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

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.
KEYWORDS
biological pathways, magnetic resonance imaging, polygenic risk, preclinical Alzheimer’s disease

Highlights
• Polygenic risk for Alzheimer’s disease encompasses six biological pathways that can be quantified with pathway-specific genetic risk scores, and differentially relate to cerebrospinal fluid and imaging biomarkers.
• Inflammatory pathways are mostly related to cerebrovascular burden.
• White matter health is associated with pathways of clearance and membrane integrity, whereas functional connectivity measures are related to signal transduction and synaptic communication pathways.

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/ε4. 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 GWASs. 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 Aβ1-42 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
score <1). Exclusion criteria were the presence of conditions associated with neurodegeneration or affecting cognition, contraindication to MRI or lumbar puncture, and cancer or history of cancer in the preceding 5 years. A total of 1835 participants were included in the EPAD study. Demographic, cognitive, neuroimaging, fluid biomarker, and genetic outcome data were collected. For this work, we excluded participants with unavailable or low-quality (see below) genetic data, resulting in a final sample of n = 1738.

### 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 ($p < 1 \times 10^{-10}$) 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 GRSs 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:

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

where $K$ represents the full set of genetic variants, dosage$_k$ 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, GRSs were computed both with and without the two alleles (rs7412 and rs429358) from the APOE gene (denoted as GRS$_{APOE}$ and GRS$_{noAPOE}$, respectively).

### 2.4 | Pathway-GRS

In order to construct pathway-specific GRSs, 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.
FIGURE 1  Results of the pathway analysis. The upper part of the figure shows the results of the clustering performed on identified pathways relating to the selected set of genes. The most frequent words from the pathways description within each cluster of pathways are visualized using word clouds. The lower part of the figure shows the contribution of each gene to each identified cluster, expressed as a log of odds ratios.

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 distance25 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:

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

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, Mön ndal, Sweden.20 Concentrations of \(A\beta_{1-42}\) were determined using the manufacturer’s guidelines. Following a previous study on the same cohort,26 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 in27 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 comissural (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 (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_{noAPOE} and the pathway-GRSs as predictors were further corrected for APOE ε4 allele carrier status 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 Aβ_{1-42} and p-tau_{181} 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-tau_{181} were further corrected for Aβ_{1-42}. 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 Aβ_{1-42} and p-tau_{181} 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 sub-
sequent analyses that focused on AD-related processes, resulting in a
final sample of 1612 individuals. Imaging-derived phenotype dis-
tributions 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.1 We assigned two global GRSs to each participant, one
including the weighted effect of all the 85 SNPs (GRS\textsubscript{APOE}), and a
second one excluding the effect of the two APOE SNPs (rs429358 and
rs429358; GRS\textsubscript{noAPOE}). The variant-pathway mapping yielded six sig-
ificant clusters (Figure 1), referred to as (1) immune activation (no.
of SNPs = 27), (2) signal transduction (no. of SNPs = 48), (3) inflam-
ma
tion (no. of SNPs = 52), (4) migration (cholesterol and lipid related, no.
of SNPs = 47), (5) amyloid (no. of SNPs = 50), and (6) clearance (no. of
SNPs = 70; Figure 1). Individual pathway-GRSs were derived for each
of the identified clusters. The correlation between scores in differ-
ent pathway-GRSs with age and sex is 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

Higher GRS\textsubscript{APOE} was significantly related to decreased CSF A\beta\textsubscript{1-42}
($\beta = -0.48$; FDR adjusted $p < 0.001$) and increased CSF p-tau\textsubscript{181}
($\beta = 0.36$; FDR adjusted $p < 0.001$). GRS\textsubscript{noAPOE} showed a reduced, but
still significant, association with decreased A\beta\textsubscript{1-42} levels ($\beta = -0.07$;
FDR adjusted $p < 0.001$), and with higher levels of p-tau\textsubscript{181} ($\beta = 0.11$;
FDR adjusted $p < 0.001$). All pathway-GRSs were associated with CSF
A\beta\textsubscript{1-42} (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-tau\textsubscript{181},
even when correcting for CSF A\beta\textsubscript{1-42} (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\textsubscript{APOE}
with CSF A\beta\textsubscript{1-42}, while most pathways were more strongly associated with
CSF p-tau\textsubscript{181} in A+T– participants.

We then compared the global and pathway-GRSs between the AT
groups using multinomial logistic regressions. Compared to the ref-
ter group (A–T–), all AT groups showed higher GRS\textsubscript{APOE} values
(all $p < 0.001$). Moreover, higher GRS\textsubscript{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 signifi-
cantly 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 inflammatory (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–.

**TABLE 1** Cohort characteristics.

<table>
<thead>
<tr>
<th>Overall</th>
<th>A–T–</th>
<th>A–T+</th>
<th>A+T–</th>
<th>A+T+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean ± SD</td>
<td>65.72 ± 7.31</td>
<td>64.58 ± 7.05</td>
<td>69.54 ± 6.56</td>
<td>65.75 ± 7.41</td>
</tr>
<tr>
<td>Sex, male, N (%)</td>
<td>767 (44.1)</td>
<td>425 (41.8)</td>
<td>55 (43.7)</td>
<td>208 (47.7)</td>
</tr>
<tr>
<td>MMSE, mean ± SD</td>
<td>28.41 ± 1.87</td>
<td>28.74 ± 1.47</td>
<td>28.27 ± 1.71</td>
<td>28.38 ± 1.85</td>
</tr>
<tr>
<td>CDR = 0.5, N (%)</td>
<td>474 (27.4)</td>
<td>185 (18.3)</td>
<td>49 (38.9)</td>
<td>131 (30.0)</td>
</tr>
<tr>
<td>GRS\textsubscript{APOE}, mean ± SD</td>
<td>0.22 ± 0.72</td>
<td>0.04 ± 0.63</td>
<td>0.30 ± 0.74</td>
<td>0.38 ± 0.75</td>
</tr>
<tr>
<td>GRS\textsubscript{noAPOE}, mean ± SD</td>
<td>−0.14 ± 0.36</td>
<td>−0.16 ± 0.35</td>
<td>−0.06 ± 0.37</td>
<td>−0.14 ± 0.37</td>
</tr>
<tr>
<td>A\beta\textsubscript{1-42}, mean ± SD</td>
<td>1378.99 ± 720.93</td>
<td>1672.06 ± 496.43</td>
<td>2181.13 ± 1320.89</td>
<td>724.48 ± 186.27</td>
</tr>
<tr>
<td>p-tau\textsubscript{181}, mean ± SD</td>
<td>19.76 ± 10.61</td>
<td>16.44 ± 4.02</td>
<td>35.09 ± 10.10</td>
<td>15.49 ± 5.72</td>
</tr>
<tr>
<td>t-tau, mean ± SD</td>
<td>226.85 ± 99.33</td>
<td>198.27 ± 45.87</td>
<td>392.01 ± 101.13</td>
<td>181.01 ± 58.60</td>
</tr>
</tbody>
</table>

Abbreviations: A\beta, amyloid beta; CDR, Clinical Dementia Rating (scale); MMSE, Mini-Mental State Examination; N, number; GRS, Genetic risk score;
p-tau, phosphorylated tau; SD, standard deviation.9

First, we assessed the influence of the GRSs on the CSF measures
and the AT group classification using linear models. All models’ coef-
ficients 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.

**TABLE S1** Terms and relative assigned clusters are reported in Table S3. The pathway-GRSs is illustrated in Figure S3. Mapped Gene Ontology
of the identified clusters. The correlation between scores in differ-
ent SNPs = 27), (2) signal transduction (no. of SNPs = 48), (3) inflamma-
tion (no. of SNPs = 52), (4) migration (cholesterol and lipid related, no.
of SNPs = 47), (5) amyloid (no. of SNPs = 50), and (6) clearance (no. of
SNPs = 70; Figure 1). Individual pathway-GRSs were derived for each
of the identified clusters. The correlation between scores in different
pathway-GRSs is illustrated in Figure S3. Mapped Gene Ontology
terms and relative assigned clusters are reported in Table S3. The per-
centages of contribution of each SNP to each pathway are reported in Table S4.

**TABLE S2** Cohort characteristics.
DISCUSSION

Pathways of inflammation determine cSVD

Distinct genetic pathways regulate quantitative imaging biomarkers

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 showed a mild association with higher pathway-GRs of migration and clearance, which did not survive multiple testing corrections. For WMH volumes, higher clearance pathway-GRs 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_APOE 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-GRs of signal transduction. The association of ventral DMN FC with the inflammatory pathway-GRs did not survive multiple testing corrections.

Higher GRS_APOE 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-GRs. 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-GRs. Lower MD in the splenium of the corpus callosum also exhibited a significant association with higher scores in the immune activation and inflammation pathway-GRs.

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.1,37–39 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.40 This confirms previous studies that reported the contribution of inflammation-related genetic variants to the development of AD,41 with a particular interest in the genes regulating microglial function, such as TREM2 and PLCG2,42 suggesting that these pathways may constitute major non–Aβ-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.43

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,44–48 little evidence on the APOE-independent genetic influence exists.49 Pathways of clearance and cholesterol have previously been shown to relate to CSF Aβ1-42 in individuals genetically enriched for AD.11 Endocytosis and immune response pathways have in turn often been associated with clinical and cognitive status,18 and with resilience to AD.39 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-tau181.16,50–52 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.53 Furthermore, “lipid metabolism-related” genes were driving the spread of both pathologies.53 Human and animal studies have reported evidence of inflammation being present in both primary and secondary tauopathies.54,55 A state of chronic neuroinflammation and immune activation might not only be a reaction to neural death and
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—were specifically determined by the clearance pathway-GRSs. The glymphatic 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.
Higher cholesterol and lipid levels were shown to be associated with WM microstructural changes, as measured on diffusion-weighted imaging (DWI). These changes can be linked to the migration of genetic risk score (GRS) variants, linked to cholesterol and lipid dysfunctions. Previous work has indicated that levels of local cholesterol and lipid metabolism can regulate WM integrity, as measured on DWI, by regulating WM myelination.

Cholesterol dysmetabolism is thought to interact with WM demyelination to promote and worsen initial amyloid pathology. Our results provide genetic evidence for the role of early cholesterol and lipid dysmetabolism and WM injury 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 continuum, and have been proposed to be a driving event in the disease course. In this framework, large-scale reconfiguration of functional networks in aging brains would influence biological processes linked to amyloid production. 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. 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.

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 nondemented 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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REFERENCES


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