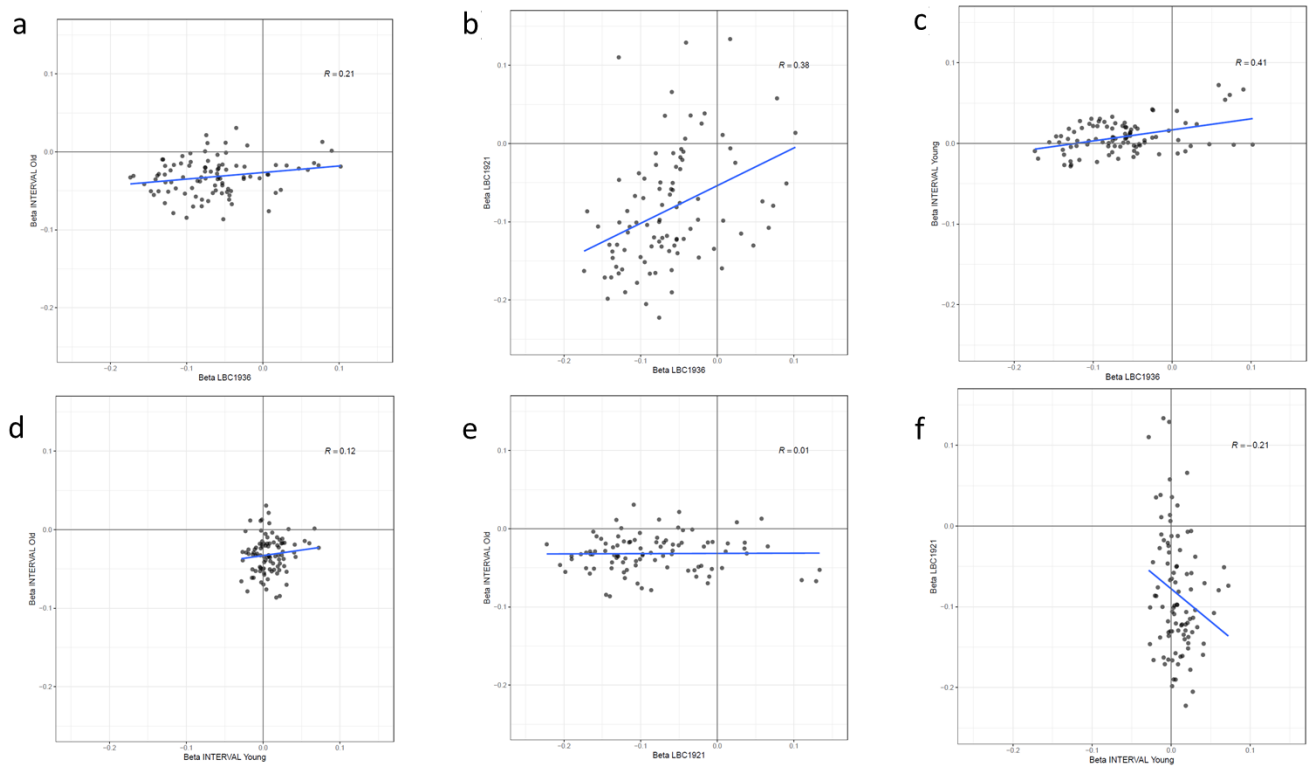

Fig. S1. Scattergraphs indicating the effect sizes (betas) for 91 proteins and protein PC1-PC3 for all four cohorts versus each other.

Association of 91 neurology-related protein biomarkers and protein-PC1-PC3 with brain MRI variables in the LBC1936

Ten, seven and six proteins plus protein-PC3 were associated with total brain, grey matter and normal appearing white matter volumes, respectively, after Bonferroni correction ($p<0.0029$). Protein-PC3, neurocan (NCAN) and contactin 5 (CNTN5) were associated with gFA (β between 0.14 and 0.18, $p<0.0029$). Secreted frizzled-related protein 3 (SFRP-3), CNTN5 and cadherin 6 (CDH6) were associated with gMD (β between -0.12 and -0.13, $p<0.0029$) (Supplementary Table 7). No proteins or protein-PCs were associated with WMH or PVS score (all $p>0.0029$). Twenty-two proteins and protein-PC1 were

associated with general cognitive function in LBC1936; some of these were also associated with brain volume (total brain [5], grey matter [4], normal appearing white matter [2]), and gMD [1] ($p<0.0029$).

Higher levels of EDA2R were associated with smaller total brain volume ($\beta = -0.21$, $p= 3.9x10^{-7}$), smaller grey matter volume ($\beta = -0.16$, $p= 7.4x10^{-5}$), and less normal appearing white matter volume ($\beta = -0.2$, $p= 1.8x10^{-4}$). The strongest associations with total brain, grey matter and normal appearing white matter volumes were with NCAN and brevican (BCAN). Higher levels of NCAN and BCAN were associated with larger brain volumes (β between 0.16 and 0.28); higher levels were also associated with higher fluid cognitive ability in INTERVAL-Young ($\beta=0.07$, $p=2.0x10^{-5}$; $\beta=0.06$, $p=4.0x10^{-4}$). Protein-PC3 was the only principal component associated with brain volumes (β between 0.15 and 0.23); it was also associated with fluid cognitive ability in INTERVAL-Young ($\beta=0.07$, $p=8.1x10^{-5}$).

Mediation analysis in LBC1936

Mediation analyses were performed in the LBC1936 to investigate if brain MRI phenotypes mediated the association between the 23 proteins/protein-PC1 and general fluid cognitive ability. Total brain volume corrected for intracranial volume significantly and partially mediated the association between 10 of these proteins and general fluid cognitive ability (FDR corrected, percentage attenuation between 16.2% and 35.9%) (Table 4). The most significant mediation was identified for EDA2R, where the association between higher EDA2R and poorer cognitive ability was partially (30.6%; β reduced from -0.157 to -0.109) mediated via total brain volume (Figure 2). Multiple brain MRI measures mediated the association between half (5/10) of the proteins and general fluid cognitive ability (FDR corrected, percentage attenuation between 22.0% and 36.4%) (Table 5). The most significant mediation was identified for EDA2R, where the association between higher EDA2R and poorer cognitive ability was partially (36.42%; β reduced from -0.162 to -0.103) mediated via brain variables (Figure 3). Figure 4 and Supplementary Table 8 show that the greatest unique contributions to this mediation effect were consistently from normal appearing white matter and grey matter volumes.

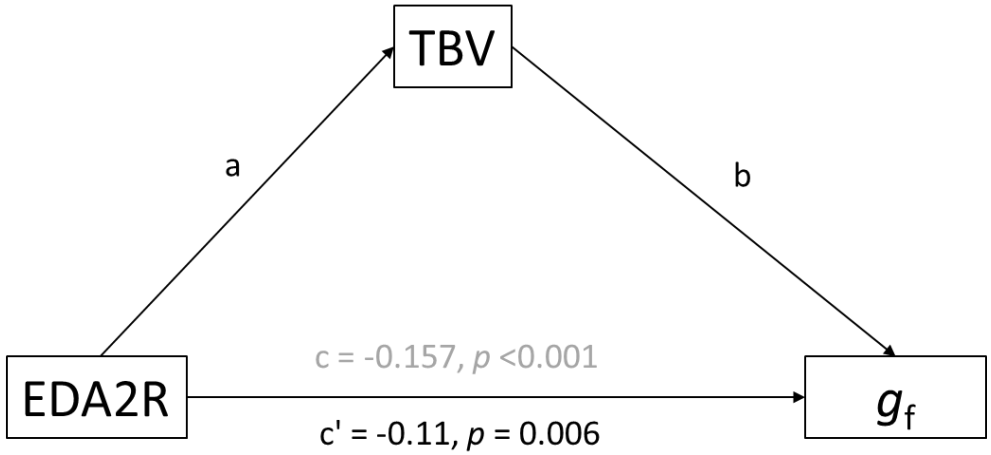

Fig. 2. Mediation analysis showing that the association between EDA2R and gf was significantly partially mediated (31%) by total brain volume in LBC1936. Indirect effect (a x b) = -0.048, p < 0.001.

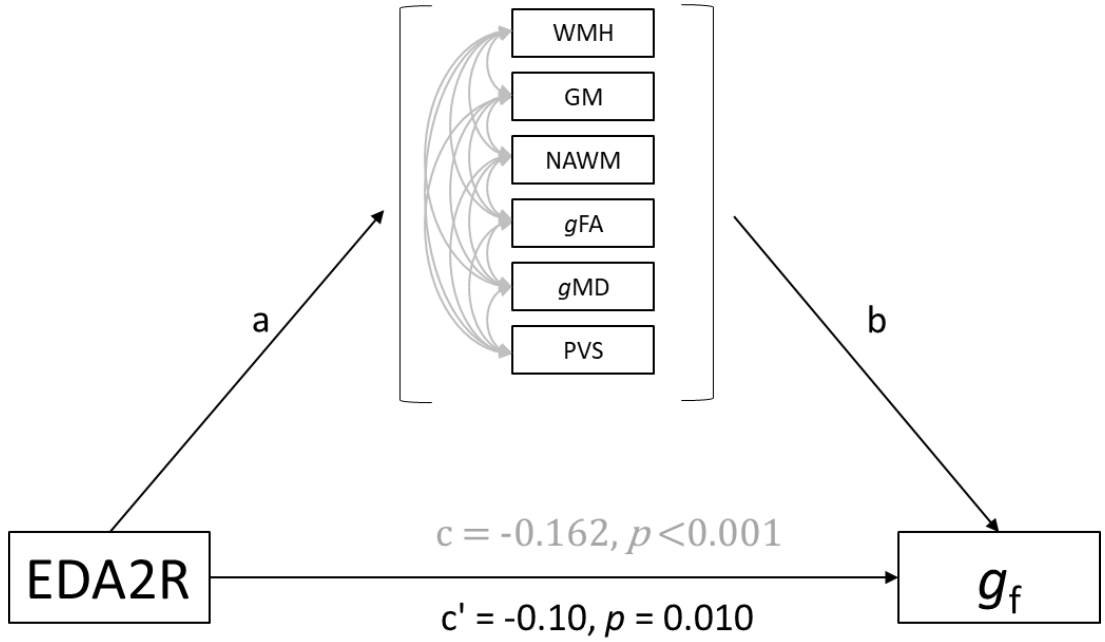

Fig. 3. Mediation analysis showing that the association between EDA2R and gf was significantly partially mediated (36%) by brain MRI variables in LBC1936. Indirect effect (a x b) = -0.059, p < 0.001.

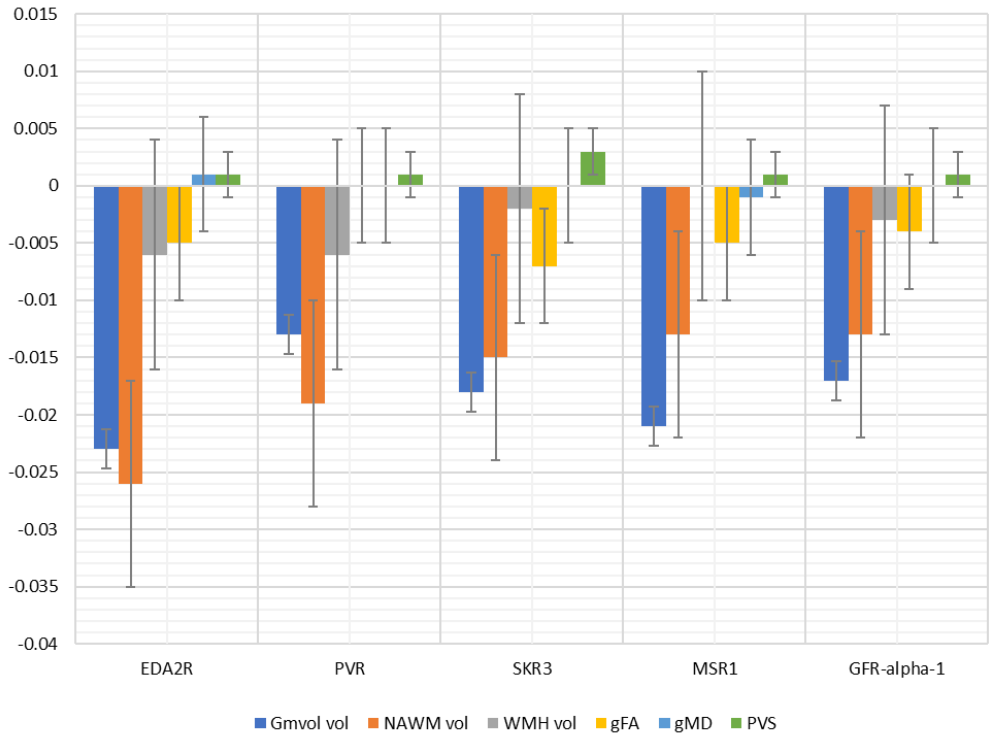

Fig. 4. Mediation analysis in LBC1936 separated by brain variable. Unique contributions from each of the brain variables are indicated. Gmvol=grey matter volume, NAWM vol=normal appearing white matter volume, WMH vol=white matter hyperintensity volume, gFA=general fractional anisotropy, gMD=general mean diffusivity PVS=perivascular spaces.

Discussion

This study investigated associations between 91 neurology-related proteins and general fluid cognitive ability in the LBC1936, LBC1921 and INTERVAL. Twenty-two proteins were associated with general fluid cognitive ability in the LBC1936 and in a meta-analysis of LBC1936 and an age-matched INTERVAL sample. Effect sizes, although smaller in INTERVAL-Old, were all in the same direction as in the LBC1936. Another study that measured proteins in two different populations, using an Olink panel, showed similar differences in the effects sizes of associations with insulin resistance [20]. Differences in

effect sizes may be due to blood from the two cohorts being collected in different tube types (citrate for LBC1936, EDTA for INTERVAL-Old) or differences in the selection bias between the two cohorts. Similar effect sizes to LBC1936 were found for the majority of these 22 proteins in the older LBC1921. Mediation analysis showed that brain volume mediated the association between 10 of the proteins and general fluid cognitive ability. The two proteins that showed the strongest association with total brain, grey matter and normal appearing white matter volumes (NCAN, BCAN) were not associated with general fluid cognitive ability in the LBC1936, LBC1921 or INTERVAL-Old groups, but were associated in the INTERVAL-Young sample.

The EDA2R protein showed the strongest association with general fluid cognitive ability in the meta-analysis of the LBC1936 and age-matched INTERVAL-Old samples. EDA2R is the tumour necrosis factor receptor superfamily member 27 encoded by *EDA2R* on chromosome X. This protein is important in hair and tooth development [21] and levels of EDA2R have been shown to increase with age in blood [22] and lung tissue [23]. It was also associated with reactive astrogliosis in mice [24] and enriched in mouse astrocytes [25]. Other proteins that were relatively strongly associated with general fluid cognitive ability in the LBC1936 and the meta-analysis of the LBC1936 and INTERVAL-Old sample included sialoadhesion encoded by the *SIGLEC1* gene on chromosome 20, a member of the immunoglobulin family [26]; poliovirus receptor encoded by the *PVR* gene on chromosome 19; and R-spondin-1 encoded by the *RSPO1* gene on chromosome 1.

Interestingly, two chondroitin sulfate proteoglycans (CSPGs) that are common constituents of the extracellular matrix (ECM) and specific to the CNS were strongly associated with brain volume in LBC1936. CSPGs are key members of perineuronal nets (PNNs), which are ECM structures surrounding neurons, important in storage and maintenance of long-term memories [27–32]. Neurocan and brevican are encoded by *NCAN* (chromosome 19) and *BCAN* (chromosome 1), respectively, and are expressed in astrocytes and neurons. BCAN is also expressed in oligodendrocytes. These were the only CSPGs on the Olink assay. Neurocan inhibits neuronal adhesion and neurite outgrowth in vitro [33]. Common genetic

variation in NCAN is associated with bipolar disorder [34]. NCAN is the closest relative of BCAN, and animal knockouts of BCAN and NCAN have a similar phenotype (normal development and memory with deficient hippocampal long-term potentiation [35,36]. NCAN peaks in development and declines in the adult brain. In contrast, BCAN is one of the most common CSPGs in the adult brain. It is not yet known what role CSPGs and the PNN may play in age-related cognitive decline, however our data suggest that NCAN and BCAN influence brain volume and may potentially play a neuroprotective role for general fluid cognitive ability in early adulthood.

PCA indicated that the levels of the individual proteins were not independent, with 30% of the variance explained by the first PC. The first three PCs derived from the 91 proteins were highly congruent between the four cohorts, providing cross-sample validation of the stability of the proteins' correlational structure. The first PC was associated with general fluid cognitive ability in the LBC1936, LBC1921, and a meta-analysis of LBC1936 and INTERVAL-Old samples. This association was not mediated by brain variables in the LBC1936, suggesting that the influence on general fluid cognitive ability was independent of the micro- and macrostructural brain variables measured at the global level. Protein-PC3 (like BCAN and NCAN which load highly on protein-PC3) was not associated with general fluid cognitive ability, but was associated with total brain, grey matter and normal appearing white matter volumes in the LBC1936, suggesting that although it is related to brain volume, it does not do so in a way that affects general fluid cognitive ability. A review looking at how components of PNNs, including BCAN and NCAN, control plasticity, and on their role in memory in normal aging concluded that interventions that target PNNs may allow the brain to function well, despite pathology [29]. Therefore, components of the PNN may protect against changes in brain volume.

Strengths of this study include the fact that protein levels, cognitive ability and structural brain variables were measured in the same individuals at about the same time in ~600 members of the LBC1936. Participants in the LBC1936 have a narrow age range and are an ancestrally homogeneous population, which reduces the variability compared to other cohorts. The age-matched INTERVAL cohort for

replication of associations with general fluid cognitive ability and the ability to investigate these associations in both an older (LBC1921) and younger (INTERVAL-Young) cohort were further strengths of this study, giving a total sample size larger than most other studies of this type. A key strength of the INTERVAL sample is that they are all healthy blood donors, which minimises confounding by disease status. The Olink Neurology panel was particularly well suited to this study as all proteins were chosen because of a prior link to neurology-related diseases, traits or processes and because it has high sensitivity and specificity [17].

One potential limitation of our investigation is the use of non-fasting plasma samples. However, a recent study concluded that timing of food intake only had a modest effect on the levels of the Olink neurology-related biomarkers used in this study [37]. Another limitation was the lack of a replication cohort that included brain MRI variables. A further limitation is that we investigated MRI and cognitive measures at the global level. Potentially counterintuitive findings (such as the protein-PC3 associations with brain volumes but not general fluid cognitive ability) are plausible where specific cognitive abilities are affected, or where specific but not global brain regions are associated. A further potential limitation is the use of different cognitive tests in the LBC1936, the LBC1921 and the INTERVAL sample. However, research has shown that general factors created from different cognitive batteries are highly consistent [38,39].

In conclusion we have identified a number of proteins associated with general fluid cognitive ability and brain volume. These may be useful as biomarkers of cognitive ability in later life and to identify biological pathways to potentially target therapeutically for age-related cognitive decline.

Methods

Lothian Birth Cohort 1936 (LBC1936)

LBC1936 consists of 1091 individuals, most of whom took part in the Scottish Mental Survey of 1947 at the age of ~11 years old. In the Survey, they took a validated test of cognitive ability, the Moray House Test (MHT) version 12 [40]. They were recruited to a study to determine influences on cognitive ageing at age ~70 years and have taken part in four waves of testing in later life (at mean ages 70, 73, 76 and 79 years). At each wave they underwent a series of cognitive and physical tests, with concomitant brain MRI introduced at age ~73 years [18]. For the present study, cognitive tests were performed and plasma was extracted from blood collected in citrate tubes at a mean age of 72.5 (SD 0.7) years. The cognitive tests included here were six of the non-verbal subtests from the Wechsler Adult Intelligence Scale-IIIUK (WAIS-III) [41]: matrix reasoning, letter-number sequencing, block design, symbol search, digit symbol coding, and digit span backwards. From these six cognitive tests, a general fluid cognitive component was derived. The scores from the first unrotated component of a principal components analysis were extracted and labelled as general fluid cognitive ability. This component explained 51% of the variance, with individual test loadings ranging from 0.65 to 0.76. General fluid cognitive ability was regressed onto age and sex, and residuals from these linear regression models were used in further statistical analyses. Cognitive data and neurology related protein levels were available for 798 individuals.

Whole brain structural and diffusion tensor MRI data were acquired using a 1.5T GE Signa Horizon scanner (General Electric, Milwaukee, WI, USA) located at the Brain Research Imaging Centre, University of Edinburgh, soon after cognitive testing and plasma collection. Mean age at scanning was 72.7 (SD 0.7) years. Full details are given in [42]. In brief, T1-, T2-, T2* and FLAIR-weighted MRI sequences were collected and co-registered (voxel size = 1 × 1 × 2 mm). Total brain, grey matter, normal appearing white matter volume and WMH were calculated using a semi-automated multispectral fusion method [43–45]. PVS were visually rated (5 point score in basal ganglia and centrum semiovale; the sum of the two scores was used in this study) by a trained neuroradiologist as previously described [45].

The diffusion tensor MRI protocol employed a single-shot spin-echo echo-planar diffusion weighted sequence in which diffusion-weighted volumes (b = 1000 s mm^{-2}) were acquired in 64 non-collinear

directions, together with seven T_2-weighted volumes (b = 0 s mm^{-2}). This protocol was run with 72 contiguous axial slices with a field of view of 256 × 256 mm, an acquisition matrix of 128 × 128 and 2mm isotropic voxels. Full details are included in [45].

White matter connectivity data were created using the BEDPOSTX/ProbTrackX algorithm in FSL (https://fsl.fmrib.ox.ac.uk) and 12 major tracts of interest were segmented using Tractor (https://www.tractor-mri.org.uk) scripts: the genu and splenium of the corpus callosum, and bilateral anterior thalamic radiations, cingulum bundles, uncinate, arcuate and inferior longitudinal fasciculi. Tract-average white matter FA and MD were derived as the average of all voxels contained within the resultant tract maps. General factors of FA (gFA) and MD (gMD) were derived from a confirmatory factor analysis using all 12 tracts, to reflect the well-replicated phenomenon of common microstructural properties of brain white matter in early, middle and later life [46–48].

WMH volume was log transformed, after which it showed an approximately normal distribution. Total brain, grey matter, normal appearing white matter volume and log WMH volumes were regressed onto age, sex and intracranial volume. PVS score, gFA and gMD were regressed onto age and sex. Residuals from these linear regression models were used in further statistical analyses. Brain imaging data and neurology related plasma protein levels were available for between 600 and 635 individuals.

Lothian Birth Cohort 1921 (LBC1921)

LBC1921 consists of 550 individuals, most of whom took part in the Scottish Mental Survey of 1932 at the age of ~11 years old. In the Survey, they took a validated test of cognitive ability, the MHT version 12[49]. They were recruited to a study to determine influences on cognitive ageing at age ~79 years and have taken part in 5 waves of testing in later life (at ages 79, 83, 87, 90 and 92 years). For this study, cognitive tests were performed and plasma was extracted from blood collected in citrate tubes at a mean age of 86.6 years (SD 0.4) [18]. Cognitive tests included Raven's Standard Progressive Matrices [50], letter-number sequencing [41] and digit symbol coding [41]. From these three cognitive tests, a general fluid

cognitive component was derived. The scores from the first unrotated component of a principal components analysis were extracted and labelled as general fluid cognitive ability. This component explained 68% of the variance, with individual test loadings ranging from 0.78 to 0.83. General fluid cognitive ability was regressed onto age and sex and residuals from these linear regression models were used in further statistical analyses. Cognitive data and neurology related plasma protein levels were available for 165 individuals.

INTERVAL

INTERVAL is a randomised trial of ~45,000 blood donors from the National Health Service Blood and Transplant Centres in England [19]. The trial was designed to determine whether the interval between donations could be safely reduced. Cognitive function tests were taken ~two years into the trial at which point plasma was extracted from blood collected in EDTA tubes. Cognitive tests adapted from the Cardiff Cognitive Battery [51] were assessed: Stroop Test (part 1, measures attention and reaction times in milliseconds); Trail Making Test (duration of part B in milliseconds, measures executive function); Pairs Test (participants were asked to memorize the positions of six card pairs, and then match them from memory while making as few errors as possible), and Reasoning Test (a task with 13 logic/reasoning-type questions and a two-minute time limit). Scores on the Pairs Test are for the number of errors that each participant made; higher scores reflect poorer episodic memory. The Reasoning Test is known as the 'Fluid Intelligence' test in UK Biobank [10]. The scores from the first unrotated component of a PCA of the four tests were extracted and labelled as general fluid cognitive ability. This component explained 48% of the variance, with individual test loadings ranging from 0.35 to 0.60. General fluid cognitive ability was regressed onto age and sex and residuals from these linear regression models were used in further statistical analyses. Cognitive data and neurology related protein biomarkers were available for 4,451 individuals. For the purposes of this study, INTERVAL was split into individuals aged ≥67 years (INTERVAL-Old, N=975, mean age=70.3 years, SD=2.4 years), and individuals ≤66 years (INTERVAL-

Young, N=3476, mean age=58.4 years, SD=5.0 years). The former subsample was formed to be approximately matched in mean age with the LBC1936.

Neurology-related protein biomarker measurement

92 neurology-related protein biomarkers were measured by the Proximity Extension Assay technique using the Proseek Multiplex Neurology I 96 × 96 reagents kit by Olink® Proteomics [16]. The data were pre-processed by Olink® using NPX Manager software. Normalised protein expression levels were transformed by inverse-rank normalisation and then regressed onto age and sex. Residuals from these linear regression models were used in further statistical analyses. LBC1936 and LBC1921 used a newer version of the kit which included microtubule associated protein tau (MAPT) rather than brain derived neurotrophic factor (BDNF). BDNF (in INTERVAL) and MAPT (in the LBCs) both failed Olink quality control and were therefore excluded from all analyses. See Supplementary Table 2 for the 91 proteins analysed.

Statistical analyses

We conducted a PCA of the 91 proteins for each cohort to establish the common variance among these markers. We used the coefficient of factor congruence to assess the consistency with which the individual proteins loaded on each component across groups. We used PCA results to inform our threshold for multiple testing of independent tests (number of components with eigenvalues >1). PCA on the transformed levels of the 91 neurological markers revealed that 17 components explained the majority (70%) of the variance in the data in the LBC1936. Based on PCA, a Bonferroni corrected p value of 0.0029 (0.05/17 independent proteins) was used to indicate statistical significance [52].

Next, linear regression models were used to test the associations of each of the 91 neurology-related protein biomarkers with: general fluid cognitive ability (LBC1936, LBC1921 and INTERVAL-Old and Young), total brain, grey matter, normal appearing white matter and WMH volumes; and PVS, gFA and gMD (LBC1936 only). We also extracted the first three components from the PCA of all 91 proteins, that

showed acceptable stability across cohorts, i.e. those with a coefficient of factor congruence > 0.70. We then examined their associations with cognitive and brain variables, as above. Linear regression analyses were performed in R [53]. Results from LBC1936 and the approximately age-matched INTERVAL-Old cohort were inverse variance weighted meta-analysed using (METAL) [54].

Finally, we performed mediation analysis in a structural equation modelling framework to identify if the significant (Bonferroni-corrected) protein-cognitive ability associations were mediated by the brain MRI variables in the LBC1936. Two analyses were performed. The first included total brain volume corrected for intracranial volume. The second included multiple brain structural mediators (grey matter, normal appearing white matter and WMH volumes; all corrected for intracranial volume), PVS, gFA and gMD. For these analyses no selection for brain imaging variables was made on the basis of their association with the proteins. Mediation analyses were carried out using the lavaan package, using bootstrapping to calculate the standard errors, in R [53].

Data Availability

Data supporting the findings of this manuscript are available from the corresponding author upon reasonable request.

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Author contributions

SEH, SRC, SB, REM, IJD participated in the design and/or interpretation of the reported experiments or results. SEH, SRC, SB, REM, BPP, AP, JC, SMM, MVH, ZM, SJ, PGB, EMTD, JMS, MEB, JMW, ASB, IJD participated in the acquisition and/or analysis of data. SEH, SRC, SB, REM, BPP, AP, JC, SMM, MVH, ZM, SJ, PGB, EMTD, MEB, JMW, ASB, IJD participated in drafting and/or revising the manuscript.

Competing interests

No authors declared any competing interests.

Variable	**Mean (SD, range)**	**N (with OLINK data)**
Age at cognitive testing and plasma collection (years)	72.5 (0.7, 70.9-74.2)	805
General fluid cognitive ability	-0.0 (1.0, -3.5-3.2)	798
Age at brain scan (years)	72.7 (0.7, 71.0-74.2)	684
Total brain volume (cm^3)	989.1 (90.4, 730.8-1247.0)	600
Grey matter volume (cm^3)	471.7 (45.1, 366.4-616.2)	600
Normal appearing white matter volume (cm^3)	474.6 (51.0, 301.7-636.0)	600
White matter hyperintensity volume (cm^3)	12.1 (13.0, 0.0-98.4)	617
Intracranial volume (cm^3)	1438.7 (135.3, 1059.3-1857.9)	618
general fractional anisotropy	0.0 (0.02, -0.07-0.05)	621
general mean diffusivity	-0.0 (29.2, -76.6-92.7)	621
Sex	Male	423 (52.5%)
	Female	382 (47.5%)

Table 1 Summary descriptive data for LBC1936.

	INTERVAL-Old		INTERVAL-Young		LBC1921	
Variable	Mean (SD, range)	N (with OLINK data)	Mean (SD, range)	N (with OLINK data)	Mean (SD, range)	N (with OLINK data)
Age (years)	70.3 (2.4, 67.0- 77.8)	975	58.4 (5.0, 48.9-66.9)	3476	86.6 (0.4, 85.7-87.4)	175
General fluid cognitive ability	-1.2 (1.4, -8.9-3.2)	975	-0.5 (1.3, -6.6-3.4	3476	0.0 (1.0, -2.6-3.4)	165
Sex	Male	646 (66.3%)	Male	1975 (56.8)	Male	83 (47.4%)
	Female	329 (33.7%)	Female	1501 (43.2%)	Female	92 (52.6%)

Table 2 Summary descriptive data for INTERVAL-Old, INTERVAL-Young and LBC1921.

Protein	**Beta**	**SE**	**P**
EDA2R	-0.17	0.035	$7.32x10^{-7}$
PVR	-0.17	0.035	$1.44x10^{-6}$
EFNA4	-0.16	0.035	$1.03x10^{-5}$
DDR1	-0.15	0.035	$3.43x10^{-5}$
RSPO1	-0.14	0.035	$5.70x10^{-5}$
SKR3	-0.14	0.035	$6.49x10^{-5}$
TNFRSF12A	-0.14	0.035	$8.77x10^{-5}$
VWC2	-0.14	0.035	$1.02x10^{-4}$
Siglec-9	-0.14	0.035	$1.10x10^{-4}$
SCARB2	-0.13	0.035	$1.86x10^{-4}$
LAYN	-0.13	0.035	$2.03x10^{-4}$
UNC5C	-0.13	0.035	$2.77x10^{-4}$
CLM-6	-0.13	0.035	$2.82x10^{-4}$
CPM	-0.13	0.035	$2.90x10^{-4}$
MSR1	-0.13	0.035	$3.08x10^{-4}$
Protein-PC1	-0.12	0.035	$4.44x10^{-4}$
N2DL-2	-0.12	0.035	$5.90x10^{-4}$
GFR-alpha-1	-0.12	0.035	$6.49x10^{-4}$
SIGLEC1	-0.12	0.035	$9.52x10^{-4}$
CDH6	-0.12	0.035	$9.80x10^{-4}$
THY 1	-0.11	0.035	0.0012
SCARA5	-0.11	0.035	0.0024
PLXNB1	-0.11	0.035	0.0029

Table 3 Proteins and principal components (PCs) associated with general fluid cognitive ability in LBC1936 at $p<0.0029$.

Protein	**total Beta**	**total SE**	**total P**	**Total Brain vol IDE Beta**	**Total Brain vol IDE SE**	**Total Brain vol IDE P**	**% attn**	**c'**
EDA2R	-0.157	0.04	<0.001	-0.048	0.012	**<0.001**	30.57%	-0.109
PVR	-0.173	0.04	<0.001	-0.028	0.011	**0.009**	16.18%	-0.145
EFNA4	-0.135	0.041	0.001	-0.027	0.011	**0.015**	20.00%	-0.108
DDR1	-0.138	0.04	0.001	-0.01	0.01	0.35	7.25%	-0.128
RSPO1	-0.141	0.041	0.001	-0.02	0.011	0.057	14.18%	-0.121
SKR3	-0.126	0.041	0.002	-0.036	0.012	**0.002**	28.57%	-0.090
TNFRSF12A	-0.114	0.04	0.005	-0.027	0.011	**0.014**	23.68%	-0.087
VWC2	-0.124	0.04	0.002	-0.01	0.01	0.354	8.06%	-0.114
Siglec-9	-0.143	0.04	<0.001	-0.014	0.01	0.184	9.79%	-0.129
SCARB2	-0.131	0.041	0.002	-0.033	0.011	**0.004**	25.19%	-0.098
LAYN	-0.121	0.041	0.003	-0.026	0.011	**0.019**	21.49%	-0.095
UNC5C	-0.101	0.04	0.012	-0.015	0.011	0.147	14.85%	-0.086
CLM-6	-0.12	0.04	0.003	-0.026	0.011	0.055	21.67%	-0.094
CPM	-0.116	0.04	0.004	-0.006	0.01	0.56	5.17%	-0.110
MSR1	-0.106	0.039	0.007	-0.038	0.011	**0.001**	35.85%	-0.068
PC1	-0.113	0.04	0.005	-0.01	0.01	0.338	8.85%	-0.103
N2DL-2	-0.11	0.04	0.006	-0.019	0.011	0.079	17.27%	-0.091
GFR-alpha-1	-0.106	0.041	0.009	-0.031	0.011	**0.006**	29.25%	-0.075
SIGLEC1	-0.12	0.04	0.003	-0.028	0.011	**0.011**	23.33%	-0.092

CDH6	-0.091	0.04	0.023	-0.006	0.01	0.572	6.59%	-0.085
THY 1	-0.105	0.041	0.01	-0.017	0.011	0.118	16.19%	-0.088
SCARA5	-0.096	0.041	0.018	-0.004	0.011	0.722	4.17%	-0.092
PLXNB1	-0.079	0.041	0.052	-0.012	0.011	0.277	15.19%	-0.067

Table 4 Mediation of association between protein-PC1-PC3 and proteins and general fluid cognitive ability by total brain volume in LBC1936.

Total effect sizes (total betas) differ from those in table 3 as the mediation analysis included fewer individuals. Significant mediations (FDR corrected) are indicated in bold. IDE=indirect effect, %attn=percentage attenuated.

Protein	**total Beta**	**total SE**	**total P**	**Sum IDE Beta**	**Sum IDE SE**	**Sum IDE P**	**% attn**	**c'**
EDA2R	-0.162	0.041	<0.001	-0.059	0.015	**<0.001**	36.42%	-0.103
PVR	-0.173	0.041	<0.001	-0.038	0.014	**0.006**	21.97%	-0.135
EFNA4	-0.136	0.042	0.001	-0.028	0.014	0.049	20.59%	-0.108
DDR1	-0.136	0.041	0.001	-0.014	0.014	0.308	10.29%	-0.122
RSPO1	-0.136	0.041	0.001	-0.027	0.014	0.055	19.85%	-0.109
SKR3	-0.125	0.042	0.003	-0.038	0.015	**0.01**	30.40%	-0.087
TNFRSF12A	-0.117	0.041	0.005	-0.032	0.015	0.027	27.35%	-0.085
VWC2	-0.129	0.041	0.001	-0.012	0.013	0.342	9.30%	-0.117
Siglec-9	-0.141	0.041	0.001	-0.018	0.014	0.187	12.77%	-0.123
SCARB2	-0.136	0.042	0.001	-0.024	0.015	0.104	17.65%	-0.112
LAYN	-0.126	0.042	0.003	-0.025	0.014	0.074	19.84%	-0.101
UNC5C	-0.101	0.041	0.014	-0.01	0.014	0.438	9.90%	-0.091
CLM-6	-0.118	0.041	0.004	-0.031	0.015	0.033	26.27%	-0.087
CPM	-0.109	0.041	0.008	-0.014	0.013	0.299	12.84%	-0.095
MSR1	-0.109	0.04	0.006	-0.039	0.015	**0.007**	35.78%	-0.070
PC1	-0.111	0.041	0.007	-0.006	0.013	0.651	5.41%	-0.105
N2DL-2	-0.109	0.041	0.008	-0.018	0.014	0.218	16.51%	-0.091
GFR-alpha-1	-0.107	0.042	0.01	-0.036	0.014	**0.01**	33.64%	-0.071
SIGLEC1	-0.112	0.041	0.007	-0.021	0.014	0.12	18.75%	-0.091
CDH6	-0.088	0.041	0.032	0.002	0.014	0.862	-2.27%	-0.090
THY 1	-0.104	0.041	0.012	-0.021	0.014	0.121	20.19%	-0.083
SCARA5	-0.098	0.041	0.018	-0.005	0.014	0.718	5.10%	-0.093
PLXNB1	-0.081	0.041	0.050	-0.006	0.014	0.636	7.41%	-0.075

Table 5 Mediation of association between PC1-PC3 and proteins and general cognitive fluid ability by MRI brain variables in LBC1936: grey matter volume, normal appearing white matter volume, white matter hyperintensity volume, perivascular spaces, general fractional anisotropy and general mean diffusivity.

Total effect sizes (total betas) differ from those in table 3 as the mediation analysis included fewer individuals. Significant mediations (FDR corrected) are indicated in bold. IDE=indirect effect, %attn=percentage attenuated.