



Featured Article

Disentangling the biological pathways involved in early features of Alzheimer's disease in the Rotterdam Study

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Abstract

Introduction: Exploring the role of Alzheimer's disease (AD) implicated pathways in the pre-dementia phase may provide new insight for preventive and clinical trials targeting disease specific pathways.

Methods: We constructed weighted Genetic risk scores, first based on 20 genome-wide significant AD risk variants and second clustering these variants within pathways. Risk scores were investigated for their association with AD, mild cognitive impairment, and brain magnetic resonance imaging phenotypes including white matter lesions, hippocampal volume, and brain volume.

Results: The risk score capturing *endocytosis* pathway was significantly associated with mild cognitive impairment ($P = 1.44 \times 10^{-4}$). *Immune response* ($P = .016$) and *clathrin/AP2 adaptor complex* pathway ($P = 3.55 \times 10^{-3}$) excluding apolipoprotein E also showed modest association with white matter lesions but did not sustain Bonferroni correction ($P = 9.09 \times 10^{-4}$).

Discussion: Our study suggests that the clinical spectrum of early AD pathology is explained by different biological pathways, in particular, the *endocytosis*, *clathrin/AP2 adaptor complex*, and *immune response* pathways, that are independent of apolipoprotein E (*APOE*).

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Keywords:

Genetic risk score; Alzheimer's disease; White matter lesions; Mild cognitive impairment; Endocytosis; Immune response

1. Introduction

Alzheimer's disease (AD) is a heterogeneous and genetically complex disease with high heritability (56–79%) [1]. It has been known since the end of the

previous century that a polymorphism in the apolipoprotein E (*APOE*) gene is the strongest common genetic risk factor [2–4]. This finding fueled speculations on the role of the *lipid metabolism* and *cholesterol transport* pathway in AD in addition to the *amyloid cascade* and *tau phosphorylation* mechanism [5,6]. Furthermore, large-scale genome-wide association studies have discovered more than 20 novel common genetic variants that influence the risk of late-onset AD [7–13]. These

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common genetic variants have been mapped to eight biological pathways including *immune response*, *endocytosis*, *cholesterol transport*, *hematopoietic cell lineage*, *protein ubiquitination*, *hemostasis*, *clathrin/AP2 adaptor complex*, and *protein folding*, each having a distinct biological function [14–16]. These eight pathways are not independent in a way that genes may be part of more than one biological pathway. For instance, *APOE* is part of four of the eight pathways namely *cholesterol transport*, *hematopoietic cell lineage*, *clathrin/AP2 adaptor complex*, and *protein folding* pathways; clusterin (*CLU*) encoding for apolipoprotein J is involved in six pathways; phosphatidylinositol binding clathrin assembly protein (*PICALM*) and complement factor 1 (*CRI*) are involved in two pathways [14–16].

These diverse biological pathways may be responsible for the clinically heterogeneous manifestation of AD [17–19], which include endophenotypes such as changes in structural and functional magnetic resonance imaging (MRI) phenotypes, most notably hippocampal volume, total brain volume, and white matter lesions [20–23]. Furthermore, these biological pathways may also modulate the prodromal stages of AD such as mild cognitive impairment (MCI) [24–26]. Owing to heterogeneity during the predementia phase, one important unanswered question is whether the different biological pathways that are implicated in AD relate to the pleiotropy of clinical endophenotypes. We hypothesized that some biological pathways are involved in distinct clinical endophenotypes, whereas others may be involved in multiple or even all. Disentangling the connection of biological pathways to various aspects of AD-related early pathology may be a crucial step toward improving our understanding of the pathogenesis of AD during the predementia stage and a first step toward a more informative and powerful readout for preventive and therapeutic trials targeting specific pathways.

The present study aims to capture the different biological pathways involved in AD using genetic risk scores to evaluate their role in AD and predementia endophenotypes including MCI, white matter lesions, and total brain and hippocampal volume.

2. Methods

2.1. Study population

This study included samples from the Rotterdam Study (RS). The RS is a prospective population-based study [27] designed to investigate the etiology of age-related disorders. At the baseline examination in 1990 to 1993, study recruited 7983 subjects ≥ 55 years of age from the Ommoord district of Rotterdam (RS-I). At the baseline entry and after every 3 to 4 years, all the study participants were extensively interviewed and physically examined at the dedicated research center. During 2000 to 2001, the baseline cohort (RS-I) was expanded by adding 3011 subjects ≥ 55 years

of age, who were not yet part of RS-I (RS-II). Second expansion of RS was performed by recruiting 3932 persons having ≥ 45 years of age during 2006 to 2008 (RS-III). The study has been approved by the Medical Ethical Committee of Erasmus Medical Center and by the Ministry of Health, Welfare and Sport of the Netherlands. Written informed consents were also obtained from each study participant to participate and to collect information from their treating physicians. Details of AD, dementia, and MCI diagnosis are provided in the [Supplementary Information](#). In the present study for AD cross-sectional analysis, we included in total 1270 late-onset AD cases and 7623 controls (age at last follow-up ≥ 65 years and dementia free) whose follow-up information is complete until 2009 to 2013 in RS-I, RS-II, and RS-III cohorts. This AD sample includes 1057 incident and 213 prevalent AD cases. For prospective AD analysis, 10,370 dementia-free (normal) participants were also included in the study from all three RS cohorts at their baseline and were subsequently followed until 2009 to 2013, to analyze their progression into AD (average 11 years of follow-up). In the MCI data set, we included 360 MCI cases and 3245 cognitively normal controls from the first extensive cognitive assessment conducted between 2002 and 2005 in RS-I and RS-II cohorts. MRI was implemented in 2005 in RS cohorts, and 5899 persons came for MRI scanning until 2015. After excluding subjects with stroke and/or dementia ($n = 251$) at time of scanning, poor imaging quality ($n = 313$), and missing genotyping information ($n = 814$), we retained 4521 cognitively normal individuals in the MRI sample (Table 1).

2.2. Genotyping

Blood was drawn for genotyping from participants of RS cohorts during their first visit, and DNA genotyping was performed at the internal genotyping facility of the Erasmus Medical Center, Rotterdam. All samples were genotyped with the 550K, 550K duo, or 610K Illumina arrays. Genotyping quality control criteria include call rate $< 95\%$, Hardy-Weinberg equilibrium $P < 1.0 \times 10^{-6}$, and minor allele frequency $< 1\%$. Moreover, study samples with excess autosomal heterozygosity, call rate $< 97.5\%$, ethnic outliers, and duplicate or family relationships were excluded during quality control analysis. Genetic variants were imputed from the Haplotype Reference Consortium reference panel (version 1.0) [28], using the Michigan imputation server [29]. The server uses SHAPEIT2 (v2.r790) [30] to phase the genotype data and performs imputation with Minimac 3 software [31]. For this study, we used genetic variants that had imputation quality (R^2) > 0.5 .

2.3. MRI scanning

2.3.1. Image acquisition

MRI scanning is assessed on a 1.5-T MRI unit with a dedicated eight-channel head coil (Signa HD platform; GE

Table 1
Cohort characteristics

Characteristics	RS-I	RS-II	RS-III	Total
AD data set (N)	5854	2062	977	8893
Late-onset AD	1118	134	18	1270
AD free controls	4736	1928	959	7623
Age-of-onset (SD), years	84.58 (6.8)	82.75 (6.7)	78.54 (9.5)	84.30 (6.8)
Age of controls (SD), years	82.87 (6.9)	76.52 (6.4)	69.15 (5.7)	79.53 (8.2)
Female (%)	3526 (60)	1133 (55)	569 (58)	5228 (59)
MCI data set (N)	2178	1427		3605
MCI cases	235	125	-	360
Controls	1943	1302	-	3245
Age (SD), years	74.79 (5.7)	67.53 (6.9)	-	71.9 (7.2)
Female (%)	1271(58.4)	786 (55.1)	-	2057 (57%)
MRI data set (N)	968	1068	2485	4521
Age (SD), years	78.89 (4.9)	69.34 (5.9)	57.21 (6.4)	64.72 (10.8)
Female (%)	556 (58)	565 (53)	1390 (56)	2511 (56)

Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment; MRI, magnetic resonance imaging; RS, Rotterdam Study (cohort I, II, and III); SD, standard deviation.

Healthcare, Milwaukee, WI) since the induction of a dedicated MRI machine in the RS. The MRI protocol was based on several high-resolution axial sequences, including a T1-weighted (slice thickness 0.8 mm), T2-weighted (1.6 mm), and fluid-attenuated inversion recovery sequence (2.5 mm). A detailed description of the MRI protocol is described previously [32].

2.3.2. Image processing

We excluded 251 persons with stroke and/or dementia from the total 5899 subjects because this may affect image processing. All T1 images were segmented into the supratentorial gray matter, white matter, and cerebrospinal fluid using a *k*-nearest neighbor algorithm [33]. White matter lesions were segmented based on T1 tissue maps and an automatically detected threshold for the intensity of fluid-attenuated inversion recovery scans [34]. The hippocampus was segmented using a fully automated method, as described previously [35]. Semiquantitative MRI postprocessing software was used to measure intracranial volume and brain volume, which included Elastix and custom-built software [36]. To calculate intracranial volume, non-brain tissues (skull, eyes, and dura) were removed by nonlinearly registering all brain scans to a manually created template in which non-brain tissues were masked [33,36,37]. In all MRI scans, after visual inspection of all segmentations, additional 313 subjects were excluded because of poor quality.

2.4. Statistical analysis

2.4.1. Genetic risk score computation

To construct the risk score, we selected late-onset AD-associated single-nucleotide polymorphisms (SNPs) reaching genome-wide significance level ($P < 5.0 \times 10^{-8}$; Supplementary Table 1), including one rare *TREM2* variant [7,38]. In common variants, we considered only variants identified by the International Genomics of Alzheimer's

Project (IGAP) meta-analyses. In addition, we considered *APOE* $\epsilon 4$ (rs429358) variant for risk score construction. From a total of 21 SNPs, the *HLA-DRB1-HLA-DRB5* (rs9271192) variant was excluded from risk score calculation because of its low imputation quality ($R^2 = 0.31$) in the RS. This led to a final selection of 20 independent genome-wide significant AD-associated variants. Weighted genetic risk score was constructed using the effect sizes (log of odds ratio) of the genome-wide significant variants from the IGAP meta-analysis [7] as weights and their respective allele dosages from imputed genotype data of our study cohorts. Risk score was constructed as the sum of the products of SNP dosages and their corresponding weights in R software (<https://www.R-project.org/>). We constructed genetic risk score in two ways: (1) combining all 20 selected variants and (2) clustering the variants into their respective pathways.

2.4.1.1. Combined genetic risk score

Combined genetic risk score (GRS1) was constructed in two ways, that is, (1) using all the 20 selected SNPs and (2) excluding the *APOE* $\epsilon 4$ variant to identify the joint independent effect of all other genome-wide significant SNPs.

2.4.1.2. Pathway-specific genetic risk score

For pathway-specific genetic risk score (GRS2), the genome-wide significant AD SNPs were divided into pathways (*immune response, endocytosis, cholesterol transport, hematopoietic cell lineage, protein ubiquitination, hemostasis, clathrin/AP2 adaptor complex, and protein folding pathway*) identified by Jones et al. [16] (Supplementary Table 2). Classifying genome-wide significant AD SNPs into pathways, we also used information from Guerreiro et al. [14], in which the authors reviewed the possible division of known AD-associated genes into biological pathways [14]. Furthermore, the GeneNetwork database (<http://genenetwork.nl/>) was used to confirm the allocated pathways. Of the 20 SNPs, 14 could be clustered into seven nonmutually exclusive pathways

Table 2
Results of association of AD with risk scores

SNP cluster*	Including <i>APOE</i>			Excluding <i>APOE</i>		
	β	SE	<i>P</i> value	β	SE	<i>P</i> value
GRS1 (combined)	0.73	0.040	6.53×10^{-74}	0.69	0.101	1.12×10^{-11}
Immune response	-	-	-	0.69	0.166	3.20×10^{-5}
Endocytosis	-	-	-	0.75	0.171	1.28×10^{-5}
Cholesterol transport	0.71	0.042	3.22×10^{-64}	0.39	0.219	.077
Hematopoietic cell lineage [†]	0.73	0.042	5.16×10^{-66}	-	-	-
Hemostasis	-	-	-	0.50	0.292	.090
Clathrin/AP2 adaptor complex	0.72	0.042	4.68×10^{-65}	0.50	0.236	.036
Protein folding [†]	0.72	0.042	2.96×10^{-64}	-	-	-

Abbreviations: β , regression coefficient; *APOE*, apolipoprotein E; GRS1, combined genetic risk score; GRS2, pathway-specific genetic risk score; RS, Rotterdam Study; SE, standard error; SNP, single-nucleotide polymorphism.

NOTE. Multiple testing correction by Bonferroni [$0.05/(5 \text{ phenotypes} \times 11 \text{ risk scores})$; $P < 9.09 \times 10^{-4}$] was considered significant.

*Logistic regression model adjusted for age and sex in the RS ($N = 1270$ cases).

[†]Only one SNP available in excluding *APOE* GRS2.

(Supplementary Table 2). Similar to GRS1, we also constructed GRS2 with and without the *APOE* $\epsilon 4$ variant. The *APOE* $\epsilon 4$ variant was grouped under four pathways including *cholesterol transport* [14], *hematopoietic cell lineage*, *clathrin/AP2 adaptor complex*, and *protein folding* [16]. GRS2 was constructed for only those pathways which could be assigned at least two SNPs; therefore, *protein ubiquitination* pathway, which contained only one SNP, was excluded from all analyses, while *hematopoietic cell lineage* and *protein folding* pathways were also not considered in the analyses excluding the *APOE* $\epsilon 4$ variant.

2.4.2. Association analyses of GRS1 and GRS2

To test the association of AD and MCI with the risk scores, we used logistic regression analysis in R software (www.R-project.org), using disease status as the outcome, risk scores as predictor, and age and sex as covariates. To assess the possible inflation of association results between AD and risk scores, we repeated the association analysis excluding 625 AD cases who were part of the IGAP meta-analysis [7] from total 1270 AD cases of the RS cohort. Furthermore, we performed prospective analysis using the Cox-proportional hazards model

($N = 1057$ incident AD cases) in R software using “survival” package [39] and reported results as hazard ratio (HR) per 1–standard deviation increase in risk score and 95% confidence interval. The association of single variants with AD and MCI was assessed using a logistic regression model adjusted for age and sex. Results of association analyses were reported as unstandardized regression coefficient and *P* values.

To test the association of MRI phenotypes including total brain volume, white matter lesions, and hippocampal volume with the risk scores, we used linear regression adjusted for age, sex, and intracranial volume in MRI scans. Single-variant association analysis was also performed for MRI phenotypes. Bonferroni correction [$0.05/(11 \text{ risk scores} \times 5 \text{ phenotypes})$; $P = 9.09 \times 10^{-4}$] was used to correct for multiple testing.

3. Results

3.1. Association of the GRS1 with AD, MCI, and MRI endophenotypes

The risk score containing all SNPs, that is, GRS1 both including *APOE* $\epsilon 4$ (effect = 0.73, $P = 6.53 \times 10^{-74}$)

Table 3
Results of association of MCI with risk scores

SNP cluster*	Including <i>APOE</i>			Excluding <i>APOE</i>		
	β	SE	<i>P</i> value	β	SE	<i>P</i> value
GRS1 (combined)	0.19	0.075	.012	0.59	0.179	9.51×10^{-4}
Immune response	-	-	-	0.46	0.295	.116
Endocytosis	-	-	-	1.16	0.305	1.44×10^{-4}
Cholesterol transport	0.11	0.082	.164	0.39	0.392	.322
Hematopoietic cell lineage [†]	0.09	0.084	.269	-	-	-
Hemostasis	-	-	-	-0.08	0.524	.872
Clathrin/AP2 adaptor complex	0.12	0.082	.128	0.72	0.423	.089
Protein folding [†]	0.10	0.083	.218	-	-	-

Abbreviations: β , regression coefficient; *APOE*, apolipoprotein E; GRS1, combined genetic risk score; GRS2, pathway-specific genetic risk score; SE, standard error; SNP, single-nucleotide polymorphism; RS, Rotterdam Study.

NOTE. Multiple testing correction by Bonferroni [$0.05/(5 \text{ phenotypes} \times 11 \text{ risk scores})$; $P < 9.09 \times 10^{-4}$] was considered significant.

*Logistic regression model adjusted for age and sex in the RS ($N = 360$ cases).

[†]Only one SNP available in excluding *APOE* pathway-based GRS2.

Table 4
Results for association of risk scores with MRI phenotypes

SNP cluster*	Including <i>APOE</i>									Excluding <i>APOE</i>								
	White matter lesions			Hippocampal volume			Brain volume			White matter lesions			Hippocampal volume			Brain volume		
	β	SE	P	β	SE	P	β	SE	P	β	SE	P	β	SE	P	β	SE	P
GRS1 (combined)	0.012	0.016	.448	−0.001	0.016	.929	0.002	0.007	.806	0.059	0.037	.114	−0.009	0.038	.810	−0.006	0.016	.724
Immune response	-	-	-	-	-	-	-	-	-	0.149	0.062	.016	−0.024	0.062	.706	−0.010	0.026	.692
Endocytosis	-	-	-	-	-	-	-	-	-	0.071	0.062	.254	−0.046	0.063	.462	0.004	0.026	.865
Cholesterol transport	0.005	0.017	.785	0.001	0.017	.964	0.004	0.007	.574	0.063	0.080	.434	0.013	0.080	.875	0.023	0.033	.497
Hematopoietic cell lineage [†]	0.002	0.017	.901	0.001	0.017	.976	0.004	0.007	.556	-	-	-	-	-	-	-	-	-
Hemostasis	-	-	-	-	-	-	-	-	-	0.228	0.108	.034	−0.077	0.109	.479	−0.009	0.045	.835
Clathrin/AP2 adaptor complex	0.011	0.017	.507	−0.002	0.017	.924	0.003	0.007	.658	0.258	0.088	3.55×10^{-3}	−0.077	0.109	.479	−0.009	0.045	.835
Protein folding [‡]	0.007	0.017	.700	−0.001	0.017	.970	0.004	0.007	.619	-	-	-	-	-	-	-	-	-

Abbreviations: β , regression coefficient; *APOE*, apolipoprotein E; GRS1, combined genetic risk score; GRS2, pathway-specific genetic risk score; MRI, magnetic resonance imaging; SE, standard error; SNP, single-nucleotide polymorphism; RS, Rotterdam Study.

NOTE. Multiple testing correction by Bonferroni [$0.05/(5 \text{ phenotypes} \times 11 \text{ risk scores})$; $P < 9.09 \times 10^{-4}$] was considered significant.

*Linear regression model with MRI phenotype as outcome and risk score as predictor, adjusted for age at MRI scan and sex in the RS (N = 4521).

[†]Only one SNP available in excluding *APOE* pathway-based GRS2.

and excluding *APOE* $\epsilon 4$ (effect = 0.69, $P = 1.12 \times 10^{-11}$), was significantly associated with an increased risk of AD (Table 2). This association remained significant (*APOE* excluding; effect = 0.66, $P = 8.45 \times 10^{-7}$) after removing the patients who were included in the IGAP meta-analysis [7] (Supplementary Table 3). GRS1 was also significantly associated with progression from normal subjects into AD patients both including (HR = 1.69, $P = 6.64 \times 10^{-83}$) and excluding *APOE* $\epsilon 4$ (HR = 1.27, $P = 4.88 \times 10^{-15}$; Supplementary Table 4). GRS1 was associated with MCI when *APOE* $\epsilon 4$ was included (effect = 0.19, $P = .012$), but the association was stronger when *APOE* $\epsilon 4$ was excluded from the analysis (effect = 0.59, $P = 9.51 \times 10^{-4}$; Table 3); however, these associations did not pass multiple testing correction. No association of GRS1 was observed with any of the MRI phenotypes: white matter lesions, hippocampal volume, and total brain volume (Table 4).

3.2. Association of the GRS2 with AD

Among GRS2 of which *APOE* $\epsilon 4$ is a part, *cholesterol transport*, *hematopoietic cell lineage*, *clathrin/AP2 adaptor complex*, and *protein folding* were significantly associated with AD (effect ≥ 0.71 , $P < 3.22 \times 10^{-64}$) only when *APOE* $\epsilon 4$ was included in the risk scores. Among the non-*APOE* pathways, AD was significantly associated with GRS2 capturing *immune response* (effect = 0.69, $P = 3.20 \times 10^{-5}$) and *endocytosis pathway* (effect = 0.75, $P = 1.28 \times 10^{-5}$; Table 2), and association sustained (*immune response*: effect = 0.68, $P = 2.22 \times 10^{-3}$, and *endocytosis*: effect = 0.79, $P = 5.37 \times 10^{-4}$) even after removing the patients who were included in the IGAP meta-analysis [7] (Supplementary Table 3). GRS2 capturing *immune response* (HR = 1.14, $P = 1.19 \times 10^{-5}$),

endocytosis (HR = 1.19, $P = 5.16 \times 10^{-8}$), and *APOE* $\epsilon 4$ -excluded *clathrin/AP2 adaptor complex* (HR = 1.09, $P = 5.98 \times 10^{-3}$) pathway showed association with progression from normal into AD. Both *Immune response* and *endocytosis* pathways were significant after correcting for multiple testing. GRS2 including *APOE* $\epsilon 4$ were also significantly associated with normal-to-AD progression (HR ≥ 1.60 , $P \leq 1.44 \times 10^{-69}$; Supplementary Table 4). Comparatively, except for *APOE* $\epsilon 4$, and the variants in *CR1* and *BIN1* genes, no single variant showed significant evidence of association with AD (Supplementary Table 5). The variant rs6733839 in the *BIN1* gene partially explains the association between the *endocytosis* pathway and AD, whereas *APOE* $\epsilon 4$ mainly explains the association of all pathways of which *APOE* $\epsilon 4$ is a part.

3.3. Association of the GRS2 with MCI

In GRS2, only the *endocytosis* pathway showed significant evidence for association (effect = 1.16, $P = 1.44 \times 10^{-4}$; Table 3) with MCI. Although the significance of the association is similar to that of the overall risk score (GRS1), the effect estimate is considerably higher (1.16 vs. 0.59 overall). In the single-variant analysis, the strongest association of MCI was observed with rs6733839 in the *BIN1* gene (effect = 0.262, $P = 1.12 \times 10^{-3}$; Supplementary Table 5). Although this association was not significant after correcting for multiple testing, however, it partially explains the association between MCI and GRS2 capturing *endocytosis*.

3.4. Association of the GRS2 with MRI phenotypes

White matter lesions were associated with GRS2 capturing *immune response* (effect = 0.15, $P = .016$) and

clathrin/AP2 adaptor complex excluding *APOE* $\epsilon 4$ (effect = 0.26, $P = 3.55 \times 10^{-3}$). If we consider multiple testing, both these associations lose significance after accounting for all tested phenotypes and risk scores. Of note is that no association of white matter lesions with the GRS2 capturing the *clathrin/AP2 adaptor complex* is observed when *APOE* $\epsilon 4$ is included in the GRS2 (effect = 0.011, $P = .507$; Table 4). We did not observe association of GRS2 with hippocampal volume and total brain volume. In the single-variant analysis, association of white matter lesions is seen with variants in *PICALM* and *CLU* genes ($P \leq .05$). Hippocampal volume shows association with variants in *BINI* and *CELF1* genes ($P < .05$; Supplementary Table 6). None of the single-variant association sustained Bonferroni correction for multiple testing.

4. Discussion

Combined risk score is significantly associated with AD and normal-to-AD progression but not with any of the early features of AD tested in our study including MCI and MRI markers. However, our pathway-based risk score analysis shows that the *endocytosis* pathway significantly associates with MCI in addition to AD and normal-to-AD progression (Supplementary Fig. 1).

The association of GRS1 with AD is consistent with other similar studies on AD [40–42]. However, while others observed significant association of combined risk score with MCI [43,44], in our study the association of GRS1 with MCI was not significant after correcting for multiple testing. We did not find association of GRS1 with any of the studied MRI endophenotypes. These findings are consistent with those of Mormino et al. [45] and Lupton et al. [46]; both studies did not find association of hippocampal volume with combined GRS1 based on genome-wide significant AD variants, but Mormino et al. [45], observed this association only with risk score based on non-genome wide significant AD variants. The largest study so far that included the RS, however, reported significant evidence of association of risk score based on all genome-wide significant AD variants with hippocampal volume and total brain volume [47].

This is the first study that addressed the role of specific pathways in AD and its early clinical manifestations, that is, MCI and MRI phenotypes. Our study shows that GRS2 based on the *immune response* pathway was significantly associated with AD and normal-to-AD progression. Furthermore, we observed association of *immune response* with white matter lesions at MRI, but this association did not survive Bonferroni correction. The genes clustered in the *immune response* pathway (*CLU*, *CRI*, *INPP5D*, *MS4A6A*, *TREM2*, *MEF2C*, and *EPHA1*) are mainly expressed in microglial cells and play a part in the innate *immune response* in the central nervous system [48–52]. Microglial cells are also thought to play a role in amyloid

plaque clearance [53,54]. It has been hypothesized that the activation of the immune system and the subsequent inflammatory response are involved in neuronal damage including axonal loss and white matter pathology due to demyelination [55]. White matter lesions are associated with increased risk of cognitive decline, developing dementia [21] and AD [22,56]. White matter lesions are also more frequently observed in AD patients than controls [57,58].

The most interesting finding of the present study is that the genes capturing the *endocytosis* pathway significantly associate to MCI, AD, and with progression from normal (dementia free) to AD. This pathway is independent of *APOE* and includes the *BINI*, *PICALM*, *CD2AP*, and *SORL1* genes. We show that the association of GRS1 with MCI status is mainly attributed to the genes involved in the *endocytosis* pathway. Omitting the AD genes not related to the *endocytosis* pathway makes the association of the pathway with MCI even stronger. This suggests that the *endocytosis* pathway plays a critical role in an early prodromal phase of AD. Our findings are in line with previous studies suggesting the activation of the *endocytic* pathway is the earliest reported intracellular manifestation of AD [59–61]. Furthermore, the effect estimate of the *endocytosis* pathway was larger for MCI (1.16) compared with AD (0.75), suggesting a stronger association with MCI; however, this difference in effect estimates was not significant ($P = .12$). The *endocytosis* pathway is involved in neuronal uptake of macromolecules and secretory vesicles during synaptic transmission. As efficient uptake of extracellular cholesterol is critical for neuronal functions such as repair, synapse formation, and exon elongation [62], normal neuronal work needs smooth functioning of *endocytosis* pathway [63]. Postmortem studies have also demonstrated reduced brain cholesterol levels in the brain areas responsible for memory and learning, among late-onset AD cases and age-matched controls [64]. These facts suggest that defects in *endocytosis*, which derive the cholesterol uptake, could lead to impaired neurotransmitter release and synaptic function [65]. Dysfunction in *endocytosis* can also contribute to accumulation of abnormal amyloid β (A β) peptides [66]. Based on this finding, we can suggest that the *endocytosis* pathway is a common molecular mechanism between MCI and AD that starts manifesting at early stages of disease. Risk contributed by variants clustered in this pathway at various stages of AD progression can possibly provide clue about disease trajectory.

Our study further shows association of the *clathrin/AP2 adaptor complex* pathway with white matter lesions. Although the association failed to pass the multiple testing, it is interesting to note that no association was detected with the combined risk score either in our study or a larger study performed earlier by Chauhan et al. [47] that included up to 11,550 individuals. This suggests that pathway-based risk scores may be more sensitive in picking association signals

that may be relevant for specific AD pathologies. Two variants tagging *PICALM* and *CLU* genes cluster in the *clathrin/AP2 adaptor complex* pathway. Each variant independently shows nominal association with white matter lesions in our analyses, but combining their effect are additive and improve the strength of the association. There is a strong evidence that the two proteins encoded by the genes interact at molecular level [67,68]. *PICALM* is involved in VAMP2 trafficking that is a crucial process to maintain functional integrity of synapses, which are crucial to cognitive function [69,70]. *PICALM* is also found to be expressed in the white matter, and immunolabeling of human brain tissue shows that *PICALM* is mainly found in blood vessel walls [71]. *CLU* clustered in the *clathrin/AP2 adaptor* is involved in efflux of free insoluble A β peptides through blood-brain barrier [72]. Increased plasma levels of *CLU* were associated with increased burden of A β peptides in healthy elderly population and brain atrophy in AD [73,74] and decreased integrity of white matter in young adults [75]. Demyelination of white matter is reported to occur even before the accumulation of A β plaques and neurofibrillary tangles [76]. The findings of the present study suggest that the increased genetic burden of risk variants in the *clathrin/AP2 adaptor complex* (*clathrin-mediated endocytosis*) and *immune response* pathway may play a role in early pathogenesis of AD through white matter pathology.

Among pathways including *APOE* (*cholesterol transport, hematopoietic cell lineage, clathrin/AP2 adaptor complex, and protein folding*), significant association with AD and normal-to-AD progression suggests that *APOE* ϵ 4 appears to be the driving genetic factor for these associations.

Our study provides a readout of pathway-based risk score association with AD and its prodementia endophenotypes. Our findings are important from a clinical perspective as these will aid in determining whether a certain biological pathway is involved in a patient. This will permit targeted interventions based on predicted pathological pathways. Similar as the case of cardiovascular diseases [77], a heterogeneous disease treatment can be followed based on pathway biomarkers (e.g., glucose level, total cholesterol and high-density lipid levels, and liver enzymes in case of cardiovascular disease) [78] but rather on genetic basis. This requires reference pathways and treatment portfolio. In the meantime, the pathway-based genetic risk score will allow stratification of patients in clinical trials based on causal pathways involved in patients. This may improve both the power and efficiency of future clinical and preventive trials.

Our study is a step forward to use known genetic and pathway information for disentangling the mechanisms of AD, but it has one major limitation that pathway information is based on known AD variants identified so far. This will further improve in future with improved genetic risk information that can better capture the underlying pathways.

Another possible limitation of our study is that 625 cases of RS-I was a part of meta-analysis performed by the IGAP [7], which can contribute to possible inflation in our results of association of risk score with AD. However, excluding these patients, the results of this study largely remained unchanged.

To conclude, our study provides strong evidence that the *endocytosis* pathway is relevant in the prodromal phase of AD, that is, in subjects with MCI. Furthermore, the pathways including *immune response* and *clathrin/AP2 adaptor complex* pathways may be relevant for brain-related early endophenotypes of AD, such as white matter lesions; this, however, needs further investigation in larger samples. Interestingly, all the observed associations with early AD pathology are shown by *APOE*-excluding pathways. Future findings from genomic research will improve the quality of the pathway-specific genetic scores.

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Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jalz.2018.01.005>.

RESEARCH IN CONTEXT

1. Systematic review: For constructing genetic risk scores, we classified genome-wide significant Alzheimer's disease risk variants into seven diverse biological pathways based on literature identified using Google Scholar and PubMed resources.
2. Interpretation: In a large prospective population-based cohort, we evaluated the role of pathway-based genetic risk scores in predementia endophenotypes including mild cognitive impairment and brain magnetic resonance imaging phenotypes. Genetic risk scores capturing the *endocytosis* pathway was significantly associated with mild cognitive impairment, and the *clathrin/AP2 adaptor complex* pathway also showed evidence of modest association with white matter lesions. This study provides a differential role of biological pathways during the clinically heterogeneous predementia phase of Alzheimer's disease.
3. Future directions: In addition to replication of these findings in a larger independent population, this research can be extended to include all non-genome wide significant variants in these pathways. Furthermore, the results of this study could help to prioritize targets for early intervention in the disease process.

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