Genetic effects on longitudinal cognitive decline during the early stages of Alzheimer's disease

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Abstract

Background: Cognitive decline in early-stage Alzheimer's disease (AD) may depend on genetic variability.

Methods: In the Swedish BioFINDER study, we used polygenic scores (PGS) (for AD, intelligence and educational attainment) to predict longitudinal cognitive change (measured by MMSE) over a mean of 4.2 years. We included 260 b-amyloid (Ab) negative cognitively unimpaired (CU) individuals, 121 Ab-positive CU (preclinical AD), 50 Ab-negative mild cognitive impairment (MCI) patients, and 127 Ab-positive MCI patients (prodromal AD).

Results: The polygenic score (PGS) for intelligence (p = 2.9e-02, beta = 0.1) was protective in CU and MCI participants regardless of Ab status, while polygenic risk score for AD (p = 8.2e-03, beta = -0.12) was correlated with rate of cognitive impairment and was partially mediated by Ab-pathology (mediation effect 20 %). There was no influence of education PGS, implying that educational achievement and rate of cognitive impairment are unrelated.

Conclusions: Genetic variants associated with intelligence mitigate cognitive decline independent of Ab-pathology, but the effects of genetic variants associated with AD are partly mediated by Ab-pathology.

Background

The rate of cognitive decline in Alzheimer's disease (AD) is highly variable [1, 2]. Greater understanding of factors underlying this variability may provide tools for better prognostics and give clues to novel treatments to modulate cognitive decline. Several potential factors may contribute to variability in cognitive decline, for example burden of AD pathology, cognitive reserve, concomitant pathologies, therapies and genetic variance [3]. Several genes contribute to heritability of AD [4, 5], but studies testing genetic contributions to rate of cognitive decline in AD are rare [6, 7, 8]. There is especially a lack of studies testing associations between genetic variants and cognitive decline during the early stages of AD, before dementia [9, 10, 11]. For example, it is unclear if genetic variants that are associated with AD diagnosis [12], as well as with intelligence [13] and education attainment [14], may modulate cognitive decline during the earliest stages of AD. This is relevant to study given the increased focus on early disease stages (including preclinical AD) for potential disease-modifying treatments. Genetic variants that are linked to differences in trajectories of cognitive decline during the early stages of disease may be explored to identify novel treatment targets.

The relationship between genetic risk factors and cognitive decline can be tested both for individual genetic variants, and for multiple genetic variants simultaneously using the polygenic scoring (PGS) method [15]. For PGS, the cumulative effect of a large number of genetic variants across the entire genome is calculated by summing the weighted effect size of each variant on the target feature (e.g. AD) from a genome-wide association analysis (GWAS) multiplied by the number of effect alleles (0, 1, or 2) in that individual [16, 17]. For a risk trait (e.g. AD), the term polygenic risk score (PRS) is used, while PGS can be used for a benign or undecided trait (e.g. intelligence).

In this study, we explored genetic predictors of cognitive decline in early stages of AD, including individuals with preclinical AD or mild cognitive impairment (MCI) due to AD. We used longitudinal cognitive data from the BioFINDER study and utilized three polygenic predictors: (1) a PRS for AD (PRS-Alz), (2) a PGS for intelligence (PGS-Int) and (3) a PGS for educational attainment (PGS-Edu) in order to ascertain the role of genetic variants in cognitive decline of these patients given an understanding of the roles education and intelligence play in this outcome.

Methods

Study participants

The study population consisted of 381 cognitively unimpaired (CU) elderly participants and 177 patients with mild cognitive impairment (MCI) from the prospective and longitudinal Swedish BioFINDER sample (clinical trial no. NCT01208675; www.biofinder.se), for which baseline cognitive tests, age, education, gender, Aβ status and at least three time-points for MMSE (including baseline) were available. Details on recruitment, exclusion and inclusion criteria have been presented previously [18, 19] and also detailed in the supplementary file. Among consecutively included patients with mild cognitive symptoms, some were classified as MCI and some as subjective cognitive decline (SCD). Control participants and patients with SCDs were combined to make a group of CU individuals, following research guidelines [20].

Endophenotype

Longitudinal change in Mini-mental state examination (MMSE) score was used as the main endophenotype in this study. The MMSE is a cognitive examination that explores five cognitive function areas: orientation, registration, attention and calculation, word recall, and language, with score ranging from 0 to 30 [21].

Genotyping and preparation of genetic data

Genotyping was conducted using the Illumina platform GSA-MDA v2. Subject-level and SNP level quality control (QC) was conducted as per accepted guidelines [22]. In brief; person based QC included compatibility between chip-inferred gender and self-reported gender, call rates (1% cut-off), and extreme heterozygosity. High-quality variants (autosomal, bi-allelic variants with Hardy–Weinberg Equilibrium (HWE) $P > 5 \times 10^{-8}$, Minor Allele Frequency [MAF] $\geq$ 5% [rather than e.g. MAF > 1 % due to the relatively small sample size of the cohort] and with a call rate of > 99%) were used. Further information on the imputation and QC process is detailed in supplementary methods.

Polygenic Score Calculation

The PRS / PGS was calculated with PLINK2 [23] using the weighted effects for each SNP. Prior to PRS/PGS calculation, SNPs were pruned using PLINK's clump function with an $r^2 < 0.1$ over 1000 kilo basepair (KBP). The *APOE* gene is the largest known AD risk factor, with strong linkage disequilibrium (LD) levels in the region surrounding the locus. SNPs falling within the *APOE* gene region

(chr19:44400000–46500000; GRCh37 / hg19 assembly) were therefore omitted from the dataset while constructing the PRS for AD. Publicly accessible summary statistics from reported GWAS studies (not overlapping with our dataset) of AD [12], intelligence [13] and educational attainment [14] were used to define PRS for Alzheimer's, PGS for intelligence and educational attainment, respectively. We iterated over a variety of values ($P < 0.05$ to $P < 5 \times 10^{-8}$) to evaluate the appropriate p-value threshold and created models named PRS/PGS 1–7 (details given in supplementary method).

Aβ status

Cerebrospinal fluid (CSF) was analysed for β-amyloid42 (Aβ42) and Aβ40 using Euroimmun (EI) immunoassay (EUROIMMUN AG, Lübeck, Germany). For each individual, Aβ status was defined as negative or positive using the CSF Aβ42/Aβ40 ratio, with Aβ + defined as ratio less than 0.09 [24].

Bioinformatics Analysis

We performed gene ontology (GO) enrichment analysis, protein-protein interaction (PPI) analysis and pathway analysis for the unique set of genes that were contributing to the PRS/PGS in each of the most significant PRS/PGS. We mapped each SNP to its nearest corresponding protein coding gene (PCG) within 1 mega base pair (1MBP) of distance. If for a SNP there was no PCG within 1MBP of distance it was excluded from this analysis. We used Gonet [25], STRING [26] and Reactome database [27] for the GO enrichment, PPI and pathway analysis respectively.

Identification of the Aβ independent PRS-Alz variants

To identify the Aβ independent variants in the most significant PRS-Alz we applied a heuristic approach. We generated "n" different PRSs (where n = number of variants in the most significant PRS-Alz) using only "n-1" variants of the most significant PRS-Alz and removing 1 specific variant in an order from each of these "n" different PRSs. We tested the effect of each of these reduced PRS on Aβ using the mediation analysis. Each of the "n" PRSs were ranked in ascending order of p-value significance of association between PRS and Aβ. Next, we again generated "n" different PRSs using this ranked list of variants with each PRS consisting of ascending number of variants in it (e.g. First PRS only First variant, second PRS first top 2 variants, third PRS first top 3 variants and so on) and tested effect of each of these reduced PRS on Aβ using the mediation analysis. This step was further repeated by ranking the variants in descending order of p-value significance of association between PRS and Aβ.

Statistical Analyses

Using the lmer function (lme4 package), mixed-effect models were fitted through maximum likelihood, using longitudinal MMSE data as dependent variable. Random slopes and intercepts were extracted and were rank-based inverse normal transformed (INT) to be used as dependent variable in linear regression models to test the association between PGS/PRS and cognition. The models were adjusted for age, gender, education, baseline MMSE (not for intercept), *APOE* ε2 and ε4 count and the top 10 principal components (PC) from the principal component analysis (PCA) on the entire set of genotype data. Each set of association analysis was corrected family wise error rate (FWER) using Bonferroni correction. All

associations below Bonferroni corrected p-value of 0.05 were considered significant. For mediation analysis we tested the significance of the indirect effect using bootstrapping procedures. For each of 1000 bootstrapped samples, unstandardized indirect effects were measured, and the 95 percent confidence interval was calculated by calculating the indirect effects at the 2.5th and 97.5th percentiles. All the statistical analysis was conducted in R programming (version 4.0.2). Further details about statistical analysis can be found in supplementary methods.

Results

Table 1 shows the characteristics of the individuals and Figure S1 (supplementary file) shows the spaghetti plot of the longitudinal MMSE values for each individual.

Table 1
Demographics and baseline characteristics

	CU (Aβ-)	CU (Aβ+)	MCI (Aβ-)	MCI (Aβ+)	Total	P-Value
N	260	121	50	127	558	
Female (%)	154 (59.2)	64 (52.9)	16 (32)	62 (48.8)	296 (57.1)	0.5
Age (years)	71.5 (5.3)	73.1 (4.7)	68.6 (5.4)	73 (5)	71.9 (5.2)	0.14
Education (years)	12.5 (3.5)	12.1 (3.9)	10.7 (3.2)	11.3 (3.3)	12 (3.6)	0.0003
APOE **ε4 (0/1/2)**	204/54/2	45/57/19	36/11/3	38/68/21	323/190/45	< 2e-16
APOE **ε2 (0/1/2)**	214/42/4	114/6/1	41/8/1	115/12/0	484/68/6	0.04
MMSE (IQR) (Median)	(30 – 29) (29)	(30 – 28) (29)	(29 – 26) (28)	(28 – 25) (27)	(30 – 27) (29)	< 2e-16

Age and education data are mean (standard deviation). CU = Cognitively unimpaired; MCI = mild cognitive impaired. The group mean difference was calculated based on ANOVA.

Alzheimer's PRS and Intelligence PGS are associated with cognitive decline

We first tested effects of the three polygenic scores, i.e., PRS for Alzheimer's (PRS-Alz), PGS for intelligence (PGS-Int) and educational attainment (PGS-Edu), on longitudinal cognitive decline in the cohort. As explained above, we tested seven different levels of each score (e.g PRS-Alz 1–7) with fewer SNPs for higher levels. PRS-Alz 4, 5 and 6 (p-value threshold 5×10^{-5}, 5×10^{-6} and 5×10^{-7}respectively) had significant associations with both lower intercept (Bonferroni corrected p-values = 4.02e-02, 2.53e-02 and 1.69e-02 respectively) and steeper decline of slope (Bonferroni corrected p-values = 1.58e-02, 8.19e-

03 and 4.71e-02 respectively) for MMSE, meaning that the polygenic predictor of these three PRSs was associated with lower MMSE score at baseline and accelerated cognitive decline. PRS-Alz 2 was significantly associated with lower random intercept (lower MMSE score at baseline, p = 1.37e-02 [Bonferroni corrected]) but not with random slope (p = 2.24e-01 [Bonferroni corrected]). PRS-Alz 1, 3 and 7 had no significant associations with intercept or slope of MMSE (Fig. 1a and 1b, supplementary table S1, S2).

For polygenic score of intelligence, PGS-Int 7 had a strong association with MMSE slope (p = 2.91e-02 [Bonferroni corrected]) but not with intercept (p = 1.59e-01 [Bonferroni corrected]). Multiple PGS-Int (PGS 1, PGS 2, PGS 3 and PGS 4) had significant associations with MMSE intercept (Fig. 1c and 1d, supplementary table S3, S4). None of the PGS-Edu (Fig. 1e and 1f, supplementary table S5, S6) were significantly associated with MMSE intercepts or slopes.

As an exploratory analysis, we tested interactions between PRS-Alz 4, 5, 6 and 7 and PGS-Int 7 to predict MMSE slopes. None of the interactions were significant (p = 0.3, for PRS-Alz 4 x PGS-Int 7 interaction, p = 0.1, for PRS-Alz 5 x PGS-Int 7 interaction, p = 0.2, for PRS-Alz 6 x PGS-Int 7 interaction and p = 0.1 for PRS-Alz 7 x PGS-Int 7 interaction) (supplementary table S7) suggesting that the polygenic contributions to AD and intelligence do not influence each other's effect on cognitive decline.

Significant PGS/PRS is associated with cognitive decline irrespective of Aβ-status

The models described above were not adjusted for Aβ-status. To test if the effects of the identified PGS/PRSs on cognition depended on Aβ-positivity (indicating underlying AD pathology), we tested the models for the significant PRG/PRSs with the additional adjustment for Aβ status. The associations were attenuated when adjusting for Aβ-status. However, two PRS-Alz (PRS-Alz 5 and PRS-Alz 6) and all the PGS-Int still showed significant association with either slope or intercept of longitudinal MMSE after Bonferroni correction. PRS-Alz 5 showed significant association with the random slope of longitudinal MMSE (p = 1.42e-02 [Bonferroni corrected]) (Fig. 2a, supplementary table S8), and PRS-Alz 6 with the random intercept (p = 5e-02 [Bonferroni corrected]) (Fig. 2b, supplementary table S9).

PGS-Int 7 remained significantly associated with the random slope of longitudinal MMSE (p = 1.49e-02) (Fig. 2c, supplementary table S10) and PGS-Int 1, 2, 3 and 4 with the random intercept (p = 3.06e-02, 2.55e-02, 1.86e-02 and 4.96e-02 respectively [Bonferroni corrected]) (Fig. 2d, supplementary table S11).

No interaction between PRS/PGS association and Aβ-status for cognitive decline

To further test if the effects were specific to an Aβ subgroup (positive or negative), we next included an interaction term with the Aβ status and PRS/PGS in the model. The interaction term was non-significant for any of the PRS/PGS models (supplementary table S12-S17).

Mediation analyses

As described above, we observed significant effect of PRS/PGS on cognitive decline when not adjusting for Aβ-status, and attenuated effects when adjusting for Aβ-status. We further tested for the association of the significant PRS/PGS with the dichotomized Aβ-status. PRS-Alz 5 showed a significant association with the Aβ-status (p = 0.03) whereas PRS-Alz 6 showed a marginal association with Aβ-status (p = 0.051). PGS-Int 7 and PGS-Int 3 did not showed any significant association with the Aβ-status (p = 0.08 and p = 0.3 respectively). Given these findings, we performed a mediation analysis for PRS-Alz 5 to quantify the extent to which the effects of PRS/PGS on cognitive decline were mediated by Aβ in the cohort. Figure 3 illustrates that the rate of change of cognition that is significantly predicted by PRS-Alz 5 is partly but significantly mediated by Aβ pathology (20% mediation effect) (supplementary table S18).

PRS-variants independent of Aβ

The results above suggested that the effect of PRS-Alz 5 on longitudinal cognition was significantly but only partly mediated by Aβ pathology. We therefore hypothesized that this PRS may be heterogenous and contain some genetic components that did exert their effect through accumulation of Aβ pathology, and others that were truly independent of Aβ. Using a heuristic approach (method section for details) we therefore explored PRS-Alz 5 and identified 16 variants that might be independent of Aβ, as the p-value of association of PRS with Aβ reduced relative to PRS-Alz 5 in the absence of the respective variant. We also found 17 variants whose removal from the respective PRSs resulted in an increase in the p-value of association of PRS with Aβ relative to PRS-Alz 5 suggesting a possibility of these variants to be Aβ dependent (supplementary table S19). On further analysis of this ranked variant list, we found that the PRS model (here after called as PRS-Alz 5-I, where I stand for independent) consisting of the top 23 variants to be the best model that is independent of Aβ status. For PRS-Alz 5-I there was no mediation effect shown by Aβ, whereas there was a significant effect of PRS on cognitive decline both when not adjusting for Aβ (beta = -0.07, p = 4.9 e-02) and when adjusting for Aβ (beta = -0.08, p = 1.5e-02). These findings and increase in the effect size and significance of association when adjusting for Aβ suggests that the set of these 23 variants is possibly Aβ independent (supplementary table S20). Further to test for the Aβ dependent set of variants we analyzed these variants by ranking them in the reverse order. PRS model (here after called as PRS-Alz 5-D, where D stand for dependent) with the top 10 variants (reverse order ranking) was found to be the best Aβ dependent model as the association between PRS model and slope of cognitive decline was lost when adjusting for A (beta = -0.06, p = 8.5e-02) compared to when not adjusting for A (beta = -0.11, p = 2e-03) (supplementary table S21). Among these 10 variants, there were 3 variants (rs3851179, rs11257240 and rs9649710) that made the Aβ adjusted effect significant when added in the models. We tested the models by removing these SNPs (anyone at a time) and found that even after removal of rs11257240 and rs9649710 the Aβ not adjusted model remained significant (beta = -0.09, p = 1.5e-02) and Aβ adjusted model was non-significant (beta = -0.04, p = 0.2). These findings suggest that 8 out 10 variants are totally dependent on Aβ whereas reaming 2 variants may be partially depending on Aβ. To further confirm the variants that are dependent on Aβ we performed the association between each SNP weighted with their risk score with Aβ. We found 2 SNPs (rs3851179 and rs6733839) to be significantly associated with Aβ (uncorrected p-value = 2.6e-02 and 2.4 e-02 respectively). We also

found other 8 SNPs from the Aβ dependent variant set showing strong effect size (beta > 0.15) though non-significant due to insufficient power (supplementary table S22).

Bioinformatics Analysis of the Most significant PRS/PGS

To elucidate the biological pathways that contributed to altered risk for cognitive decline, we performed bioinformatics analyses of the key PRS (PRS-Alz 5) and PGS (PGS-Int 7) results that were associated with cognitive decline. 33 SNPs of “PRS-Alz 5” mapped to 28 unique PCG (supplementary table S23). Out of these 28, 10 PCGs did not show any interaction with other PCGs at an interaction score of 0.4 (medium confidence) [23] (figure S2). Figure 4 shows the PPI network of the 18 PCGs enriched at p-value < 1e-16. Such an enrichment indicates that the proteins are at least partially biologically connected, as a group. The GO enrichment analysis enriched the 4 genes (*ABCA7*, *BIN1*, *CLU* and *PICALM*) to be involved in regulation of Aβ formation as the most top GO terms in the Biological Process category (p value ≤ 1.98e-03 [FDR corrected]) (Fig. 5a, supplementary table S24). In the pathway analysis we could not find any significant pathway enrichment for the PCGs after the FDR correction a p < 0.05. The top pathway enrichment term was “clathrin-mediated endocytosis pathway”, that involved *BIN1*, *PTK2B*, *PICALM* and *HBEGF* (9.85e-02 [FDR corrected]) (supplementary table S25).

For the intelligence PGS, we found that 176 SNPs of “PGS-Int 7” (were mapped to 170 unique PCGs (supplementary table S26). Making a PPI network with such large number of gene set was beyond the scope of this study. For “PGS-Int 7” GO enrichment analysis returned 6 GO terms enriched for the 170 PCGs at FDR corrected p-value < 0.05. “modulation of chemical synaptic transmission” was the top term in this category (p = 6.26e-04 [FDR corrected]) followed by 3 other terms related to “neuronal activity” (“regulation of trans-synaptic signaling”, “regulation of synaptic plasticity” and “regulation of neuron projection development”) for biological process at p ≤ 4.26e-02 (FDR corrected) (Fig. 5b, supplementary table S27). In the pathway enrichment analysis, we identified only one term “MECP2 regulates transcription factors” as the top category with FDR corrected p-value = 6.94e-03 (supplementary table S28).

Discussion

In this study of genetic contributions to cognitive decline in the earliest stages of AD, we investigated whether a priori defined PRS/PGS for AD, intelligence and educational attainment were associated with cognitive decline in CU and MCI individuals depending upon their Aβ status. Our main findings were that the PRS for AD was associated with rate of cognitive decline and was partly mediated through Aβ-pathology, while an intelligence PGS was protective in CU and MCI participants irrespective of Aβ status. Taken together, these findings suggest that a priori defined genetic risk factors for AD may influence the disease by affecting the rate of cognitive decline for people with the early AD, while genetic factors linked to intelligence may affect the overall rate of decline in cognition. It is possible that treatments that mimic or interfere with specific biological pathways, identified through these genetic analyses, may have a potential to affect the rate of disease progression in early-stage AD.

Notably, in our exploratory analysis of individual genetic variants and rate of cognitive decline adjusting with and without *APOE* ε4 burden resulted the same variant rs10492328 as a top hit with similar p-value threshold (p = 4.4e-07) (supplementary results, supplementary table S29, S30). This finding suggests that in early stages of AD the genetic control of the rate of cognitive decline is largely unrelated to *APOE*. This agrees with our recent finding that effects of Aβ-burden on cognition did not vary by *APOE* genotype in very early stages of AD [28].

Previous studies of genetic risk factors for cognitive decline in early stages of AD using the Alzheimer's Disease Neuroimaging Initiative (ADNI) subjects [29] and Australian Imaging, Biomarkers and Lifestyle (AIBL) [30] found significant effects of *APOE* ε4 and PGSs associated with AD [29, 30]. A study focused on preclinical AD subjects from AIBL study used episodic memory PRS and predicted rates of cognitive decline in domains typically affected in the preclinical stages of AD [31]. Unlike those studies [29, 30, 31] our findings suggest that a well-defined AD PRS can predict cognitive decline over and above *APOE* ε4 at an early stage of AD. This is consistent with one study that used polygenic hazard score (PHS) over and above *APOE* ε4 to predict longitudinal clinical decline in older individuals with moderate to high amyloid load [32]. Such PRSs may potentially be used (together with other modalities) to improve early prognostics of risk individuals. Looking for the PRS effect on the early disease process, another recent study showed the association of AD PRS with an increased probability of MCI compared to normal individuals at 50 years of age [33]. But perhaps more importantly, these genetic results may aid in understanding of the metabolic abnormalities of AD at an early stage, as discussed further below.

The intelligence PGS-Int 7 was protective in both early stage AD and in CU and MCI participants independent of Aβ status. Four intelligence PGS (PGS 1, PGS 2, PGS 3 and PGS 4) were only significantly associated with baseline cognition. The involvement of these PGSs in cognitive decline is uncertain. Previous studies have reported different results for associations between intelligence and AD. For example, one study found that intellectual enrichment was not a significant predictor of Aβ or AD-pattern neurodegeneration [34]. Two other studies found that intellectual enrichment may have marginal effects on AD biomarkers but a greater impact on delaying onset of cognitive impairment [35, 36]. Higher intelligence (and perhaps relevant genetic variants) was also associated with lower risk of AD in a twin study [37]. Taken together, these and our findings supports a model where genetic factors that contribute to higher intelligence are protective against general cognitive decline and may delay the onset of symptoms of AD (despite not affecting the underlying biological processes of the disease).

Interestingly the PGS-Int 7 consist of all the SNPs that were significant at a genome wide significant threshold of p < 5e-08. Similarly, for PRS-Alz 5, after mapping the variants to unique PCG resulted in variants that are all know genome-wide significant signals reported in previous work [12]. Hence, it suggests that the major effect in the PRS for AD and PGS for intelligence is reached when the PRS/PGS contains genome-wide significant variants.

There was no effect of education PGS, arguing against associations between educational attainment and rate of cognitive decline. This is in line with another recent study using educational attainment PGS to

predict rate of cognitive decline in non-demented individuals [38]. Another recent study agreeing with our findings showed that education and cognitive function in midlife did not affect long-term brain Aβ accumulation [39].

The effect of the PRS-Alz 5 on cognitive decline was partly mediated by Aβ pathology, indicating a symbiotic effect between Aβ pathology and genetic factors related to AD. The mediation analysis shows that around 20% of the effect of PRS-Alz on cognitive decline was mediated by Aβ pathology (both at baseline and progression). This finding is consistent with the AD pathological cascade, suggesting that Aβ biomarkers become abnormal long before cognitive decline during the development of AD [40]. While Aβ levels become pathological long before extreme cognitive impairments manifest, new research [41, 42] indicates that subtle cognitive changes can begin early, perhaps before Aβ reaches the abnormality threshold. Early intervention could be greatly aided by identifying at-risk patients before Aβ hits pathological thresholds.

Dividing PRS-Alz 5 into Aβ dependent (PRS-Alz-D) and independent component (PRS-Alz-I) identified 8 variants to be Aβ dependent, 2 variants partially dependent of Aβ and 23 variants independent of Aβ. Mapping these variants to genes resulted in 21 unique genes for the PRS-Alz-I variants and 8 genes for PRS-Alz-D. Except for *NIFKP9* all other genes were specific to Aβ independent and dependent groups. *NIFKP9* is pseudogene around 80 megabase pair (MBP) away from *BIN1* and is falling in both Aβ independent (rs6710467) as well as Aβ dependent (rs6733839) category. The two variants possibly work opposite to each other as evident from our association analysis of individual variant with Aβ (supplementary table S22). rs6733839 (beta = 0.23, p = 2.4e-02) has a significantly stronger association with Aβ compared to rs6710467 (beta = -0.02, p = 8.7e-01). Pseudogenes modulate the expression of the functional genes [43], and studies have reported that any sequence variation in the pseudogenes will have serious implication on the expression of the functional gene [44]. Taken together with these findings *NIFKP9* might regulate Aβ both positively and negatively.

Our bioinformatics analysis based on the GO enrichment analysis showed that most of the genes of PRS-Alz 5 were involved with regulation of Aβ in the brain. The lower enrichment p-value indicates that the proteins are at least partially biologically connected, and associated, with rate of cognitive decline in harmony. The pathway analysis showed “Clathrin-mediated endocytosis” as the top pathway hit (non-significant after FDR correction) in which the major genes (*BIN1, PTK2B, PICALM, HBEGF*) of the AD PRS are involved. Previous studies have shown that this pathway plays a central role in the production of Aβ in neurons [45, 46]. Our pathway analysis also showed Trges pathway as a second top hit. Dysfunction of Tregs has been reported to be associated with the neurodegenerative disease, such as AD [47].

For the Intelligence PGS-Int 7 GO enrichment term related to synaptic signaling were the top hits. Previous research has identified that the genes involved in such synaptic signaling pathways play a play an important role in cognitive ability [48] and disruption of such pathways may result in cognitive deficit [49]. The pathway analysis the “MECP2 regulates transcription factors” pathways to be top hit, where *MECP2* is involved in the regulation of gene activity. MeCP2 protein is important for the function of several types

of cells, including nerve cells. The genetic loss of *MECP2* has been identified as changing the properties of cells in the locus coeruleus the exclusive source of noradrenergic innervation to the cerebral cortex and hippocampus [50].

Limitations

Although the BioFINDER cohort has extensive phenotyping for cognition and Aβ that are necessary for a study of early stage AD, the sample size was relatively small. We therefore restricted the analysis to genes with MAF ≥ 0.05. Larger studies would be necessary in order to take into account the rarer genetic variants, which could also be important for cognitive decline.

Conclusions

The study evaluated the rate of cognitive decline based on PRS of AD and PGS of intelligence and education. PRS-Alz is associated with rate of cognitive decline and was partly mediated by Aβ, while PGS-Int was protective irrespective of Aβ status. There was no effect of PGS-Edu in any of the populations. Novel genetic associations with rate of cognitive decline in AD may provide new insights into the pathophysiology of AD and new therapeutic development targets.

Abbreviations

Alzheimer's disease: AD

Polygenic Scores: PGS

Genome-Wide Association Study: GWAS

Mini-Mental State Examination: MMSE

b-amyloid: Ab

Cognitively Unimpaired: CU

Mild Cognitive Impairment: MCI

Polygenic Risk Score: PRS

Subjective Cognitive Decline: SCD

Quality Control: QC

Hardy–Weinberg Equilibrium: HWE

Minor Allele Frequency: MAF

Linkage Disequilibrium:	LD
Cerebrospinal fluid:	CSF
Gene Ontology:	GO
Protein-Protein Interaction:	PPI

Declarations

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Author contributions

AK, NM-C, and OH developed the hypothesis and study design. SP and ES collected the patient information and blood samples for genotyping. AK, MS and JH performed the genetic data imputation. AK and NM-C performed the statistical analysis and wrote the first and successive draft of the manuscript. All authors contributed to review of manuscript and approved the final version of the manuscript to be published. NM-C and OH obtained funding. AK, NM-C, and OH had full access to the data and take responsibility for the integrity of the data and the accuracy of the data analysis.

Competing interests

AK, MS, SP, ES, JH and NMC have no disclosures. OH has acquired research support (for the institution) from AVID Radiopharmaceuticals, Biogen, Eli Lilly, Eisai, GE Healthcare, Pfizer, and Roche. In the past 2 years, he has received consultancy/speaker fees from AC Immune, Alzpath, Biogen, Cerveau and Roche.

Data availability

The summary statistics of the BIOFINDER GWAS is available from the corresponding author on reasonable request. Genome-wide summary statistics used for the generation of Alzheimer's PRS can be downloaded from the National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site (NIAGADS)—a NIA/NIH-sanctioned qualified-access data repository, under accession NG00075. Summary

statistics used for generation of Intelligence PRS are available for download from https://ctg.cncr.nl/. Summary statistics used for generation of educational attainment PRS can be downloaded from http://www.thessgac.org/data.

Ethics declarations

The Regional Ethics Committee in Lund, Sweden, approved the study. All subjects gave their written informed consent.

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Figures

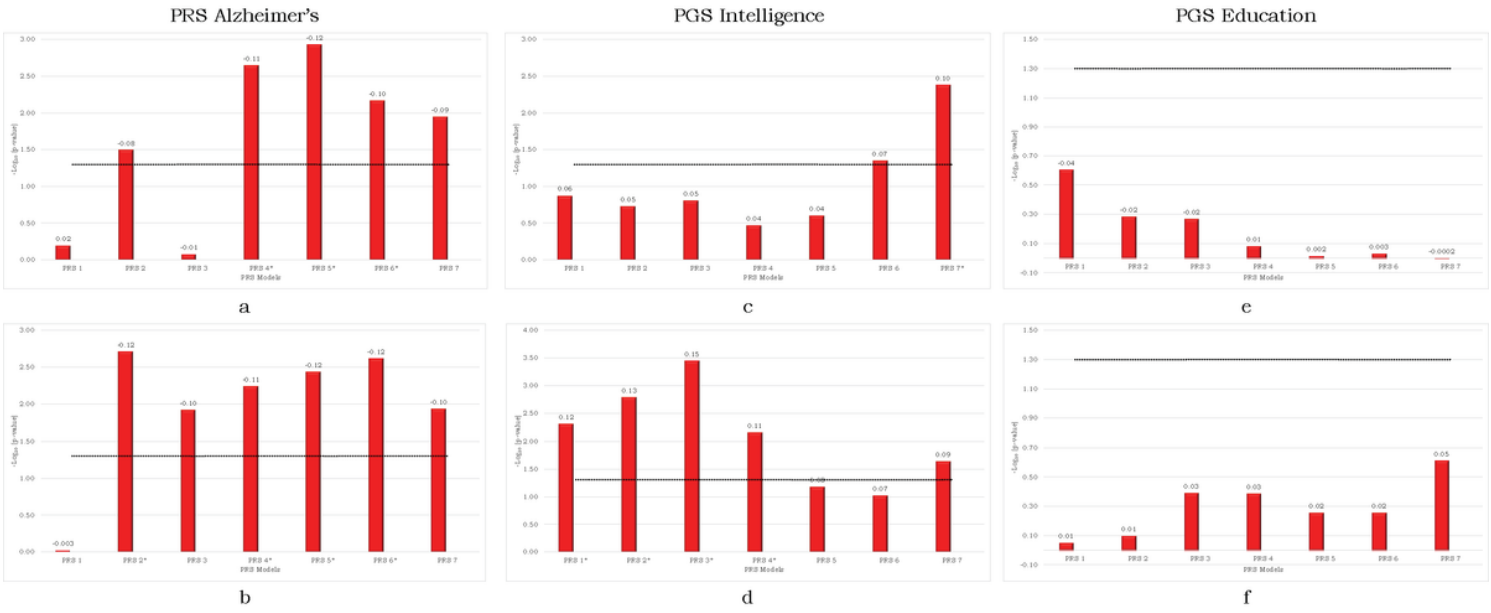

Figure 1

Associations of Polygenic Scores of AD, intelligence and education with cognition. a) PRS-Alz association with slope of longitudinal MMSE score. b) PRS-Alz association with intercept of MMSE score. c) PGS-Int association with slope of longitudinal MMSE score. d) PGS-Int association with intercept of

MMSE score. e) PGS-Edu association with slope of longitudinal MMSE score. f) PGS-Edu association with intercept of MMSE score. The x-axis represents the 7 different PRS/PGS models at different p-value thresholds based on the GWAS summary statistics (PRS1 ≤ 0.05, PRS2 ≤5e-3, PRS3 ≤ 5e-4, PRS4 ≤ 5e-5, PRS5 ≤ 5e-6, PRS6 ≤ 5e-7, PRS7 ≤ 5e-8). The models were adjusted for age, gender, education, baseline MMSE (not for intercept), APOE ε2 and ε4 count and the top 10 principal components (PC) from the principal component analysis (PCA) on the entire set of genotype data. The y-axis shows the negative log of the p-value for the significance of associations between PRS models with slope and intercept of longitudinal MMSE score. The values on the top of each bar shows the effect size (beta-coefficient) of the association. The horizontal dotted line shows the p-value threshold of 0.05. *These PRSs were significant after Bonferroni-correction at p-value < 0.05.

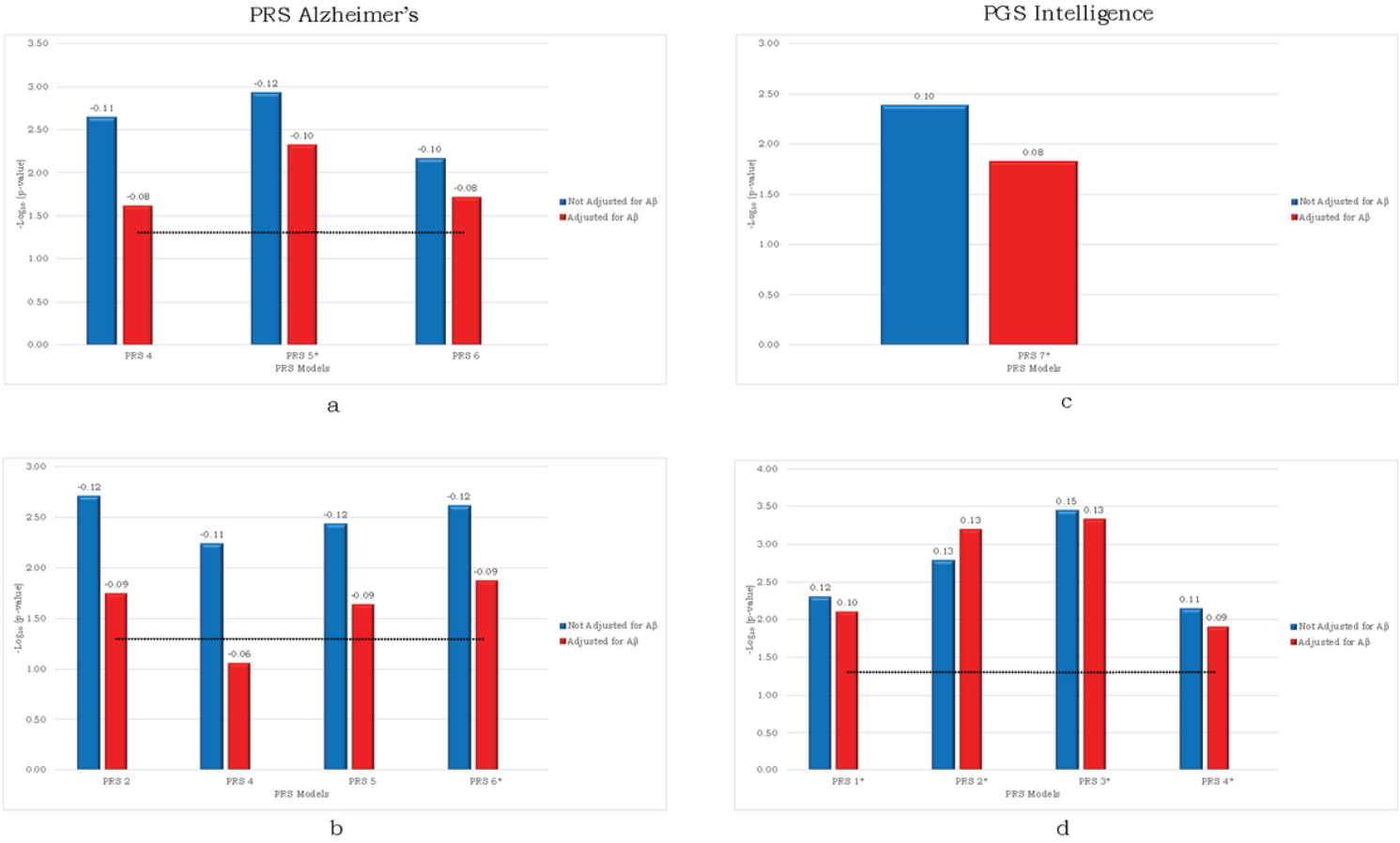

Figure 2

Associations of Polygenic Scores of AD and intelligence with cognitive decline after adjusting for Aβ-status. a) PRS-Alz association with slope of longitudinal MMSE score. b) PRS-Alz association with intercept of longitudinal MMSE score. c) PGS-Int association with slope of longitudinal MMSE score. d) PGS-Int association with intercept of longitudinal MMSE score. PRS/PGS models that showed significant associations with MMSE in base models (when not adjusted for Aβ-status) are included and shown on the x-axis. The y-axis shows the negative log of the p-value for the significance of associations between PRS models with random slope and random intercept of longitudinal MMSE score. The values on the top

of each bar shows the effect size (beta-coefficient) of the association. The horizontal dotted line shows the p-value threshold of 0.05. *These PRSs were significant after Bonferroni-correction at p-value < 0.05.

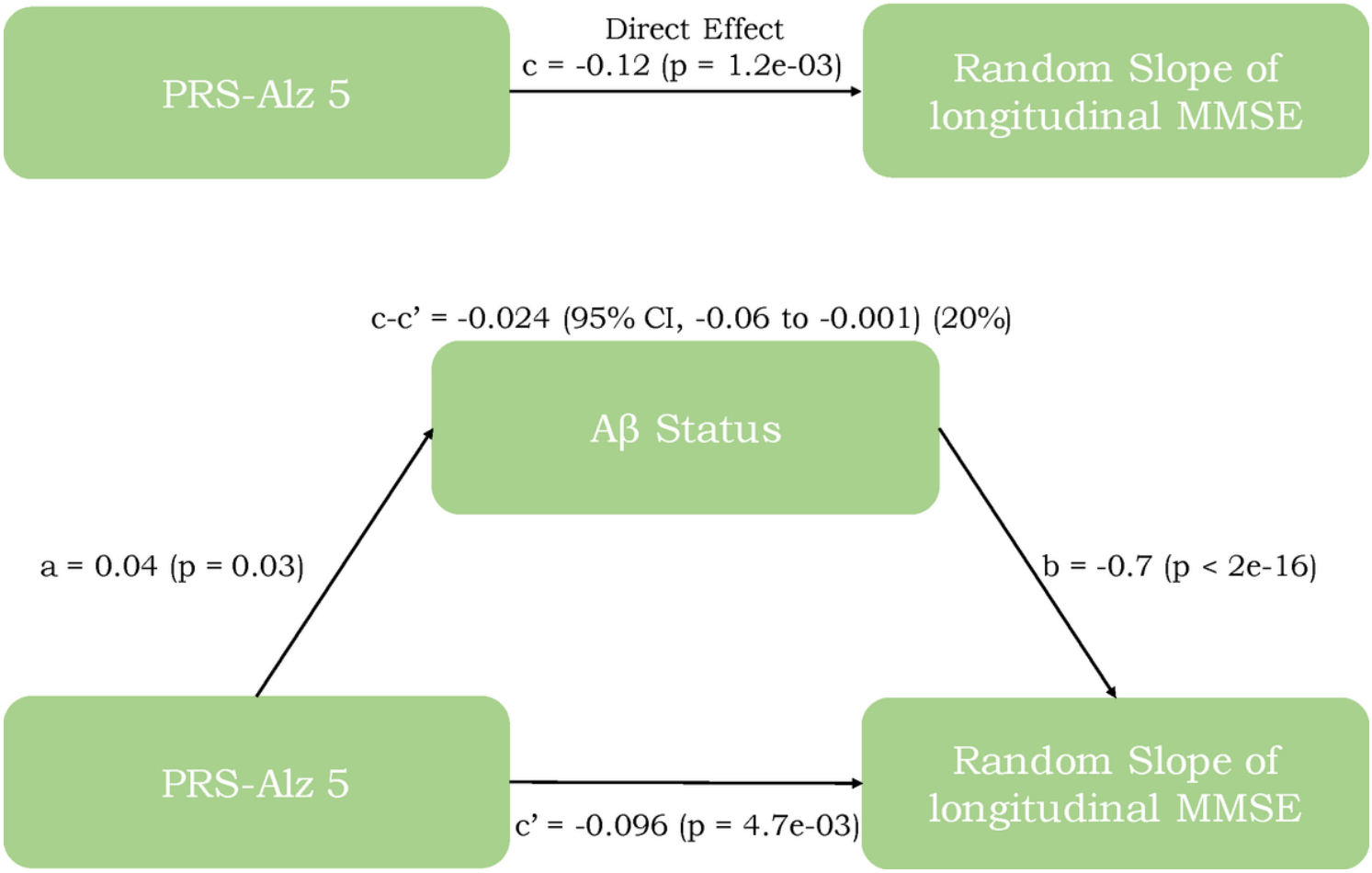

Figure 3

Mediation analysis between PRS, Aβ status and cognition. Mediation analysis with PRS-Alz 5 (predictor), mediated by Aβ status predicting the cognitive decline (slope of longitudinal MMSE). The figure includes the following standardized regression coefficients: a, the effects of PRS/PGS on Aβ; b, the effects of Aβ on random slope of longitudinal MMSE score; c, the direct association between PRS and slope of longitudinal MMSE score; c', the association between PRS and slope of longitudinal MMSE score when adjusting Aβ; and c-c', the mediated effect on slope of longitudinal MMSE (with % mediation)

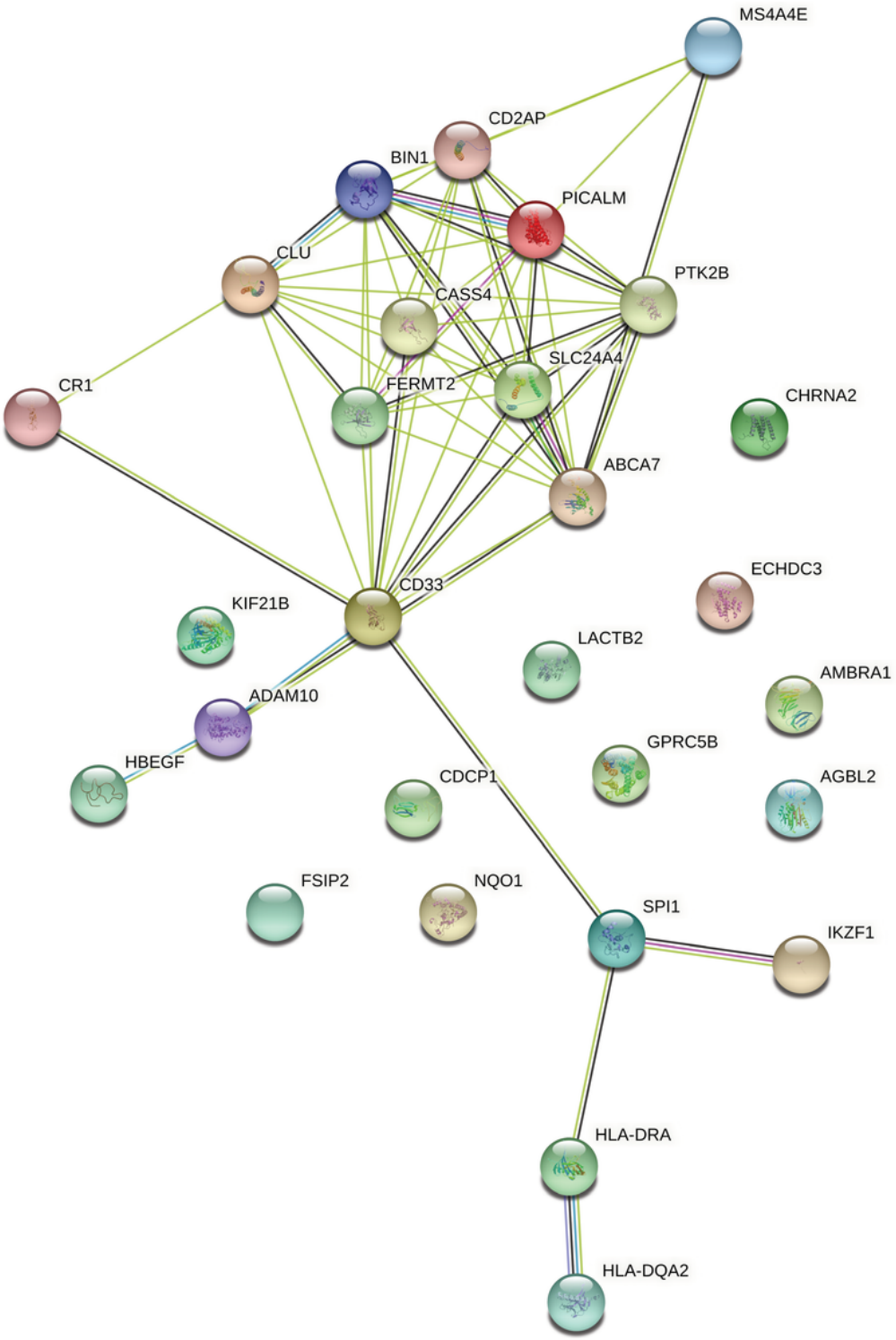

Figure 4

Bioinformatics analysis of Alzheimer's disease PRS-Alz 5: Protein-Protein Interaction network for the protein coding genes of the SNPs of PRS-Alz 5 (PPI enrichment p-value:< 1.0e-16). Nodes represent the proteins and edges indicate both functional and physical protein association. The edge line thickness indicates the strength of data support at an interaction score ≥ 0.4.

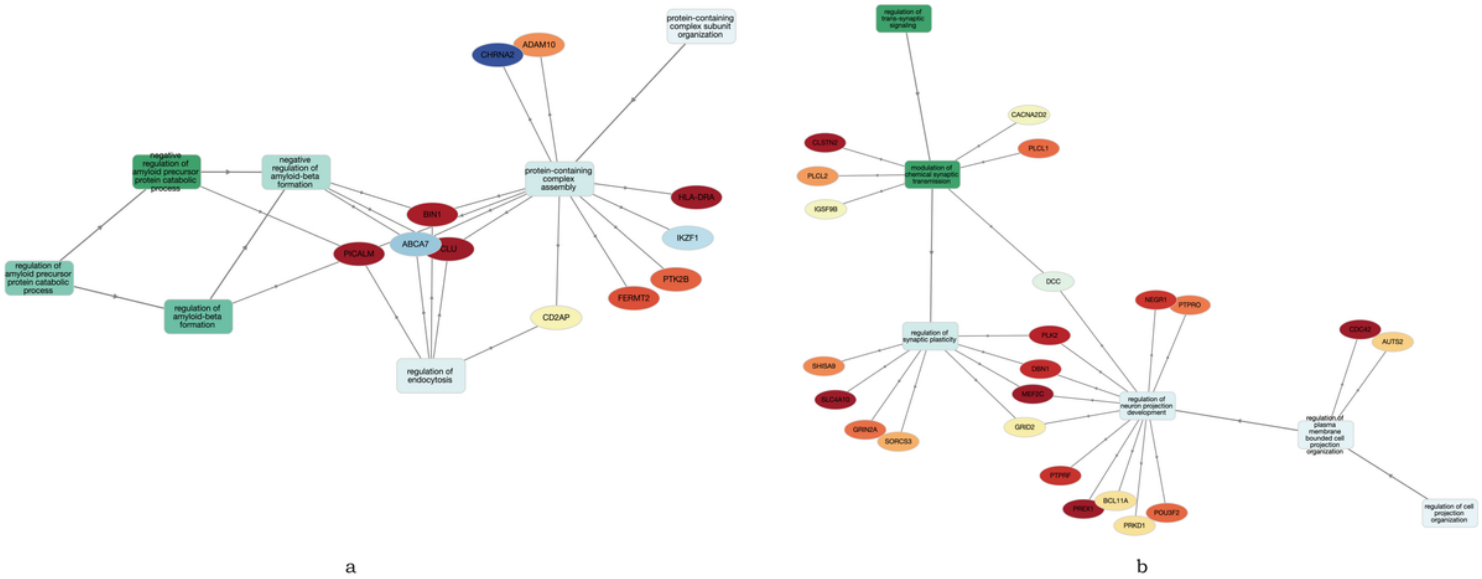

Figure 5

Gene ontology term enrichment of genes of the variants involved in a) PRS-Alz 5 and b) PGS-Int 7 for biological process category. Oval shaped boxes represent the protein coding genes and the rectangular boxes represent the GO term for the biological category. The oval boxes are colored based on protein expression in the cerebral cortex as per Human protein Atlas dataset.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFile.pdf
- SupplementaryTables.xlsx