

Original Article

# A Non-*APOE* Polygenic Risk Score for Alzheimer's Disease Is Associated With Cerebrospinal Fluid Neurofilament Light in a Representative Sample of Cognitively Unimpaired 70-Year Olds

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## Abstract

The effect of Alzheimer's disease (AD) polygenic risk scores (PRS) on amyloid and tau pathophysiology and neurodegeneration in cognitively unimpaired older adults is not known in detail. This study aims to investigate non-*APOE* AD-PRS and *APOE*  $\epsilon 4$  in relation to AD pathophysiology evaluated by cerebrospinal fluid (CSF) biomarkers in a population-based sample of 70-year olds. A total of 303 dementia-free individuals from the Gothenburg H70 Birth Cohort Studies were included. Genotyping was performed using the NeuroChip, and AD-PRS were calculated. CSF levels of amyloid- $\beta$  (A $\beta$ 42), total tau (t-tau), phosphorylated tau (p-tau), neurogranin (Ng), and neurofilament light (NfL) were measured with enzyme-linked immunosorbent assay. Associations were found between non-*APOE* PRS and both NfL ( $p = .001$ ) and A $\beta$ 42 ( $p = .02$ ), and between *APOE*  $\epsilon 4$  and A $\beta$ 42 ( $p = 1e^{-10}$ ), t-tau ( $p = 5e^{-4}$ ), and p-tau ( $p = .002$ ). Similar results were observed when only including individuals with CDR = 0, except for no evidence of an association between non-*APOE* PRS and A $\beta$ 42. There was an interaction between non-*APOE* PRS and A $\beta$ 42 pathology status in relation to NfL ( $p = .005$ ); association was only present in individuals without A $\beta$ 42 pathology ( $p = 3e^{-4}$ ). In relation to A $\beta$ 42, there was a borderline interaction ( $p = .06$ ) between non-*APOE* PRS and *APOE*  $\epsilon 4$ ; association was present in  $\epsilon 4$  carriers only ( $p = .03$ ). Similar results were observed in individuals with CDR = 0 ( $n = 246$ ). In conclusion, among cognitively healthy 70-year olds from the general population, genetic risk of AD beyond the *APOE* locus was associated with NfL in individuals without A $\beta$ 42 pathology, and with A $\beta$ 42 in *APOE*  $\epsilon 4$  carriers, suggesting these associations are driven by different mechanisms.

**Keywords:** Amyloid-beta, CSF biomarkers, Genetic variants, Tau

Alzheimer's disease (AD) is characterized by aggregation of amyloid- $\beta$  (A $\beta$ ) protein into plaques, hyperphosphorylation of tau protein with the formation of tangles, and brain atrophy in certain regions of the brain (1). Studies including neuropathologic series have shown that a large proportion of cognitively normal older individuals exhibit Alzheimer pathology in the brain (2). Pathological changes (brain amyloidosis, tau pathology, neurodegeneration, and synaptic dysfunction) may be reflected by cerebrospinal fluid (CSF) biomarkers (3). In the Gothenburg H70 Birth Cohort Studies, we recently reported that as much as 45% of cognitively normal 70-year olds had pathological CSF levels of A $\beta$  or tau or both (4). Such CSF pathology was associated with having at least one *APOE*  $\epsilon$ 4 allele, which is the strongest genetic risk factor for late-onset AD (5).

The contribution of AD-related genetic variants of lower effect than *APOE*  $\epsilon$ 4 is often studied through the use of polygenic risk scores (PRS), which are based on available summary data from previous large genome-wide association studies (GWAS) on AD. Studies using AD-PRS report associations with disease stage and dementia progression (6,7). Previous studies of AD-PRS in relation to AD biomarkers in CSF have mainly been performed in clinical samples or convenience samples of cognitively healthy individuals (ie, samples recruited within health care institutions or through advertising), with mixed results (6,8–15). Studies involving representative population-based samples of dementia-free individuals are very sparse.

In addition to A $\beta$  and tau, it is now possible to measure other CSF biomarkers of potential importance for preclinical AD, such as neurogranin (Ng) (16), a marker of early synaptic degeneration, and neurofilament light protein (NfL), a marker of subcortical large-caliber axonal degeneration (17). Increased levels of Ng, but not of NfL, have been associated with *APOE*  $\epsilon$ 4 carriership in subjects from the Alzheimer's Disease Neuroimaging Initiative (ADNI) (17). In the population-based Mayo Clinic Study on Aging (MCSA), presence of the *APOE*  $\epsilon$ 4 allele was associated with increased CSF levels of both biomarkers, but only in individuals with mild cognitive impairment (MCI) or dementia (18). So far, none of these markers have been studied in relation to AD-PRS.

The aim of the present study was to investigate non-*APOE* AD-PRS and *APOE*  $\epsilon$ 4 status in relation to CSF biomarkers of AD and neurodegeneration (ie, A $\beta$ 42, total tau [t-tau], phosphorylated tau [p-tau], NfL, and Ng) in a representative sample of 70-year olds without dementia recruited from the general population. We also aimed to examine possible interactions with A $\beta$ 42 pathology status and *APOE*  $\epsilon$ 4 carriership. Further, we wanted to study the same relationships after excluding individuals with MCI (clinical dementia rating [CDR] > 0), who do not fulfill all the criteria of a dementia diagnosis.

## Method

### Population

The sample used in the present study originates from the 2014–2016 examinations of the H70 Gothenburg Birth Cohort Studies in Gothenburg, Sweden (19). The sample was obtained from the Swedish Population Registry and included persons living in private households and in residential care. Every 70-year old in Gothenburg, Sweden, born during 1944 on prespecified birthdates, was invited to the examination, and 1203 participated (response rate 72.2%). Of these, 430 (35.8%) consented to a lumbar puncture. Contraindications (anticoagulant therapy, immunomodulated therapy, cancer therapy) were present in 108, leaving

322 (26.8%) with a CSF tap. CSF volume was insufficient for analyses in 4 participants, leaving 318 with data on the CSF biomarkers A $\beta$ 42, t-tau, and p-tau (4). Due to insufficient CSF volume, 1 person lacked data on Ng; 3 were missing NfL. One further person had an outlier value on NfL and was excluded from the analyses involving that biomarker. Ten additional individuals were excluded based on the quality control (QC) of the genetic data (described in detail below), and 5 had dementia, leaving a final sample of 303 individuals free from dementia with data on A $\beta$ 42, t-tau, and p-tau, 302 with data on Ng, and 299 with data on NfL. Characteristics of the total sample are presented in Table 1. Analyses were also performed on the subgroup with CDR = 0;  $n$  = 246 (245 for Ng and 242 for NfL).

All participants and/or their close relatives gave written informed consent. The study was approved by the Regional Ethics Review Board in Gothenburg.

### Examinations and Diagnoses

Neuropsychiatric examinations were performed by experienced psychiatric nurses. The examinations were semi-structured and included comprehensive psychiatric examinations and an extensive battery of neuropsychological tests (20). Close informant interviews were performed by psychiatric nurses or psychologists. Dementia was diagnosed according to DSM-III-R criteria (21) (which have been used in the Gothenburg studies for over 30 years). A history of stroke/transient ischaemic attack (TIA) was determined based on self- or close informant report, and on the Swedish Inpatient and Outpatient Registries (ICD codes: I60, I61, I63.0–I63.5, I63.8–I63.9, I64, G45.0, G45.1, G45.3, G45.9, I69.0, I69.1, I69.2, I69.3, I69.4, and I62).

### CSF Analyses

CSF t-tau and p-tau (tau phosphorylated at threonine 181) concentrations were measured with sandwich enzyme-linked immunosorbent assays (ELISAs) (INNOTEST htau Ag and PHOSPHO\_TAU [181P], Fujirebio [formerly Innogenetics], Ghent, Belgium) (22,23). CSF A $\beta$ 42 was measured with a sandwich ELISA (INNOTEST A $\beta$ 1–42) specifically constructed to measure A $\beta$  starting at amino acid 1 and ending at amino acid 42 (24). For NfL, an in-house sandwich ELISA with capture and detection antibodies that were directed against the central rod domain of the protein (NfL 21 and NfL 23, respectively)

**Table 1.** Sample Characteristics

Total Sample <sup>a</sup> ( $n$ = 303)	
Age at CSF sampling, mean (SD)	70.9 (0.35)
Sex: women, $n$ (%)	140 (46.2)
<i>APOE</i> $\epsilon$ 4, $n$ (%)	111 (36.6)
MMSE, mean (SD)	29.0 (1.2)
Years of education, mean (SD)	12.7 (3.9)
Stroke, $n$ (%)	14 (4.6)
Amyloid-beta 42 (pg/mL), mean (SD)	718.1 (224.1)
t-tau (pg/mL), mean (SD)	331.7 (135.2)
p-tau (pg/mL), mean (SD)	49.4 (17.4)
Ng (pg/mL) <sup>b</sup> , mean (SD)	204.6 (69.7)
NfL (pg/mL) <sup>c</sup> , mean (SD)	842.9 (605.6)

Notes: CSF = cerebrospinal fluid; MMSE = mini-mental state examination; Ng = neurogranin; NfL = neurofilament light.

<sup>a</sup>Total sample without dementia diagnosis after QC of the genotyping-data.

<sup>b</sup>Mean is based on 302 individuals. <sup>c</sup>Mean is based on 299 individuals.

was used (25). An in-house ELISA method (26) was used to measure CSF Ng.

## Genotyping

Genotyping was performed with the NeuroChip (Illumina) (27). QC included the removal of individuals due to any of the following: per-individual call rate <98%, sex mismatch, and excessive heterozygosity (F<sub>HET</sub> outside  $\pm 0.2$ ). Further, individuals were defined as non-European ancestral outliers, and removed, if their first 2 principal components (PCs) exceeded 6 SDs from the mean values of the European samples in the 1000 Genome global reference population. Closely related individuals were removed based on pairwise PL\_HAT (ie, proportion of the genome that is in identity-by-descent; calculated using --genome option in PLINK)  $\geq 0.2$ . Genetic variants were excluded due to per-single nucleotide polymorphism (SNP) call rate <98%, minor allele frequency <0.01, and Hardy–Weinberg disequilibrium ( $p < 1e-6$ ). The Sanger imputation service was used to impute post-QC, using the reference panel of Haplotype Reference Consortium data (HRC1.1). The SNPs rs7412 and rs429358, defining the *APOE* alleles  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$ , were also genotyped, using the KASPar PCR SNP genotyping system (LGC Genomics, Hoddesdon, Herts, UK).

## Construction of PRS

Among the AD-PRS constructed in this study was a 39-SNP PRS based on all SNPs (excluding the *APOE* region) that have shown genome-wide significant association with AD after combined meta-analyses in the very recent GWAS by de Rojas et al. (28) (Supplementary Table 1). Similar to the study by de Rojas et al., where the PRS was validated for the first time, the 39 SNPs were weighted using published effect sizes from IGAP (29), Sims et al. (30), and Jun et al. (31). All genetic variants included in the PRS represent independent signals (28). In addition, AD-PRS were generated using summary statistics from stage 1 of the most recently published AD GWAS including clinically defined AD (29). SNPs were selected using LD-clumping. In short, the European ancestry samples from the 1000 Genomes Project were used as reference panel to remove variants in LD; all variants 250 kb upstream and downstream of the top signal were removed ( $R^2 < .001$ ). All variants in the *APOE* region (chromosome 19, coordinates GRCh37: 44412079–46412079) were removed. In the present study, we created PRS based on 4  $p$ -value thresholds ( $p < 5e^{-8}$ ,  $p < 1e^{-5}$ ,  $p < 1e^{-3}$ , and  $p < 1e^{-1}$ ), referred to as  $5e^{-8}$  PRS,  $1e^{-5}$  PRS,  $1e^{-3}$  PRS, and  $1e^{-1}$  PRS. All AD-PRS were calculated as the sum of the  $\beta$  coefficient multiplied with the number/dosage of effect alleles of each genetic variant, and then standardized.

## Statistical Analyses

The values of t-tau, p-tau, and NfL were logarithmized due to skewed distribution. Linear regressions, adjusted for sex, age at CSF sampling, and 10 PCs (computed in PLINK) to correct for population stratification, were used to analyze non-*APOE* PRS and *APOE*  $\epsilon 4$  status in relation to levels of CSF biomarkers (A $\beta$ 42, t-tau, p-tau, Ng, and NfL), both in the total sample and in the CDR = 0 subsample.  $p$ -Values generated during these analyses were further validated against a Bonferroni corrected  $p$ -value threshold. This threshold was based on tests of 2 different “genetic risk designs” (ie, *APOE*  $\epsilon 4$  status and non-*APOE* PRS) in relation to 5 different biomarkers (ie,  $2 \times 5$  tests = 10; corrected  $p$ -value level = .005).

Analyses investigating possible interactions between A $\beta$ 42 pathology status (ie, A $\beta$ 42  $\leq 530$  pg/mL yes/no) and the non-*APOE* PRS,

and the *APOE*  $\epsilon 4$  status, in relation to the other CSF biomarkers were performed using linear regressions including the interaction terms A $\beta$ 42 pathology status  $\times$  Non-*APOE* PRS/*APOE*  $\epsilon 4$  status. Identified interactions were further investigated in analyses stratified on A $\beta$ 42 pathology status (linear regressions adjusted for sex, age at CSF sampling, and 10 PCs).

Analyses investigating possible interactions between *APOE*  $\epsilon 4$  status and non-*APOE* PRS in relation to the CSF biomarkers were performed using linear regressions including the interaction term *APOE*  $\epsilon 4$  status  $\times$  Non-*APOE* PRS. Identified interactions were further investigated in analyses stratified on *APOE*  $\epsilon 4$  status (linear regressions adjusted for sex, age at CSF sampling, and 10 PCs).

The statistical analyses were done in IBM SPSS Statistics v26 and R v4.0.0 using stats and ggplot2 packages.

## Results

Associations were found between non-*APOE* PRS and NfL (for the 39-SNP PRS:  $\beta = 0.07$ ,  $SE = 0.03$ ,  $p = .01$  and  $5e^{-8}$  PRS:  $\beta = 0.08$ ,  $SE = 0.03$ ,  $p = .001$ ) and A $\beta$ 42 ( $5e^{-8}$  PRS:  $\beta = -29.8$ ,  $SE = 13.0$ ,  $p = .02$ ) in the total sample. The association with NfL remained after Bonferroni correction for multiple testing, but the association with A $\beta$ 42 did not. Associations, surviving correction for multiple testing, were also found between *APOE*  $\epsilon 4$  status and A $\beta$ 42 ( $\beta = -171.1$ ,  $SE = 25.5$ ,  $p = 1e^{-10}$ ), t-tau ( $\beta = 0.16$ ,  $SE = 0.05$ ,  $p = 5e^{-4}$ ), and p-tau ( $\beta = 0.13$ ,  $SE = 0.04$ ,  $p = .002$ ). Further, a borderline association was found between the  $\epsilon 4$  allele and increased levels of Ng in the total sample ( $\beta = 15.5$ ,  $SE = 8.6$ ,  $p = .07$ ). Apart from an association between the non-*APOE* PRS at level  $1e^{-5}$  and NfL ( $\beta = 0.06$ ,  $SE = 0.03$ ,  $p = .04$ ), and a lack of evidence of an association between non-*APOE* PRS and A $\beta$ 42, similar results were observed in the CDR = 0 subsample (Table 2). No evidence of associations was found between the non-*APOE* PRS and t-tau, p-tau, and Ng, or between *APOE*  $\epsilon 4$  status and NfL (Table 2).

To study if the identified association, at a nominal  $p$ -value level, between the  $5e^{-8}$  PRS and A $\beta$ 42 levels was beyond the effect of *APOE*, we included *APOE*  $\epsilon 4$  status as a covariate in the linear regression analysis of the relation between the  $5e^{-8}$  PRS and A $\beta$ 42. The result showed that the  $5e^{-8}$  PRS and *APOE*  $\epsilon 4$  status were both significantly associated ( $p = 2e^{-10}$  and  $p = .04$ ) with A $\beta$ 42 (ie, both variables had an independent effect in relation to A $\beta$ 42). Moreover, the explanatory value (adjusted  $r^2$ ) of a model including *APOE*  $\epsilon 4$  status increased slightly, from 0.13 to 0.14, when the non-*APOE* PRS ( $5e^{-8}$ ) was added.

There was an interaction between A $\beta$ 42 pathology status ( $\leq 530$  pg/mL yes/no) and non-*APOE* PRS in relation to NfL levels, both in the total sample (39-SNP PRS:  $p = .005$ ,  $5e^{-8}$  PRS: .04) and in the CDR = 0 subsample (39-SNP PRS:  $p = .005$ ,  $5e^{-8}$  PRS: .05). Associations between the PRS and CSF NfL were only found among individuals with normal A $\beta$ 42 pathology levels, that is, in those without evidence of brain amyloidosis (total sample: 39-SNP PRS:  $\beta = 0.11$ ,  $SE = 0.03$ ,  $p = 3e^{-4}$  [Figure 1] and  $5e^{-8}$  PRS:  $\beta = 0.12$ ,  $SE = 0.03$ ,  $p = 2e^{-4}$ ; CDR = 0 subsample: 39-SNP PRS:  $\beta = 0.12$ ,  $SE = 0.03$ ,  $p = 3e^{-4}$  and  $5e^{-8}$  PRS:  $\beta = 0.11$ ,  $SE = 0.03$ ,  $p = .001$ ). The associations did not change when adjusting for history of stroke (including TIA) (results not shown). There were no interactions between non-*APOE* PRS and CSF A $\beta$ 42 (brain amyloidosis) status in relation to any of the other CSF-markers (ie, t-tau, p-tau, and Ng), either in the total sample, or in the CDR = 0 subsample (results not

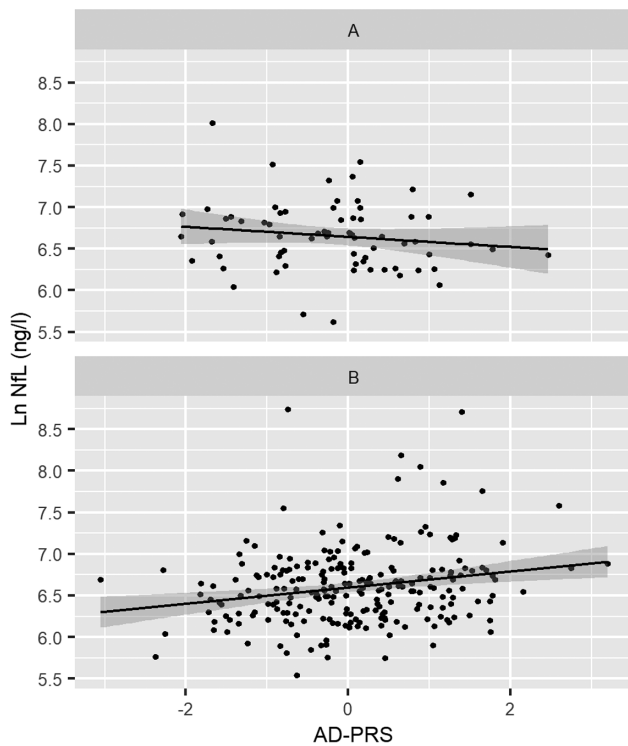
**Table 2.** Non-APOE PRS and APOE Score in Relation to CSF Biomarkers

	Total Sample													
	Total Sample							CDR = 0 Subsample						
	39-SNP PRS	5e-8 PRS	1e-5 PRS	1e-3 PRS	1e-1 PRS	APOE $\epsilon$ 4	39-SNP PRS	5e-8 PRS	1e-5 PRS	1e-3 PRS	1e-1 PRS	APOE $\epsilon$ 4 <sup>a</sup>		
A $\beta$ 42	$\beta$	16.9	-29.8	-8.2	1.1	6.3	-171.1	-23.2	-0.7	-3.1	6.1	-166.8		
	SE	13.1	13.0	13.3	13.4	13.4	25.5	14.2	14.7	15.6	14.4	29.5		
	<i>p</i>	.2	.02	.5	.9	.6	<b>1e-10</b>	.1	.96	.8	.7	<b>5e-8</b>		
Ln t-tau	$\beta$	-0.02	0.03	0.03	0.02	0.007	0.16	0.02	0.05	0.002	0.006	0.18		
	SE	0.02	0.02	0.02	0.02	0.02	0.05	0.03	0.03	0.03	0.03	0.05		
	<i>p</i>	.4	.2	.2	.5	.8	<b>5e-4</b>	.3	.08	.9	.8	.001		
Ln p-tau	$\beta$	-0.02	0.02	0.02	0.02	0.003	0.13	0.02	0.03	0.001	-0.001	0.14		
	SE	0.02	0.02	0.02	0.02	0.02	0.04	0.02	0.02	0.02	0.02	0.05		
	<i>p</i>	.4	.2	.3	.4	.9	<b>.002</b>	.5	.2	.96	.96	<b>.003</b>		
Ng <sup>b</sup>	$\beta$	-5.3	0.17	2.4	3.3	-2.6	15.5	-0.8	5.2	1.9	-3.3	20.1		
	SE	4.1	4.2	4.2	4.2	4.2	8.6	4.7	4.8	5.2	4.7	10.2		
	<i>p</i>	.2	.97	.6	.4	.5	.07	.99	.3	.7	.5	.05		
Ln NFL <sup>c</sup>	$\beta$	0.07	0.08	0.05	-0.02	-0.03	0.01	0.08	0.06	-0.04	-0.03	0.02		
	SE	0.03	0.03	0.03	0.03	0.03	0.05	0.03	0.03	0.03	0.03	0.06		
	<i>p</i>	<b>.01</b>	<b>.001*</b>	.09	.4	.3	.8	<b>.003*</b>	<b>.04</b>	.2	.3	.7		

Notes: CDR = clinical dementia rating; CSF = cerebrospinal fluid; Ng = neurogranin; NFL = neurofilament light; PRS = polygenic risk score; SNP = single nucleotide polymorphism.

<sup>a</sup>Similar results for associations between APOE  $\epsilon$ 4 and A $\beta$ 42, t-tau, and p-tau in the CDR = 0 subsample have been published previously (4). Due to removal of samples during the quality control of the genome-wide association study data, the number of individuals were slightly lower in the present study. <sup>b</sup>Results for Ng are based on 302 individuals in the total sample, and 245 in the CDR = 0 subsample. <sup>c</sup>Results for NFL are based on 299 individuals in the total sample, and 242 individuals in the CDR = 0 subsample.

\*Surviving Bonferroni correction for multiple testing (based on a corrected *p*-value level of .005). Significant *p*-values (*p* < .05) are presented in bold.



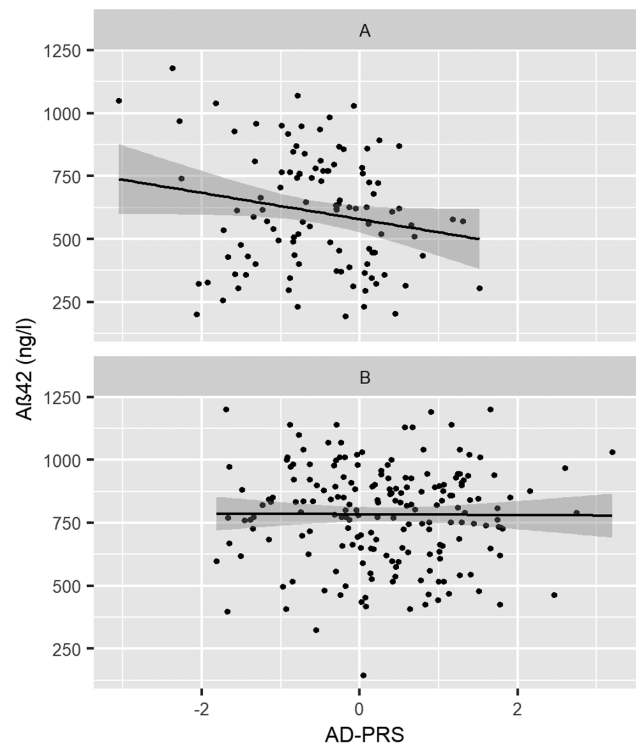
**Figure 1.** Non-*APOE* 39-SNP AD-PRS in relation to CSF NfL in individuals with (A) and without (B)  $A\beta_{42}$  pathology levels, in the total sample without dementia. AD = Alzheimer's disease; CSF = cerebrospinal fluid; NfL = neurofilament light; PRS = polygenic risk score; SNP = single nucleotide polymorphism.

shown). Further, there were no interactions between *APOE*  $\epsilon 4$  status and CSF  $A\beta_{42}$  status in relation to any of the biomarkers (results not shown).

There was a trend towards an interaction between *APOE*  $\epsilon 4$  status and the 39-SNP PRS in relation to  $A\beta_{42}$  in the total sample ( $p = .06$ ), and an interaction in the CDR = 0 subsample ( $p = .04$ ). An association between the PRS and CSF  $A\beta_{42}$  was only found among *APOE*  $\epsilon 4$  carriers in the total sample ( $\beta = -62.6$ ,  $SE = 27.7$ ,  $p = .03$ ) (Figure 2). In the CDR = 0 subsample, there was a borderline association in  $\epsilon 4$  carriers ( $\beta = -57.9$ ,  $SE = 30.0$ ,  $p = .06$ ). The interactions between *APOE*  $\epsilon 4$  status and the  $5e^{-8}$  PRS in relation to  $A\beta_{42}$  only approached significance (total sample:  $p = .09$ , CDR = 0 subsample:  $p = .1$ ), but similar to results for the 39-SNP PRS, stratification based on  $\epsilon 4$  status showed an association in  $\epsilon 4$  carriers in the total sample ( $\beta = -56.1$ ,  $SE = 22.6$ ,  $p = .02$ ), and a borderline association in the CDR = 0 subsample ( $\beta = -48.3$ ,  $SE = 25.1$ ,  $p = .06$ ). No interactions were observed between *APOE*  $\epsilon 4$  status and non-*APOE* PRS in relation to the other CSF-markers in the total sample, and the same was the case for the CDR = 0 subsample (results not shown).

## Discussion

In a representative sample of 70-year olds free from dementia, non-*APOE* PRS was associated with CSF levels of NfL and  $A\beta_{42}$ . The association with NfL remained after correction for multiple testing, while the association with  $A\beta_{42}$  did not. Associations surviving correction were also found between *APOE*  $\epsilon 4$  status and  $A\beta_{42}$ , t-tau, and p-tau, while Ng was associated at a borderline level. Stratified



**Figure 2.** Non-*APOE* 39-SNP AD-PRS in relation to CSF  $A\beta_{42}$  in *APOE*  $\epsilon 4$  carriers (A) and noncarriers (B), in the total sample without dementia. AD = Alzheimer's disease; CSF = cerebrospinal fluid; PRS = polygenic risk score; SNP = single nucleotide polymorphism.

analyses, based on identified interactions, showed associations between the non-*APOE* PRS and CSF levels of NfL only in individuals without CSF biomarker evidence of brain amyloid pathology. In addition, the non-*APOE* PRS was associated with CSF levels of  $A\beta_{42}$  among *APOE*  $\epsilon 4$  carriers, but not in those without this allele.

Previous studies of AD-PRS in relation to the CSF biomarkers  $A\beta_{42}$ , t-tau, and p-tau in representative population-based samples of cognitively healthy individuals are lacking. Studies performed among cognitively healthy and MCI individuals from convenience and clinical samples show discrepant results. A possible explanation could be heterogeneity of the samples, regarding both age and diagnostic status. Considering  $A\beta_{42}$ , several studies, including ours, show association with either *APOE*  $\epsilon 4$  status or *APOE* PRS (10,12,15), but association with non-*APOE* PRS, or *APOE* PRS adjusted for *APOE*  $\epsilon 4$  status, is rare (14). In our study, we see an association between a non-*APOE* PRS at the genome-wide significance level, but the finding is not strong enough to survive correction for multiple testing and should therefore be interpreted with caution. A recent study reported that both *APOE* and non-*APOE* PRS predicted MCI and AD, while only *APOE* predicted amyloid deposition based on positron emission tomography (PET), suggesting that genetic risk for AD can differ from genetic risk for amyloid deposition (32). Considering tau levels, reports on association with *APOE* in cognitively healthy individuals are rare (4). Apart from the AIBL study, which used a small CSF biomarker sample, associations between non-*APOE* PRS and tau levels have only been reported in analyses including individuals with MCI (12,14). Few studies report results for non-*APOE* PRS in relation to CSF biomarkers stratified by *APOE*  $\epsilon 4$  status. We found an association between non-*APOE* PRS and  $A\beta_{42}$  in  $\epsilon 4$  carriers. This type of association could not be seen for the other biomarkers.

In contrast to our results, a study on MCI reported an association between non-*APOE* PRS and CSF t-tau and p-tau, which became stronger in  $\epsilon 4$  carriers, while no association was found with A $\beta$ 42 in  $\epsilon 4$  carriers (12).

Due to discrepant results, studies in larger samples are needed to sort out the relationship between AD-PRS and CSF biomarkers in cognitively healthy individuals. Large population-based samples with data on CSF- biomarkers are rare, but combining data from several smaller studies would enable meta-analyses, or even pooled analyses if the data can be homogenized in an appropriate way. Further, discrepant findings among studies could probably to some extent also be explained by differences in the PRS used. Among the PRS employed in our study, it was apparent that those including SNPs based on a genome-wide significance level performed better than the broader versions of PRS including SNPs based on higher significance levels.

To the best of our knowledge, this is the first study investigating the relation between AD-PRS and novel CSF biomarkers (ie, Ng and NfL) suggested to be involved in the AD disease process. CSF NfL predicts progression to MCI and dementia among cognitively normal individuals with preclinical AD (33). Although it is a valuable marker of early neurodegeneration, it is not specific for AD pathobiology (17,33). In patients in the AD spectrum, NfL is more closely linked to concomitant cerebrovascular disease (34–36). Indeed, we found that NfL was associated with non-*APOE* PRS, but the association was only present among individuals without pathological levels of A $\beta$ 42. These results are to some extent in line with the findings by Mattsson et al. (17), showing that although NfL associates with AD, the association was strongest in individuals without A $\beta$  pathology. Moreover, the authors found that the association between NfL and other AD traits, such as cognitive decline, brain atrophy, brain hypometabolism, and white matter hyperintensities, often was stronger in individuals without A $\beta$ 42 pathology.

We also found an association between the non-*APOE* PRS and A $\beta$ 42 in *APOE*  $\epsilon 4$  carriers. It may be that the associations we see with the non-*APOE* PRS reflect two different pathways in the process of AD, and that the association with NfL indicates a pre-amyloid phase. The influence of polygenic scores during the prodromal phase of AD has been discussed in previous literature (6,37). An association between the non-*APOE* PRS and preclinical, and prodromal, disease independent of amyloid pathology is reasonable, since many of the genetic variants included in the PRS are involved in non-amyloidogenic pathways, such as immune response and inflammation, lipid transport, and endocytosis (28). Alternative explanations include that the association between AD-PRS and NfL among cognitively unimpaired individuals without amyloid pathology reflects brain processes also involved in accelerated aging or other neurodegenerative diseases (eg, Lewy body disease, frontal lobe dementia), in inflammatory diseases, as well as in cerebrovascular disease. A genetic overlap between these types of disorders has been suggested in several previous studies (38–40). The association between the non-*APOE* PRS and NfL in our sample did not change after adjusting for stroke (including TIA). However, the number of individuals with stroke in our cohort was low. Moreover, a relation between the non-*APOE* PRS and types of pathophysiology other than plaques and tangles characteristic of AD is further supported by the finding that elevated levels of NfL in our sample were not driven by *APOE*, the strongest genetic factor modulating risk for AD.

We also found a borderline association between *APOE*  $\epsilon 4$  carriership and increased CSF levels of Ng in cognitively unimpaired 70-year olds. This result contrasts the finding in a previous study in one of the other cohorts included in the Gothenburg H70 Birth

Cohort Studies, where no association was seen in 129 cognitively healthy individuals with a mean age of 82 years (41). A similar result was seen in the population-based MCSA, showing no association between *APOE*  $\epsilon 4$  and Ng in cognitively unimpaired older individuals ( $n = 687$ ) (18). One reason for the discrepancy may be the high frequency of *APOE*  $\epsilon 4$  carriers (37%) in our sample. However, as mentioned, the association seen in the present study is relatively weak and has to be further investigated in other samples before any conclusions could be drawn.

Among the strengths of our study are the comprehensive examinations and the homogeneous, and relatively large, CSF biomarker sample of cognitively unimpaired individuals originating from a representative population-based study. All individuals were dementia-free, and analyses including only those with CDR = 0 did not change the results, indicating that individuals with CDR above 0 are relatively similar to the rest of the sample. There are also some limitations. Even if the number of individuals with CSF data was relatively large, the overall number is low, at least for a genetic study, which influences the statistical power. Considering the findings for non-*APOE* PRS, only the association with NfL survives Bonferroni correction for multiple testing. Similarly, among the stratified analyses, the NfL-related findings seem to be more robust. Further, the cross-sectional design of the study makes it impossible to identify individuals who will stay cognitively healthy over time for subgroup analyses. Moreover, the study involves a Caucasian 70-year-old population and generalization of the results to other populations should be done with caution.

In conclusion, we found that *APOE* genotype was associated with CSF A $\beta$ 42, t-tau, and p-tau among cognitively healthy 70-year olds recruited from the general population. We also found that genetic risk of AD beyond the *APOE* locus was associated with NfL and A $\beta$ 42. However, the association with NfL was only seen in individuals without evidence of A $\beta$ 42 pathology, and the association with A $\beta$ 42 was only seen in *APOE*  $\epsilon 4$  carriers, suggesting that associations between the non-*APOE* AD-PRS and these markers of neurodegeneration and brain amyloidosis are driven by different mechanisms.

## Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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## Conflict of Interest

H.Z. has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, and CogRx; has given lectures in symposia sponsored by Fujirebio, Alzecure, and Biogen; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). K.B. has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. All other authors declared no conflict of interest.

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## Author Contributions

A.Z. and I.S. designed the study; A.Z., I.S., S.K., and J.N. took part in the acquisition of subjects and data; A.Z. analyzed the data; A.Z., I.S., S.K., J.N., R.G., J.B., M.W., H.Z., and K.B. took part in the interpretation of the data; I.S. and A.Z. drafted the manuscript; S.K., J.N., R.G., J.B., M.W., H.Z., and K.B. revised it critically for important intellectual content; A.Z., I.S., S.K., J.N., R.G., J.B., M.W., H.Z., and K.B. approved the final version of the manuscript; and I.S., A.Z., M.W., H.Z., and K.B. funded the study.

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