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Alzheimer’s disease genetic pathways impact cerebrospinal fluid biomarkers and imaging endophenotypes in non-demented individuals

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**Abstract**

**INTRODUCTION:** Unraveling how Alzheimer’s disease (AD) genetic risk is related to neuropathological heterogeneity, and whether this occurs through specific biological pathways, is a key step toward precision medicine.

**METHODS:** We computed pathway-specific genetic risk scores (GRSs) in non-demented individuals and investigated how AD risk variants predict cerebrospinal fluid (CSF) and imaging biomarkers reflecting AD pathology, cardiovascular, white matter integrity, and brain connectivity.

**RESULTS:** CSF amyloid beta and phosphorylated tau were related to most GRSs. Inflammatory pathways were associated with cerebrovascular disease, whereas quantitative measures of white matter lesion and microstructure integrity were predicted by clearance and migration pathways. Functional connectivity alterations were related to genetic variants involved in signal transduction and synaptic communication.

**DISCUSSION:** This study reveals distinct genetic risk profiles in association with specific pathophysiological aspects in predementia stages of AD, unraveling the biological substrates of the heterogeneity of AD-associated endophenotypes and promoting a step forward in disease understanding and development of personalized therapies.
1 | BACKGROUND

Recent genome-wide association studies (GWASs) of sporadic Alzheimer’s disease (AD) and related dementias have identified more than 70 genetic variants that modify the risk of developing AD, beyond apolipoprotein E (APOE) ε2/4. These risk variants are involved in several pathophysiological pathways, such as amyloid-beta 1-42 (Aβ1-42) production and clearance, lipid metabolism, endocytosis, immune function, and inflammatory response. The multitude of pathophysiological processes involved in AD pathogenesis may explain heterogeneity in neuropathological features of AD that are already present in the pre-dementia stage. For example, individuals along the AD clinical spectrum can present with heterogeneous profiles of brain functional, structural, and cerebrovascular alterations observed through magnetic resonance imaging (MRI) techniques. Neuropathological heterogeneity further exacerbates disease complexity and may contribute to the partial efficacy of anti-amyloid compounds investigated in clinical trials for AD. Individuals in the early stages of the AD continuum might indeed present alterations in different biological pathways, eventually leading to heterogeneous neuroimaging and clinical manifestations. Characterizing how genotype influences heterogeneity in these imaging phenotypes is essential for understanding individual differences in disease cause, presentation, trajectory, and response to treatment, thus will be necessary for patients’ selection and stratification in clinical trials.

One way to link genetic variants to biological pathways and neuropathological features is through determining total and pathway-specific genetic risk scores (GRSs). GRSs are weighted scores that quantify the individual genetic predisposition to develop a disease, such as AD, calculated by computing the sum of risk alleles that an individual has, weighted by the risk allele effect sizes as estimated by a GWAS. Furthermore, by linking variants to genes, and genes to associated biological pathways, one can compute pathway-specific GRSs (pathway-GRSs), which retain information about how the burden of genetic risk varies across biological processes.

The APOE-ε4 genotype promotes amyloid deposition during the stages preceding dementia onset, and has been associated, although less consistently, with tau deposition, hippocampal atrophy, and alterations of functional connectivity (FC). In contrast, there is scant evidence linking early fluid and imaging AD-related traits to genetic pathways beyond APOE. It has been demonstrated that the cerebrospinal fluid (CSF) phosphorylated tau (p-tau) and total tau (t-tau) levels are correlated with GRSs for AD that did not include the APOE variants. Moreover, GRSs have been associated with higher rates of tau-PET (positron emission tomography) and amyloid-PET uptake in patients with AD, independently of APOE genotype. Pathway-GRSs of endocytosis and immune response have been found to be associated with AD clinical progression, and to a lower extent with imaging markers of white matter damage. Although this suggests that certain AD phenotypes may be preferentially associated with accumulated genetic risk along particular biological pathways, current research has mostly focused on specific aspects, failing to capture genetic bases and pathways that regulate the broad spectrum of imaging and molecular biomarkers changes in preclinical AD stages.

To assess the genetic vulnerability underlying early AD-associated changes in brain pathology, structure, and function, we tested whether GRSs and pathway-GRSs of Alzheimer’s disease and related dementias relate to (1) CSF levels of Aβ42 and p-tau181, (2) radiological features of cerebral small-vessel disease (cSVD), and (3) a broad set of quantitative imaging phenotypes from multimodal MRI.

2 | METHODS

2.1 | Participants

Data were drawn from the latest data release from the European Prevention of Alzheimer’s Dementia (EPAD) multicenter study. EPAD general inclusion criteria were age older than (or equal to) 50 years and no diagnosis of dementia (Clinical Dementia Rating [CDR] scale...
RESEARCH IN CONTEXT

1. Systematic review: Polygenic risk for Alzheimer’s disease (AD) comprises genetic variants that play a role in various biological processes, extending beyond amyloid production and clearance. This broader genetic influence may account for the heterogeneity of neuropathological observed from the early stages of the disease. Understanding these distinct pathophysiological pathways is crucial for comprehending the disease and tailoring treatments to individual patients.

2. Interpretation: Using data from a large multicenter cohort study, we demonstrate that distinct genetic profiles determine specific imaging abnormalities and promote disease heterogeneity, through differential biological pathways.


2.2 | Genetic data acquisition and processing

DNA samples were genotyped using Illumina Infinium Global Screening Array-24 v3.0. Standard quality control procedures were applied using PLINK (www.cog-genomics.org) and are available online (https://github.com/marioni-group/epad-gwas). Briefly, quality control ensured high-quality genotypes in all individuals (individual call rate >99%, variant call rate >99%), excluding single nucleotide polymorphisms (SNPs) with a significant departure from Hardy–Weinberg equilibrium ($p < 1x10^{-16}$) and keeping SNPs with minor allele frequency >0.5%. Before imputation, individuals of non-European ancestry ($n = 19$, based on clustering with HapMap III reference data) and individuals with a family relation ($n = 46$, identity-by-descent $>0.1875$) were excluded. Genotypes were imputed using the Michigan Imputation Server (https://imputationserver.sph.umich.edu) against European sample data from the Haplotype Reference Consortium (HRC, v1.1, GRCh37). Analyses were restricted to SNPs with imputation quality scores (RSQ) $>0.6$ and minor allele frequencies (MAFs) $>0.0005$.

2.3 | Genetic risk scores calculation

We constructed GRs using 85 variants that were previously significantly associated (genome-wide threshold) with AD and related dementias, in a sample of individuals that had no overlap with the EPAD cohort. The variant effect sizes (log of odds ratio) reported in the original work (Table S1) were used as weights for the GRS. Given a subject $s$, the GRS is defined as:

$$ PRS = \sum_{k=1}^{K} \text{dosage}_s \times \ln(OR_k) $$

where $K$ represents the full set of genetic variants, $\text{dosage}_s^k$ denotes the allele dosage from the (imputed) genotype of variant $k$ in subject $s$, and $\ln(OR_k)$ is the logarithmically transformed odds ratio of variant $k$. To investigate the effects of genetic variants beyond APOE, GRs were computed both with and without the two alleles (rs7412 and rs429358) from the APOE gene (denoted as GRSAPOE and GRSnoAPOE, respectively).

2.4 | Pathway-GRS

In order to construct pathway-specific GRs, SNPs were mapped to pathways. We used a previously developed data-driven method, which has no a priori pathway definition and consists of two fundamental steps: first, single SNPs were linked to likely affected genes (variant-gene mapping); then, identified genes were associated with biological pathways (gene-pathway mapping). This method has previously demonstrated its capability to identify canonical disease pathways as identified in prior studies. The pathway analysis was performed on the set of SNPs excluding the APOE region, to specifically evaluate APOE-independent pathways.

2.4.1 | Variant-gene mapping

To perform the first step of this procedure we relied on the variant-gene mapping reported in the reference GWAS study. Briefly, to prioritize candidate genes in the new loci, the authors integrated variant annotation, quantitative-trait-loci (QTL) (such as expression-QTL, protein-QTL, splicing-QTL, methylation-QTL, and histone acetylation-QTL), and $\beta$-amyloid precursor protein (APP) metabolism. Detailed information about the annotation procedure is reported in the original work. Prioritized genes are reported in Table S1.

2.4.2 | Gene-pathway mapping

A gene-set enrichment analysis was then performed with snpXplorer to find biological pathways enriched within the set of identified genes. The Gost function from the R package gprofiler was used with gene ontology as a reference gene source for functional profiling.
Briefly, snpXplorer calculates a semantic similarity matrix between all enriched pathways, which is then used in a hierarchical clustering framework to obtain clusters of similar pathways. Lin distance is used as a semantic similarity metric, whereas the number of clusters is estimated with a dynamic cut-tree algorithm. By counting the number of times each SNP was associated with each cluster of pathways, and dividing by the total number of associations per SNP, we obtained a weighted mapping factor of each SNP to each cluster of pathways, varying between 0 and 1 and reflecting the contribution of that SNP to that cluster of pathways (Figure 1). In case no mapping to any of the pathways was found, we excluded the gene from further analyses.

2.4.3 | Pathway-GRSs

For the pathway-GRSs, we extended the definition of the GRS by adding as a multiplicative factor the variant-pathway-mapping weight of each variant:

\[
GRS = \sum_{k=1}^{K} \text{dosage}_k \times \ln(OR_k) \times M^p_k
\]

where \( M^p_k \) is the variant-pathway mapping of variant \( k \) to pathway \( p \), thus obtaining \( N \) pathway GRSs estimates per subject, with \( N \) being the number of identified clusters.

2.5 | CSF analysis and AT classification

CSF biomarkers were quantified using a harmonized pre-analytical protocol. Analyses were performed with the fully automatized Roche cobas Elecsys System at the Clinical Neurochemistry Laboratory, Mölndal, Sweden. Concentrations of \( A_\beta_{1-42} \) were determined using the manufacturer’s guidelines. Following a previous study on the same cohort, CSF \( A_\beta_{1-42} \) levels <1000 pg/mL were used to define amyloid positivity (A+), and CSF p-tau levels >27 pg/mL were used to define tau positivity (T+). Four AT groups were derived to define A−T−, A+T−, A+T+, and A−T+ participants.

2.6 | MRI acquisition and processing

EPAD MRI acquisition and pre-processing details are given in and in supplementary materials. Briefly, at all sites the MRI protocol included acquisition of three-dimensional (3D) T1-weighted (3D T1w)
and 3D fluid-attenuated inversion recovery (FLAIR), 2D T2w, and 2D T2 star images. In a subset of sites, advanced MRI sequences were also acquired including resting-state functional MRI (rs-fMRI) and diffusion-weighted imaging (DWI). From T1w sequences, the learning embeddings for atlas propagation (LEAP) framework was used to compute gray matter (GM) volumes in the hippocampus, normalized by the total intracranial volume (TIV). White matter hyperintensities (WMHs) were computed using Bayesian model selection (BaMoS) on FLAIR sequences. Periventricular and deep WMH volumes were obtained globally and for the frontal, parietal, temporal, and occipital lobes, and corrected for TIV to account for interindividual differences in total brain size. For rs-fMRI sequences, a dual regression approach was used to compute resting-state network FC within three subsystems of the default mode network (DMN), including a medial, dorsal, and ventral component, according to a previous study. For DWI sequences, a tract-based spatial statistics (TBSS) approach was used to obtain regional values of fractional anisotropy (FA) and mean diffusivity (MD) in 10 WM tracts that have been shown previously to relate to Alzheimer’s pathology. Examined WM tracts included commissural (genu, body, and splenium of corpus callosum), limbic (cingulum and fornix), associative (superior and inferior longitudinal fasciculus and superior fronto-occipital fasciculus), and projection (corona radiata and internal capsule) fibers.

### 2.7 Radiological assessment

MRI radiological reads were centrally performed for all EPAD participants, following the STandards for Reporting Vascular changes on nEuroimaging (STRIVE) criteria to evaluate cSVD burden. Enlarged perivascular spaces (PVSs) in the basal ganglia (PVS-BG) and centrum semiovale (PVS-CS) were rated separately using a 0–4 interval scale on the 2D T2w images. Visual rating of deep and periventricular WMH volumes (DWMH and PVH, respectively) was performed using the 0–3 Fazekas scale on the FLAIR images. Cortical microbleeds (CMBs) were classified as ≥2 or <2. A more detailed description of the used scales can be found in the supplementary materials.

### 2.8 Statistical analyses

Data distributions were normalized before statistical analysis to meet linear model assumptions. Normalization steps are described in the supplementary materials. All the statistical models described below were corrected for age, sex, and population substructure (using the first five principal components computed on the genomic data). Models with the GRS of APOE and the pathway-GRSs as predictors were further corrected for APOE ε4 allele carriossry to study independent effects. Participants in the A−T+ group were only included in the analysis of GRS differences across AT stages and otherwise excluded as considered suspected non-Alzheimer’s pathology (SNAP).

#### 2.8.1 Association of GRSs with core AD features

First, we looked at the relationship between GRS and CSF biomarkers of AD. We used separate linear models to evaluate the association of global and pathway-GRSs with CSF Aβ1-42 and p-tau181 levels. Multinomial regression was used to study the association of global and pathway-GRSs with AT groups. In addition to the aforementioned corrections, models predicting CSF p-tau181 were further corrected for Aβ1-42. p-Values were corrected for multiple comparisons (Benjamini–Hochberg false discovery rate [FDR]).

#### 2.8.2 Association of GRSs with radiological imaging markers

The association of global and pathway-GRSs with radiological imaging markers, including radiological evaluation of the Fazekas score (PVH and DWMH; n = 1595), enlarged PVSs (BG and CS; n = 1595), and microbleeds (n = 1595) was investigated using multinomial and logistic (for microbleeds) regression models. The models were further adjusted for AT status.

#### 2.8.3 Association of GRSs with quantitative imaging markers

Quantitative imaging markers included hippocampal GM volumes (TIV normalized; n = 1568), global and lobar WMH volumes (10 regions; n = 1334), WM integrity (FA and MD) measures in the 10 selected tracts (n = 790), and FC within the three DMN subsystems (n = 776). Separate linear regression models were used to study the effect of global and pathway-GRSs on these variables. Besides the aforementioned corrections, models were adjusted for AT status and MRI scanner type. P-Values were corrected for multiple comparisons (Benjamini–Hochberg false discovery rate).

#### 2.8.4 Sensitivity analyses

Sensitivity analyses were performed to investigate the association between pathway-GRSs, the relationship of global and pathway-GRSs with age and sex, and the association of genetic scores with CSF biomarkers stratified by AT status.

### 3 RESULTS

#### 3.1 Participants

Baseline demographics and clinical characteristics are shown in Table 1. In total, 1738 participants were included in the study. Based on CSF Aβ1-42 and p-tau181 levels, 58.5% (n = 1016) were defined as A−T−, 25.1% (n = 436) as A+T−, 9.2% (n = 160) as A+T+ and 7.2% (n = 126) as A−T+. The 126 participants with SNAP, that is, A−T+, were used
only in the analysis comparing AT groups, and excluded from subsequent analyses that focused on AD-related processes, resulting in a final sample of 1612 individuals. Imaging-derived phenotype distributions and data availability are reported in Table S2 and Figures S1 and S2.

3.2 Pathways in Alzheimer’s disease genetic risk

Global GRSs for AD was built using 85 SNPs that were identified previously. We assigned two global GRSs to each participant, one including the weighted effect of all the 85 SNPs (GRS_APOE), and a second one excluding the effect of the two APOE SNPs (rs429358; GRS_noAPOE). The variant-pathway mapping yielded six significant clusters (Figure 1), referred to as (1) immune activation (no. of SNPs = 47), (2) signal transduction (no. of SNPs = 48), (3) inflammation (no. of SNPs = 50), (4) migration (cholesterol and lipid related, no. of SNPs = 70; Figure 1). Individual pathway-GRSs were derived for each of the identified clusters. The correlation between scores in different SNPs and the AT group classification using linear models. All models’ coefficients are illustrated in Figure 2 and reported in Table S5. Association of pathway-GRSs with age and sex is illustrated in Figure S4 and S5.

3.3 Genetic risk and pathways determine AD CSF biomarkers

First, we assessed the influence of the GRSs on the CSF measures and the AT group classification using linear models. All models' coefficients are illustrated in Figure 2 and reported in Table S5. Association of pathway-GRSs with age and sex is illustrated in Figure S4 and S5. Higher GRS_APOE was significantly related to decreased CSF Aβ1-42 (β = −0.48; FDR adjusted p < 0.001) and increased CSF p-tau181 (β = 0.36; FDR adjusted p < 0.001). GRS_noAPOE showed a reduced, but still significant, association with decreased Aβ1-42 levels (β = −0.07; FDR adjusted p < 0.001), and with higher levels of p-tau181 (β = 0.11; FDR adjusted p < 0.001). All pathway-GRSs were associated with CSF Aβ1-42 (all FDR adjusted p < 0.05), except for the migration pathway that showed a trend-level association only (FDR adjusted p = 0.08). All pathway-GRSs were also significantly associated with CSF p-tau181, even when correcting for CSF Aβ1-42 (all FDR adjusted p < 0.05), except for the inflammation pathway that showed a trend-level association (FDR adjusted p = 0.08). When stratifying this analysis per AT group (Figure S6), we observed a stage-independent association of GRS_APOE with CSF Aβ1-42, while most pathways were more strongly associated with CSF p-tau181 in A+T− participants.

We then compared the global and pathway-GRSs between the AT groups using multinomial logistic regressions. Compared to the reference group (A−T−), all AT groups showed higher GRS_APOE values (all p < 0.001). Moreover, higher GRS_noAPOE values were observed in the A−T+ (odds ratio [OR] = 1.28; confidence interval [CI] = 1.04–1.58; p = 0.016) and in the A+T+ group (OR = 1.45; CI = 1.20–1.77; p < 0.001). Regarding the pathway-GRSs, the A−T+ group had significantly higher scores in the immune activation (OR = 1.29; CI = 1.05–1.58; p = 0.015), signal transduction (OR = 1.42; CI = 1.17–1.74; p = 0.001), and inflammatorry (OR = 1.32; CI = 1.09–1.61; p = 0.014) pathway-GRSs compared to A−T−. Furthermore, the A+T+ group had significantly higher clearance pathway scores (OR = 1.12; CI = 0.99–1.26; p = 0.041), whereas the A+T− group showed significantly higher pathway-GRSs for the migration (OR = 1.26; CI = 1.04–1.53; p = 0.007), amyloid (OR = 1.45; CI = 1.19–1.76; p < 0.001), clearance (OR = 1.35; CI = 1.11–1.63; p < 0.001), and signal transduction scores (OR = 1.38; CI = 1.13–1.67; p = 0.004) compared to A−T−.
3.4 | Pathways of inflammation determine cSVD radiological indices

We then investigated whether global and pathway-GRSs were related to radiological indices of cSVD, independently of AT stage. The cSVD indices included Fazekas deep (DWMH) and periventricular (PVH) enlarged perivascular spaces in basal ganglia (PVS-BG) and centrum semiovale (PVS-CS), and CMBs (supplementary materials). All model coefficients are reported in Tables S6 and S7, and illustrated in Figure 3.

Briefly, participants with a Fazekas DWMH score of 2 had higher scores in GRS_{noAPOE} (OR = 1.24; CI = 1.03–1.47; \( p = 0.02 \)) and in pathway-GRSs of signal transduction (OR = 1.26; CI = 1.05–1.50; \( p = 0.01 \)), inflammation (OR = 1.29; CI = 1.08–1.53; \( p < 0.01 \)), and amyloid (OR = 1.24; CI = 1.04–1.49; \( p < 0.01 \)), compared to Fazekas DWMH = 0. A score of 1 and 2 of PVS-BG (compared to 0) was related to higher pathway-GRSs of immune activation (PVS-BG1: OR = 1.36; CI = 1.04–1.79; \( p = 0.02 \); PVS-BG2: OR = 1.40; CI = 1.02–1.92; \( p = 0.04 \)); and a score of 3 of PVS-CS (compared to 0) was related to higher GRS_{APOE} (OR = 1.58; CI = 1.09–2.31; \( p = 0.02 \)). Finally, CMBs (>2) were significantly higher in GRS_{noAPOE} (OR = 1.54; CI = 1.02–2.32; \( p = 0.04 \)) and in pathway-GRSs of immune activation (OR = 1.32; CI = 1.03–1.70; \( p = 0.02 \)) and signal transduction (OR = 1.31; CI = 1.02–1.68; \( p = 0.04 \)). GRS_{APOE} (OR = 1.94; CI = 1.38–2.72; \( p = 0.07 \)) and pathway-GRSs inflammation (OR = 1.28; CI = 1.01–2.62; \( p = 0.07 \)) showed a statistical trend in these groups.

3.5 | Distinct genetic pathways regulate quantitative imaging biomarkers

Next, we assessed whether global and pathway-GRSs determine alterations in quantitative MRI-derived phenotypes, independently of AT stage. Model coefficients of T1w and FLAIR MRI-derived phenotypes are illustrated in Figure 4 and Table S7. Lower hippocampal volumes showed a mild association with higher pathway-GRSs of migration and clearance, which did not survive multiple testing corrections. For WMH volumes, higher clearance pathway-GRSs were associated with higher WMH volumes in most regions. The effect was most pronounced in global, frontal, and temporal periventricular and parietal deep white matter. The association of higher GRS_{noAPOE} with higher burden of WMHs in temporal (periventricular and deep) and parietal (deep) WMHs did not survive FDR correction.

Model coefficients of rs-fMRI and DWI-derived phenotypes are illustrated in Figures 5 and 6, respectively. Lower FC within the ventral DMN was associated with higher scores in the pathway-GRSs of signal transduction. The association of ventral DMN FC with the inflammatory pathway-GRSs did not survive multiple testing corrections.

Higher GRS_{noAPOE} was associated with higher FA in the genu and lower MD in the splenium of the corpus callosum. Moreover, FA and MD were distinctively related to the migration pathway-GRSs. Specifically, increases in FA in all commissural regions of interest (ROIs; genu, body, and splenium of corpus callosum) and in the corona radiata, and decreases of MD in the cingulum, genu, and splenium of corpus callosum associated significantly with higher migration pathway-GRSs. Lower MD in the splenium of the corpus callosum also exhibited a significant association with higher scores in the immune activation and inflammation pathway-GRSs.

4 | DISCUSSION

We identified and quantified global and pathway-GRSs from genetic data in a large cohort of non-demented individuals and assessed their association with AD biomarkers. Our findings confirm the involvement of several biological pathways beyond APOE within the genetic risk of AD and demonstrate their influence on fluid and imaging biomarkers. APOE-dependent genetic risk of AD is mostly related to core AD CSF biomarkers. Beyond APOE, pathways of inflammation...
FIGURE 3  Association of global and pathway-specific genetic risk scores (pathway-GRSs) with radiological visual scores of cerebral small vessel disease (cSVD). Boxplots represent the association of GRS (with and without apolipoprotein E) and the six pathway-GRSs with Fazekas deep white matter hyperintensities (DWMHs; upper-row), microbleeds (middle-row), perivascular spaces (PVSs) in the basal ganglia (lower-row). APOE, apolipoprotein E.
and immune activation are specifically related to vascular imaging markers, whereas WM integrity and functional connectivity measures are mostly determined by membrane-related and signal transduction pathways, respectively.

The pathway analysis used in this work for the quantification of pathway-GRSs identified six biological pathways that are known to occur in the pathogenesis of AD from other previous studies. These pathways could be grouped into two high-level clusters (Figure 1). The first cluster, comprising immune activation, signal transduction, and inflammation pathways, mostly represents processes linked to neuroinflammatory and chronic immune activation states. This confirms previous studies that reported the contribution of inflammation-related genetic variants to the development of AD, with a particular interest in the genes regulating microglial function, such as TREM2 and PLCG2, suggesting that these pathways may constitute major non-APOE-dependent polygenic vulnerability to AD. The second cluster, comprising the migration (related to membrane integrity and lipids), amyloid, and clearance pathways, could be representative of more AD-specific processes. Genes mostly expressed in these pathways, such as APP, BIN1, and SORL1, regulate processes linked to Aβ production, metabolism, and endocytosis. The results of our pathway enrichment analysis provide genetic evidence of the two major pathological components in AD, namely, inflammatory and amyloid-related processes. These results are particularly interesting in light of recent clinical trials effort, which mostly comprise Aβ-targeting drugs but also see an increase of anti-inflammatory agents.

We found that global GRSs, irrespective of APOE genstatus, and most pathway-GRSs were related to CSF Aβ1-42 burden. Whereas the early influence of the APOE ε4 allele and AD GRSs on amyloid burden is known, little evidence on the APOE-independent genetic influence exists. Pathways of clearance and cholesterol have previously been shown to relate to CSF Aβ1-42 in individuals genetically enriched for AD. Endocytosis and immune response pathways have in turn often been associated with clinical and cognitive status, and with resilience to AD. We showed that all GRSs and pathway-GRSs were significantly associated with CSF p-tau181 levels, independently of CSF Aβ1-42. Furthermore, pathway-GRSs of inflammation were specifically higher in the SNAP group, that is, the A−T+ participants, having only high CSF p-tau181 levels and not CSF Aβ1-42. Recent studies have demonstrated that AD GRSs excluding APOE are associated with higher CSF p-tau181. A combined tau and amyloid PET study showed that the spread of tau pathology was regulated by "axon-related" genes, whereas the spread of amyloid was linked to "dendrite-related" genes. Furthermore, "lipid metabolism-related" genes were driving the spread of both pathologies. Human and animal studies have reported evidence of inflammation being present in both primary and secondary tauopathies. A state of chronic neuroinflammation and immune activation might not only be a reaction to neuronal death and...
misfolded proteins, but also a driver in neurodegenerative diseases. In light of these previous studies, our findings suggest that amyloid and tau deposition might be driven by alterations in several biological processes, through correlated but independent pathways, and further demonstrate that AD risk genes regulating these processes might act in parallel and upstream of both amyloid and tau. Results of our sensitivity analysis (Figure S6), further suggest that genetic pathways might have a stage-dependent influence on AD CSF biomarkers, with APOE driving initial amyloid deposition and non-APOE pathways regulating downstream processes such as tau deposition. Future longitudinal studies should better investigate temporal dynamics of genetic vulnerability.

Concomitant cSVD pathology is observed in 60%–80% of patients with AD. We showed the involvement of AD-related inflammatory pathways, namely, immune activation, signal transduction, and inflammation, in promoting brain vascular damage, providing evidence of a genetic overlap between cSVD and AD, and suggesting an intrinsic relationship between the two. Animal studies have demonstrated that genes coding for pro-inflammatory cytokine production led to endothelium dysfunction and damage to the brain vasculature. The vulnerability of the blood–brain barrier (BBB) to the effects of chronic immune activation and inflammation results in alterations of the neurovascular unit with advancing age. This observation suggests that innate inflammatory processes might foster AD pathology by promoting vascular damage and BBB disruption, from the early stages of the disease. Of note, these associations were stronger for intermediate radiological scores. This could be due to the limited number of participants with significant cerebrovascular burden. However, specific inflammation-related pathways may play a role in the initial onset of cSVD, whereas a combination of various altered biological processes could be at play in later stages.

Using quantitative MRI markers, we found that specific imaging biomarkers might be influenced by distinct genetic pathways. Recent research has linked several pathway-GRSs with cortical thinning and in several brain regions, including the hippocampus. We found that lower volumes in the hippocampus were mildly associated with higher scores in pathway-GRSs of migration and clearance. In addition, WM lesion volumes—reflecting demyelination and axonal loss—commonly considered a result of heterogeneous causes, primarily cSVD—were specifically determined by the clearance pathway-GRSs. The glycophatic system plays a central role in maintaining WM integrity by preserving the flow of interstitial fluid and exchanging metabolic waste. In previous works, the CLU gene, associated with the clearance of cellular debris and apoptosis, and the PICALM gene, involved in clathrin-mediated endocytosis, were associated with WMHs.
Cholesterol dysmetabolism is thought to metabolism can regulate WM integrity, as measured on DWI, by regulating the migration GRSs, linked to cholesterol and lipid dysfunctions. Previous work has shown that levels of local cholesterol and lipid imaging—reflecting WM microstructural integrity—were mostly determined by the continuum,64,65 and have been proposed to be a driving event in the pre-dementia stages of AD.

The signal transduction pathway-GRS, involving synaptic function and intracellular communication, was related to rs-fMRI as reduced FC in the dorsal portion of the DMN. Among the genes mostly contributing to this pathway, SORL1, BIN1, and CD2AP are functionally expressed in pre- and post-synaptic compartments and promote synaptic formation, transmission, and plasticity. Alterations of synaptic function (and functional connectivity on fMRI) are observable early in the AD disease course.32 In this framework, large-scale reconfiguration of functional networks in aging brains would influence biological processes linked to amyloid production.64 We, therefore, showed that a specific cluster of variants could promote AD pathology by acting on functional brain alterations and neuronal activity.

Some limitations should be noted. First, the computation of GRSs is based on a reference GWAS that used “Alzheimer’s disease and related dementias” as a phenotype. However, this method was shown to be sensitive and effective in increasing the number of included participants in the GWAS, thereby increasing the sensitivity (more loci) and precision of the obtained estimates. Second, the method used to identify pathways-GRSs did not constrain genes’ contribution to only one cluster (one gene could contribute to multiple pathways). As such, some pathway-GRSs were more related to each other (supplementary materials). Other methods exist for computation of pathway-GRSs, often assigning genes to a priori selected sets of pathways.59,67 However, single genes can contribute to multiple biological processes. Moreover, the observation that GRSs had different profiles of associations with outcome biomarkers advocates for distinct underlying processes. Future studies should assess the independent contribution of pathway-GRSs to imaging phenotypes. Moreover, future works should also investigate the cell-type expression profiles of at-risk genes.68,69 However, some GRSs could contribute to multiple biological processes. Finally, we only considered one gene per SNP, as reported in the original GWAS. Although SNPXplorer is robust in pathway identification over a wide range of neuropathological features in non-demented individuals that can be tracked in vivo through neuroimaging techniques, and that distinct AD biomarkers are preferentially associ-
ated with specific genetic profiles. Our findings are a step forward in the understanding of the biological alterations that determine brain functional and structural dysregulation in the early stages of the AD continuum. Moreover, these results provide genetic evidence of the biological pathways promoting disease heterogeneity and offer novel insights into the use of individual risk profiles for patient selection in clinical trials and personalized interventions, encompassing a combination of strategies targeting modifiable risk factors, alongside non-amyloid-targeting drugs.

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**CONFLICT OF INTEREST STATEMENT**

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CONSENT STATEMENT

All EPAD participants provided written informed consent.

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SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.