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 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, second clustering these variants within pathways. Risk scores were investigated for their association with AD, mild cognitive impairment (MCI) 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 MCI (P = 1.44×10^{-4}). *Immune response* (P = 0.016) and *clathrin/AP2 adaptor complex* pathway (P = 3.55×10^{-3}) excluding apolipoprotein E (*APOE*) also showed modest association with white matter lesions but did not sustain Bonferroni correction (P = 9.09×10^{-4}).

DISCUSSION,

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

Keywords: Genetic Risk Score, Alzheimer's disease, White matter lesions, Mild cognitive impairment, Endocytosis, Immune response

Introduction

Alzheimer's disease (AD) is heterogeneous and genetically complex disease with a 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 *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 (GWAS) have discovered over 20 novel common genetic variants that influence the risk of late-onset AD [7-13]. These 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 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 (*CR1*) are involved in 2 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, 21]. Furthermore, these biological pathways may also modulate the prodromal stages of AD such as mild cognitive impairment (MCI) [22-

24]. Owing to heterogeneity during pre-dementia 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 while 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 towards improving our understanding of the pathogenesis of AD and a first step towards a more informative and powerful read-out for preventive and therapeutic trials targeting specific pathways.

The current study aims to capture the different biological pathways involved in AD using genetic risk scores to evaluate their role in AD and pre-dementia endophenotypes including MCI, white matter lesion, total brain and hippocampal volume.

Methodology

Study population

This study included samples from the Rotterdam study (RS). RS is a prospective population based study [25] designed to investigate the etiology of age related disorders. At the baseline examination in 1990-93, 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 2006-2008 (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 is provided elsewhere [26, 27]. In the current analyses, we included in total 1270 late-onset AD cases and 7623 controls (age at follow-up ≥ 65 years and dementia free) from RS-I (1118 cases, 4736 controls), RS-II (134 cases, 1928 controls) and RS-III (18 cases, 959 controls) cohorts, from follow-up conducted during 2009-2013. 10370 dementia free (Normal) participants were also included in study from all three RS baseline cohorts and followed for an average of 11 years to analyze their progression into incident AD. Further, we included 360 MCI cases and 3245 cognitively normal controls from RS-I (235 cases, 1943 controls) and RS-II (125 cases, 1302 controls) who were first time assessed during 2002-2005 in RS (Table 1).

Genotyping

Blood was drawn for genotyping from participants of RS cohort during their first visit and DNA genotyping was performed at the internal genotyping facility of 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 $P < 1.0x10^{-6}$ and Minor Allele Frequency (MAF) < 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 (HRC) 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 only genetic variants that had imputation quality (R-squared) > 0.5.

MRI scanning

Image acquisition

MRI scanning is assessed on a 1.5-T MRI unit with a dedicated eight-channel head coil (Signa HD platform, GE Healthcare, Milwaukee, USA) since the induction of a dedicated MRI machine in the Rotterdam Study in 2005. 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].

Image processing

we excluded 251 persons with stroke and/or dementia from the total 5899 subjects who came for MRI, since this may affect image processing. All T1 images were segmented into supratentorial gray matter, white matter and cerebrospinal fluid using a k-nearest neighbor (kNN) 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 (FLAIR) scans [34]. After visual inspection of all segmentations, an additional 313 subjects were excluded due to poor quality, leaving 5335 for the analysis. The hippocampus was segmented using a fully automated method, described previously [35]. Semi-quantitative MRI post-processing software were 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, dura) were removed by non-linearly registering all brain

scans to a manually created template in which nonbrain tissues were masked. In all scans, visual checks were performed and if needed any segmentation errors manually corrected [36-38]. After excluding subjects whose genotyping information was not available, we ended up with 4527 cognitively normal subjects collectively from RS cohorts including RS-I (968), RS-II (1074) and RS-III (2485) cohorts for our current analyses.

Statistical analysis

Genetic Risk Score computation

To construct the genetic risk score, we selected late-onset AD associated single nucleotide polymorphisms (SNPs) reaching genome-wide significance level ($P < 5.0x10^{-8}$; Supplementary Table 1), including one rare *TREM2* variant [7, 39]. In common variants, we considered only variants identified by the International Genomics of Alzheimer's Project (IGAP) meta-analyses. Additionally, we considered *APOE*4* (rs429358) variant for genetic risk score construction. From a total of 21 SNPs, *HLA-DRB1-HLA-DRB5* (rs9271192) variant was excluded from GRS calculation because of its low imputation quality (R-squared = 0.31) in 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 OR) of the genome-wide significant variants from IGAP meta-analysis [7] as weights and their respective allele dosages from imputed genotype data of our study cohorts. Genetic 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.

1-Combined Genetic Risk Score (GRS1)

GRS1 was constructed in two ways, i.e., 1) using all the 20 selected SNPs and 2) excluding the *APOE*4* variant to identify the joint independent effect of all other genome-wide significant SNPs.

2-Pathway-specific Genetic Risk Score (GRS2)

For 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*. 2014 [16] (Supplementary Table 2). Classifying genome-wide significant AD SNPs into pathways, we also utilized information from Guerreiro *et al*, 2013 [14], in which the authors reviewed the possible division of known AD associated genes into biological pathways [14]. Further, Gene Network database (http://129.125.135.180:8080/GeneNetwork/) was used to confirm the allocated pathways. Of the 20 SNPs 14 could be clustered into 7 non mutually exclusive pathways (Supplementary Table 2). Similar to GRS1, we also constructed GRS2 with and without the *APOE*4* variant. *APOE*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 *APOE*4* variant.

Association analyses of GRS1, 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. In order to assess the possible inflation of association results between AD and risk scores, we repeated the association analysis excluding 625 AD cases which were part of IGAP meta-analysis [7] from total 1270 AD cases of the RS cohort. Further, we performed prospective analysis using Cox-proportional hazards model (N=1057 incident AD cases) in R software using 'survival' package [40] and reported results as hazard ratio (HR) per 1 standard deviation (SD) increase in risk score and 95% confidence interval. The association of single variants with AD and MCI in 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 at MRI scan. Single variant association analysis was also performed for MRI phenotypes. Bonferroni correction (0.05/(11 risk scores x 5 phenotypes); $P = 9.09 \times 10^{-04}$ was used to correct for multiple testing.

Results

Association of the GRS1 with AD, MCI and MRI endophenotypes

The risk score containing all SNPs i.e., GRS1 both including *APOE*4* (effect = 0.73, $P = 6.53 \times 10^{-74}$) and excluding *APOE*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, $P = 8.47x10^{-7}$) after removing the patients that were included in the IGAP meta-analysis [7] (Supplementary Table 3). GRS1 was also significantly associated with progression of normal subjects into incident AD including (HR = 1.69, $P = 6.64x10^{-83}$) and excluding *APOE*4* (HR = 1.27, $P = 4.88x10^{-15}$; Supplementary Table 4). GRS1 was associated with MCI when *APOE*4* was included (effect = 0.19, $P = 0.012$) but the association was stronger with MCI when *APOE*4* was excluded from the analysis (effect = 0.59, $P = 9.51x10^{-4}$; Table 3), but 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).

Association of the GRS2 with AD

Among GRS2 of which *APOE**4 is a part, *cholesterol transport*, *hematopoietic cell lineage*, *clathrin/AP2 adaptor complex* and *protein folding* were significantly associated with AD (effect ≥ 0.71, $P < 3.22x10^{-64}$) only when *APOE*4* was included in the risk scores (Table 2). Among the non-*APOE* pathways, AD was significantly associated with GRS2 capturing *immune response* (effect = 0.69, $P = 3.20*10^{-5}$) and *endocytosis pathway* (effect = 0.75, $P = 1.28x10^{-5}$) and association sustained (*Immune response*; effect = 0.68, $P = 2.22x10^{-3}$ and *endocytosis*; effect = 0.79, $P = 5.37x10^{-4}$) after removing the patients that were included in the IGAP meta-analysis [7] (Supplementary Table 3). GRS2 capturing *immune response* (HR = 1.14, $P = 1.19x10^{-5}$), *endocytosis* (HR = 1.19, $P = 5.16x10^{-8}$) and *APOE*4* excluded *clathrin/AP2 adaptor complex* (HR = 1.09, $P = 5.98x10^{-3}$) pathway showed association with conversion risk from normal into incident AD. Both *Immune response* and *endocytosis* pathways were significant after multiple testing. GRS2 including *APOE*4* were also significantly associated with normal to AD conversion (HR ≥ 1.60, $P \leq 1.44x10^{-69}$; Supplementary Table 4). Comparatively, except for *APOE**4, *CR1* and

BIN1, no single variant showed significant evidence of association (Supplementary Table 5). *BIN1* as a part of *endocytosis* pathways also partially explains the association of GRS2 capturing *endocytosis* with AD.

Association of the GRS2 with MCI

In GRS2, only *endocytosis* pathway showed significant evidence for association (effect = 1.16, $P = 1.44 \times 10^{-4}$; Table 3) with MCI and it retained significance after multiple testing. Although the significance of the association is similar to that of the overall risk score (GRS1), the effect estimate is considerably higher (1.16 versus 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 Table5) but this association lost significance after Bonferroni correction. Whereas *BIN1* is part of the *endocytosis* pathway, which partially explains the association between MCI and GRS2 capturing *endocytosis*.

Association of the GRS2 with MRI phenotypes

White matter lesions were associated with GRS2 capturing *immune response* (effect = 0.15, $P = 0.016$), and *clathrin/AP2 adaptor complex* excluding *APOE*4* (effect = 0.26, $P = 3.55 \times 10^{-3}$). If we consider multiple testing, both these associations loses significance after accounting for all tested phenotypes and risk scores. Of note is that the association of the GRS2 capturing the *clathrin/AP2 adaptor complex* loses its association when *APOE*4* is included in the GRS2 (effect = 0.011, $P = 0.507$). 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, CLU* genes ($P \leq 0.05$). Hippocampal volume shows association with variants

in *BIN1* and *CELF1* genes ($P < 0.05$; Supplementary Table 6). None of the single variant association sustained Bonferroni correction.

Discussion

Combined GRS1 including and excluding *APOE*4* is significantly associated with AD but not with MCI and MRI phenotypes in our study. Our study shows that the GRS2 capturing *immune response* and *endocytosis* pathways are not only significantly associated with AD, normal to AD conversion but also its endophenotypes, for instance, the GRS2 capturing the *endocytosis* pathway also associates significantly with MCI, a group at high risk of developing AD [41, 42], while the GRS2 capturing *Immune response* and *clathrin/AP2 adaptor complex* showed modest association with the presence of white matter lesions at MRI in cognitively normal subjects in the RS cohort (Supplementary Figure 1).

In our study, the association of GRS1 with AD is consistent with other similar studies on AD [43-45]. GRS1 association with MCI did not pass Bonferroni correction while other studies observed significant association of combined risk score with MCI [46, 47]. We did not find association of GRS1 with any of the studied MRI endophenotypes. These findings are consistent with those of Mormino *et al*. 2016 [48] and Lupton *et al.* 2016 [49], both studies did not find association of hippocampal volume with combined GRS1 based on genome-wide signficant AD variants but Mormino *et al*. 2016 [48] observed this association only with risk score based on non-genome wide significant AD variants. The largest study so far that included RS, however, reported significant evidence of association of risk score based on all genome-wide signficant AD variants with hippocampal volume and total brain volume [27].

This is the first study that addressed the role of specific pathways in AD and its early clinical manifestations i.e., MCI and MRI phenotypes. Our study shows that GRS2 based on *immune response* pathway was significantly associated with AD, normal to AD conversion. We also observed evidence of association of *immune response* with white matter lesions at MRI but this did not pass Bonferroni correction, therefore should be considered carefully while interpreting results. These findings are converging with studies showing enrichment of *immune system pathway* with non-genome wide significant AD variants [16, 50]. The genes clustered in *immune response* pathway (*CLU, CRI, INPP5D, MS4A6A, TREM2, MEF2C, EPHA1*) are mainly expressed in microglial cells and play a part in the innate *immune response* in central nervous system [51-55]. Microglial cells are also thought to play a role in amyloid plaque clearance [56, 57]. It has been hypothesized that activation of immune system and the subsequent inflammatory response are involved in neuronal damage including axonal loss and white matter pathology due to demyelination [58]. This may explain the association of the AD genes involved in the *immune* response with white matter lesions that we observed in the present study [59]. Whit matter lesions are associated with increased risk of cognitive decline, developing dementia [21] and AD [60, 61]. White matter lesions are also more frequently observed in AD patients than controls [62, 63].

The present study reveals further that the genes capturing the *endocytosis pathway* not only strongly associate to AD but also to MCI. We also showed that *endocytosis* pathway is also associated with progression from normal (dementia free) to AD in average 11 years of follow-up. This pathway is independent of *APOE* and includes the *BIN1, PICALM, CD2AP, SORL1* genes. We show that the association of GRS1 with MCI status is mainly attributed to the genes

283 involved in the *endocytosis* pathway. Omitting the AD genes not related to the *endocytosis*
284 pathway makes the association of the pathway with MCI even stronger. The association
285 suggests that the *endocytosis* pathway plays a critical role in an early prodromal phase of AD
286 and converges with studies suggesting activation of *endocytic pathway* is the earliest reported
287 intracellular manifestation of AD [41, 42, 64]. Based on the effect estimates of association of
288 *endocytosis pathway* with AD and MCI (0.79 vs 1.59), we also speculated that *endocytosis* is
289 more strongly associated to MCI than to AD. MCI is considered a prodromal stage in AD
290 patients that suggest *endocytosis* pathway is associated with early pathology of AD. The
291 *endocytosis pathway* is involved in neuronal uptake of macromolecules and secretory vesicles
292 during synaptic transmission. As efficient uptake of extracellular cholesterol is critical for
293 neuronal functions such as repair, synapse formation and exon elongation [65], normal
294 neuronal work needs smooth functioning of *endocytosis* pathways [66]. Post-mortem studies
295 have also demonstrated reduced brain cholesterol levels in the brain areas responsible for
296 memory and learning, among late-onset AD cases and age matched controls [67]. These facts
297 suggest that defects in *endocytosis* which derive the cholesterol uptake could lead to impaired
298 neurotransmitter release and synaptic function [68]. Dysfunction in *endocytosis* can also
299 contribute to accumulation of abnormal Aβ peptide [69]. Based on this finding, we can suggest
300 that *endocytosis* pathway is a common molecular mechanism between MCI and AD that starts
301 manifesting at early stages of disease. Risk contributed by variants clustered in this pathway at
302 various stages of AD progression can possibly provide clue about disease trajectory.

303 Similar to the *immune response* pathway, the *clathrin/AP2 adaptor complex* pathway modestly
304 associated with white matter lesions. Although, the association failed to pass the multiple

testing, but combined AD risk score did not capture the association with white matter lesions in our and even in large studies [27]. Capturing this association in small sample indicates the importance of this pathway in explaining the white matter lesions pathology. Two variants tagging *PICALM* and *CLU* genes cluster in the c*lathrin/AP2 adaptor complex* pathway. Each variant independently shows nominally significant association with white matter lesions in our analyses but combining their effect are additive and improve the strength of association. There is a strong evidence that the two protein encoded by the genes interact at molecular level [70, 71]. *PICALM* is involved in VAMP2 trafficking that is a crucial process to maintain functional integrity of synapses which are crucial to cognitive function [72, 73]. *PICALM* is also found to be expressed in the white matter and, immune-labeling of human brain tissue shows that *PICALM* is mainly found in blood vessel walls [74]. *CLU* clustered in *clathrin/AP2 adaptor* is involved in efflux of free insoluble amyloid beta (Aβ) peptide through blood brain barrier [75]. Increased plasma levels of *CLU* were found to be associated with increased burden of Aβ peptide in healthy elderly population and brain atrophy in AD [76, 77] and decreased integrity of white matter in young adults [78]. Demyelination of white matter is reported to occur even before the accumulation of Aβ plaques and neurofibrillary tangles [79]. The findings of the present study suggest that the increased genetic burden of risk variants in *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, protein folding*), significant association with AD and normal to AD conversion suggests that *APOE*4* appear to be the driving genetic factor for these

associations. Only *the clathrin/AP2 adaptor complex* shows evidence of association (P = 0.036) to AD and normal to AD conversion ($P = 5.98 \times 10^{-3}$) when *APOE*4* variant is excluded from the analysis but did not pass multiple testing correction.

Our study provides a readout of pathway based risk score association with AD and its pre-dementia endophenotypes. Main clinical significance of our findings is that they will allow to determine whether a certain biological pathway is involved in an individual patient. This will permit targeted interventions based on predicted pathological pathways. Similar as the case of cardiovascular diseases [80], a heterogeneous disease treatment can be followed based on pathway biomarkers (e.g., glucose level, total cholesterol and high density lipid levels, liver enzymes in case of cardiovascular disease) [81] but rather on genetic basis. This require reference pathways and treatment portfolio. In the meantime, the pathway based genetic risk score will allow stratification of the high risk 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 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.

In conclusion we found different pathways are implicated in different endophenotypes of AD. *Endocytosis* pathway is involved in MCI and AD, while *immune response* associates with AD. Further, *Immune response* and *clathrin/AP2 adaptor complex* pathways are involved in white matter lesions but their association should be carefully considered due to multiple testing limitation. 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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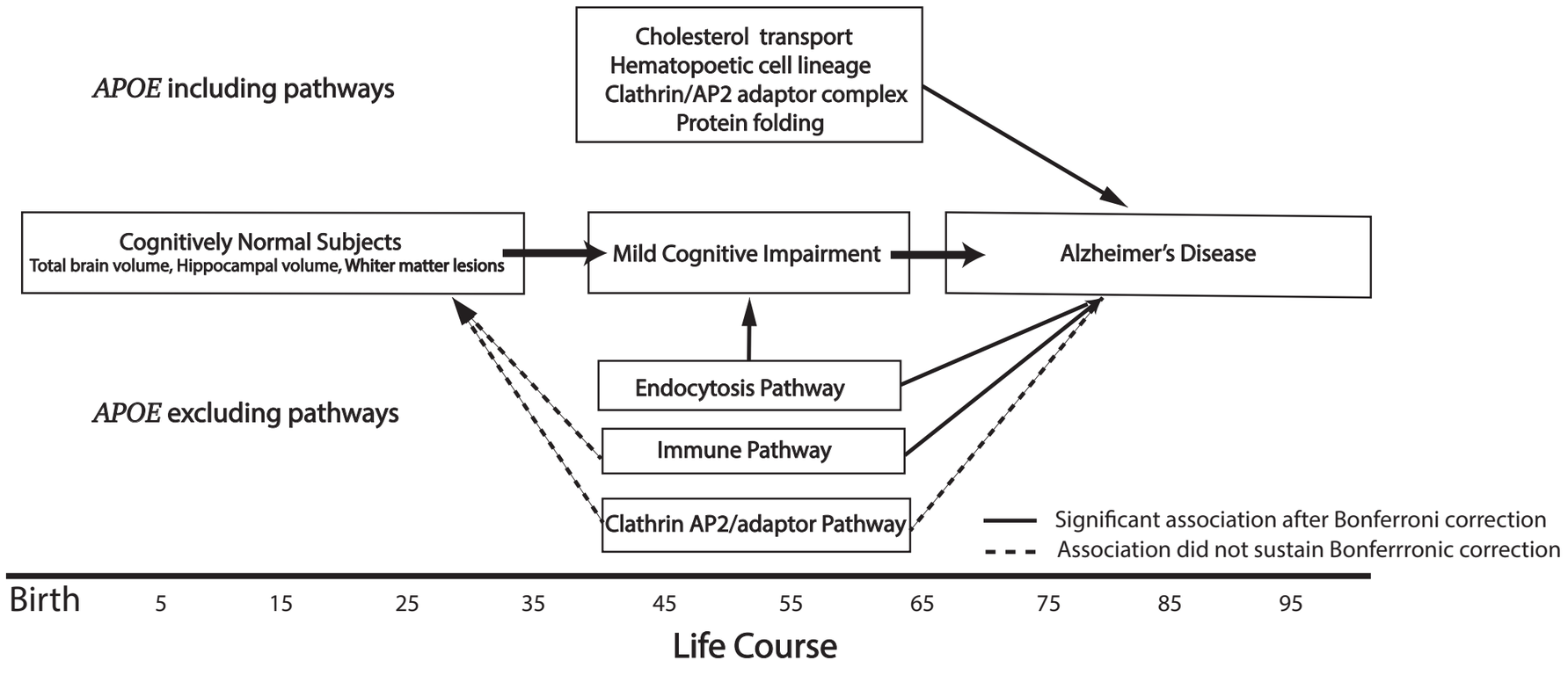
Figure caption:

Supplementary Figure 1: Diagram showing the association results of AD, MCI and WML in cognitively normal subjects with pathways-specific GRS2 both including and excluding *APOE*4*. Bonferroni correction threshold ($P = 9.09 \times 10^{-4}$)


APOE including pathways
Cholesterol transport
Hematopoetic cell lineage
Clathrin/AP2 adaptor complex
Protein folding
Cognitively Normal Subjects
Total brain volume, Hippocampal volume, Whiter matter lesions
Mild Cognitive Impairment
Alzheimer's Disease
APOE excluding pathways
Endocytosis Pathway
Immune Pathway
Clathrin AP2/adaptor Pathway
Significant association after Bonferroni correction
Association did not sustain Bonferrronic correction
Birth
5
15
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95
Life Course

Supplementary Material

Supplementary Table 1: List of Genome-wide significant variants associated with AD

Chromosome	Position (BP)	SNP	Gene	Coding Allele	Effect	MAF	RSI_Rsq	RSII_Rsq	RSIII_Rsq	Effect origin
1	207692049	rs6656401	*CR1*	G	-0.157	0.197	0.953	0.948	0.950	Lambert et al. (2013)[1]
2	127892810	rs6733839	*BIN1*	T	0.188	0.409	0.960	0.911	0.962	Lambert et al. (2013)
2	234068476	rs35349669	*INPP5D*	T	0.066	0.488	0.975	0.973	0.976	Lambert et al. (2013)
5	88223420	rs190982	*MEF2C*	A	0.08	0.408	0.979	0.934	0.978	Lambert et al. (2013)
6	41129252	rs75932628	*TREM2*	T	0.889	0.0016	0.762	0.726	0.668	Ruiz et al. (2014)[2]
6	32578530	rs9271192	*HLA-DRB5-HLA-DRB1*†	A	-0.108	0.276	0.314	0.312	0.314	Lambert et al. (2013)
6	47487762	rs10948363	*CD2AP*	G	0.098	0.266	0.998	0.998	0.998	Lambert et al. (2013)
7	100004446	rs1476679	*ZCWPW1*	T	0.078	0.287	0.995	0.996	0.995	Lambert et al. (2013)
7	143110762	rs11771145	*EPHA1*	A	-0.102	0.338	0.998	0.998	0.999	Lambert et al. (2013)
7	37841534	rs2718058	*NME8*	G	-0.07	0.373	1.000	1.000	1.000	Lambert et al. (2013)
8	27195121	rs28834970	*PTK2B*	C	0.096	0.366	0.993	0.990	0.994	Lambert et al. (2013)
8	27467686	rs9331896	*CLU*	T	0.146	0.379	0.902	0.974	0.901	Lambert et al. (2013)
11	121435587	rs11218343	*SORL1*	C	-0.27	0.039	0.998	0.995	0.998	Lambert et al. (2013)
11	47557871	rs10838725	*CELF1*	C	0.075	0.316	0.998	0.998	0.998	Lambert et al. (2013)
11	59923508	rs983392	*MS4A6A*	G	-0.108	0.403	0.989	0.990	0.991	Lambert et al. (2013)
11	85867875	rs10792832	*PICALM*	G	0.13	0.358	0.999	0.999	0.999	Lambert et al. (2013)
14	53400629	rs17125944	*FERMT2*	C	0.122	0.092	1.000	1.000	1.000	Lambert et al. (2013)
14	92926952	rs10498633	*SLC24A4-RIN3*	T	-0.104	0.217	0.999	0.999	1.000	Lambert et al. (2013)
19	45411941	rs429358	*APOE*4*	C	1.3503	0.148	0.949	0.944	0.947	Lambert et al. (2013)
19	1063443	rs4147929	*ABCA7*	G	-0.135	0.19	0.916	0.917	0.991	Lambert et al. (2013)
20	55018260	rs7274581	*CASS4*	C	-0.139	0.083	0.990	0.989	0.990	Lambert et al. (2013)

Abbreviations: AD ~ Alzheimer's disease, MAF ~ minor allele frequency, Rsq = R-squared (HRC imputation quality)

*Effect (log of odds ratio) is based on Coding Allele column

† Variants have low imputation quality in HRC imputation in our cohorts therefore excluded from genetic risk score calculation .

Supplementary Table 2: Clustering of genome-wide significant variants into their respective 8 biological pathways.

			Genes reported in pathway		Constructed GRS(Yes/No)	
Pathway	**Gene***	**Assigned SNP**	**Jones *et al***	**Guerreiro *et al***	**Including *APOE***	**Excluding *APOE***
Immune Response	*CLU*	rs9331896	Yes	Yes	**No**	**Yes**
	CR1	rs6656401	Yes	Yes		
	INPP5D	rs35349669	Yes	Yes		
	EPHA1	rs11771145	-	Yes		
	MS4A6A	rs983392	-	Yes		
	TREM2	rs75932628	-	Yes		
	MEF2C	rs190982	-	Yes		
Endocytosis	*CD2AP*	rs10948363		Yes	**No**	**Yes**
	PICALM	rs10792832	Yes	Yes		
	BIN1	rs6733839	Yes	Yes		
	SORL1	rs11218343	-	Yes		
Cholesterol transport†	*CLU*	rs9331896	Yes	Yes	**Yes**	**Yes**
	ABCA7	rs4147929	Yes	Yes		
	SORL1	rs11218343	-	Yes		
	*APOE*4*	rs429358	Yes	Yes		
Hematopoietic cell lineage†	*CR1*	rs6656401	Yes	-	**Yes**	**No**
	*APOE*4*	rs429358	Yes	-		
Protein ubiquitination	*CLU*	rs9331896	Yes	-	**No**	**No**
Hemostasis	*CLU*	rs9331896	Yes	-	**No**	**Yes**
	INPP5D	rs35349669	Yes	-		
Clathrin/AP2 adaptor complex†	*CLU*	rs9331896	Yes	-	**Yes**	**Yes**
	PICALM	rs10792832	Yes	-		
	*APOE*4*	rs429358	Yes	-		
Protein folding†	*CLU*	rs9331896	Yes	-	**Yes**	**No**
	*APOE*4*	rs429358	Yes	-		

*SNPs classification into pathways is based on the information from Jones *et al*. [3] and Guerreiro *et al[4]*.

†*APOE*4* variant (rs429358) is grouped under these pathways. *Protein folding* and *Hematopoietic cell lineage* pathways are left with one SNP after excluding *APOE*4* variant therefore were not considered for *APOE* excluding analysis.

Supplementary Table 3: Results of association of AD with risk scores

	Including *APOE*			Excluding *APOE*		
SNP Cluster*	**β**	**SE**	**P-value**	**β**	**SE**	**P-value**
GRS1 (Combined)	0.72	0.052	1.80×10^{-43}	0.66	0.135	8.45×10^{-7}
Immune response	-	-	-	0.68	0.221	2.22×10^{-3}
Endocytosis	-	-	-	0.79	0.228	5.37×10^{-4}
Cholesterol Transport	0.70	0.054	3.48×10^{-38}	0.41	0.294	0.159
Hematopoietic cell lineage†	0.72	0.055	7.19×10^{-40}		-	-
Hemostasis			-	0.35	0.390	0.364
Clathrin/AP2 Adaptor complex	0.70	0.055	6.47×10^{-38}	0.27	0.314	0.383
Protein folding†	0.71	0.055	5.18×10^{-38}		-	-

Abbreviations: GRS1 ~ Combined genetic risk score, SNP ~ single nucleotide polymorphism, β ~ regression coefficient, SE ~ Standard error.

*Logistic regression model adjusted for age and sex in RS (N=645) excluding 625 cases included in IGAP meta-analysis

†Only one SNP available in excluding *APOE* GRS2

Supplementary Table 4: Results of longitudinal analysis from normal (dementia free) to AD conversion

	Including *APOE*			Excluding *APOE*		
SNP Cluster*	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
GRS1 (Combined)	1.69	1.61-1.79	6.64×10^{-83}	1.27	1.19-1.34	4.88×10^{-15}
Immune response	-	-	-	1.14	1.07-1.21	1.19×10^{-5}
Endocytosis	-	-	-	1.19	1.12-1.26	5.16×10^{-8}
Cholesterol Transport	1.60	1.52-1.68	1.44×10^{-69}	1.07	1.01-1.14	2.45×10^{-2}
Hematopoietic cell lineage†	1.60	1.52-1.68	4.29×10^{-71}	-	-	-
Hemostasis	-	-	-	1.08	1.01-1.14	1.62×10^{-2}
Clathrin/AP2 Adaptor complex	1.61	1.52-1.69	4.21×10^{-71}	1.09	1.02-1.15	5.98×10^{-3}
Protein folding†	1.60	1.52-1.68	7.66×10^{-70}	-	-	-

Note: Multiple testing correction by Bonferroni 0.05/ (5 phenotypes x 11 risk scores); $P < 9.09 \times 10^{-4}$ was considered significant

Abbreviations: GRS1 ~ Combined genetic risk score, SNP ~ single nucleotide polymorphism,

HR ~ Hazard ratio per 1 standard deviation of risk score, CI ~ Confidence interval

*Cox proportional hazards model adjusted for age at baseline and sex in RS (N = 10370 normal at baseline)

†Only one SNP available in excluding *APOE* GRS2

Supplementary Table 5: Results of single variant association with AD and MCI

Phenotype ➔		Alzheimer's Disease			Mild Cognitive Impairment		
SNP	**Gene**	**β**	**SE**	**P-value**	**β**	**SE**	**P-value**
rs429358	*APOE*4*	0.949	0.057	8.78×10^{-63}	0.137	0.114	0.229
rs75932628	*TREM2*	0.816	0.470	0.083	1.293	0.639	0.043
rs6656401	*CR1*	-0.180	0.055	1.11×10^{-3}	0.072	0.105	0.496
rs11218343	*SORL1*	-0.161	0.110	0.142	-0.193	0.206	0.351
rs10838725	*CELF1*	0.062	0.048	0.192	0.069	0.086	0.424
rs983392	*MS4A6A*	-0.081	0.045	0.072	-0.127	0.081	0.115
rs10792832	*PICALM*	0.085	0.045	0.063	0.181	0.083	0.028
rs17125944	*FERMT2*	0.128	0.070	0.067	-0.016	0.131	0.901
rs10498633	*SLC24A4-RIN3*	-0.092	0.053	0.081	0.049	0.094	0.601
rs4147929	*ABCA7*	-0.003	0.061	0.961	-0.064	0.107	0.552
rs6733839	*BIN1*	0.149	0.045	9.74×10^{-4}	0.262	0.081	1.12×10^{-3}
rs35349669	*INPP5D*	0.055	0.044	0.216	-0.081	0.080	0.315
rs7274581	*CASS4*	-0.120	0.083	0.149	-0.034	0.143	0.810
rs190982	*MEF2C*	0.010	0.046	0.833	0.061	0.083	0.458
rs10948363	*CD2AP*	0.079	0.049	0.102	0.030	0.088	0.732
rs1476679	*ZCWPW1*	0.005	0.047	0.918	-0.037	0.085	0.662
rs11771145	*EPHA1*	-0.075	0.047	0.107	-0.117	0.085	0.168
rs2718058	*NME8*	-0.051	0.045	0.258	0.054	0.081	0.507
rs28834970	*PTK2B*	0.071	0.045	0.111	0.034	0.081	0.679
rs9331896	*CLU*	0.049	0.047	0.290	0.026	0.084	0.762

Note: Multiple testing correction by Bonferroni 0.05/ (5 phenotypes x 20 variants); $P < 5 \times 10^{-4}$ was considered significant

Abbreviations: SNP ~ Single nucleotide polymorphism, β ~ regression coefficient, SE ~ Standard error

Supplementary Table 6: Single variant association with MRI phenotypes

		White matter lesions			Hippocampal volume			Total Brain volume		
SNP	**Gene**	**β**	**SE**	**P-value**	**β**	**SE**	**P-value**	**β**	**SE**	**P-value**
rs429358	*APOE*4*	0.002	0.023	0.919	-0.001	0.023	0.973	0.004	0.010	0.676
rs75932628	*TREM2*	0.276	0.221	0.212	-0.016	0.221	0.942	-0.050	0.093	0.591
rs6656401	*CR1*	-0.003	0.022	0.893	-0.004	0.022	0.846	-0.012	0.009	0.187
rs11218343	*SORL1*	-0.001	0.040	0.978	-0.045	0.040	0.265	-0.021	0.017	0.208
rs10838725	*CELF1*	-0.005	0.018	0.765	0.036	0.018	0.041	0.008	0.007	0.275
rs983392	*MS4A6A*	-0.004	0.017	0.815	0.012	0.017	0.460	0.002	0.007	0.803
rs10792832	*PICALM*	0.036	0.017	0.030	-0.007	0.017	0.667	-0.003	0.007	0.668
rs17125944	*FERMT2*	0.009	0.027	0.723	-0.047	0.027	0.084	0.001	0.011	0.901
rs10498633	*SLC24A4-RIN3*	-0.016	0.019	0.399	0.006	0.020	0.774	0.004	0.008	0.636
rs4147929	*ABCA7*	0.023	0.022	0.285	0.010	0.022	0.664	0.006	0.009	0.488
rs6733839	*BIN1*	0.008	0.017	0.621	-0.039	0.017	0.022	-0.002	0.007	0.814
rs35349669	*INPP5D*	0.015	0.017	0.380	-0.016	0.017	0.328	-0.010	0.007	0.132
rs7274581	*CASS4*	-0.012	0.030	0.676	-0.012	0.030	0.702	0.021	0.012	0.098
rs190982	*MEF2C*	0.013	0.017	0.431	1.67×10^{-4}	0.017	0.992	-0.011	0.007	0.127
rs10948363	*CD2AP*	-0.013	0.018	0.472	0.031	0.018	0.094	1.87×10^{-4}	0.008	0.980
rs1476679	*ZCWPW1*	-0.012	0.017	0.487	0.001	0.018	0.948	-0.005	0.007	0.513
rs11771145	*EPHA1*	-0.024	0.017	0.157	-0.012	0.017	0.502	0.003	0.007	0.638
rs2718058	*NME8*	0.009	0.017	0.608	-0.029	0.017	0.089	-0.001	0.007	0.912
rs28834970	*PTK2B*	-0.029	0.017	0.082	0.018	0.017	0.274	0.007	0.007	0.288
rs9331896	*CLU*	0.034	0.017	0.051	-0.006	0.018	0.749	0.004	0.007	0.597

Note: Multiple testing correction by Bonferroni 0.05/ (5 phenotypes x 20 variants); $P < 5 \times 10^{-4}$ was considered significant

Abbreviations: MRI~ Magnetic resonance imaging, SNP ~ Single nucleotide polymorphism, β ~ regression coefficient, SE ~ Standard error

References

[1] Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet. 2013;45:1452-8.
[2] Ruiz A, Dols-Icardo O, Bullido MJ, Pastor P, Rodriguez-Rodriguez E, Lopez de Munain A, et al. Assessing the role of the TREM2 p.R47H variant as a risk factor for Alzheimer's disease and frontotemporal dementia. Neurobiol Aging. 2014;35:444 e1-4.
[3] International Genomics of Alzheimer's Disease C. Convergent genetic and expression data implicate immunity in Alzheimer's disease. Alzheimers Dement. 2015;11:658-71.
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Table 1: Cohort characteristics

Characteristics	Rotterdam Study
AD data set	
Total	8893
Late-onset AD	1270
AD free controls	84.30 (6.8)
Age-of-onset (SD)	5228 (59%)
Females (%)	
MCI data set	
Total	3605
MCI cases	360
Controls	71.9 (7.2)
Age (SD)	2063 (57%)
Females (%)	
MRI data set	
Total	4527
Age (SD)	64.74 (10.8)
Females (%)	2516 (56%)

Abbreviation: SD ~ Standard deviation, AD ~ Alzheimer's disease,
MCI ~ Mild cognitive impairment, MRI ~ Magnetic resonance imaging

Table 2: Results of association of AD with risk scores ~~GRS~~

	Including *APOE*			Excluding *APOE*		
SNP Cluster*	**β**	**SE**	**P-value**	**β**	**SE**	**P-value**
GRS1 (Combined)	0.73	0.040	$6.53x10^{-74}$	0.69	0.101	$1.12x10^{-11}$
Immune response	-	-	-	0.69	0.166	$3.20x10^{-5}$
Endocytosis	-	-	-	0.75	0.171	$1.28x10^{-5}$
Cholesterol Transport	0.71	0.042	$3.22x10^{-64}$	0.39	0.219	0.077
Hematopoietic cell lineage†	0.73	0.042	$5.16x10^{-66}$	-	-	-
Hemostasis			-	0.50	0.292	0.090
Clathrin/AP2 Adaptor complex	0.72	0.042	$4.68x10^{-65}$	0.50	0.236	0.036
Protein folding†	0.72	0.042	$2.96x10^{-64}$	-	-	-

Note: Multiple testing correction by Bonferroni 0.05/ (5 phenotypes x 11 risk scores): $P < 9.09x10^{-4}$ was considered significant

Abbreviations: GRS1 ~ Combined genetic risk score, SNP ~ Single nucleotide polymorphism, β ~ Regression coefficient, SE ~ Standard error.

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

†Only one SNP available in excluding *APOE* GRS2

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Table 3: Results of association of MCI with risk scores ~~GRS~~

	Including *APOE*			Excluding *APOE*		
SNP Cluster*	**β**	**SE**	**P-value**	**β**	**SE**	**P-value**
GRS1 (combined)	0.19	0.075	0.012	0.59	0.179	9.51×10^{-4}
Immune response	-	-	-	0.46	0.295	0.116
Endocytosis	-	-	-	1.16	0.305	1.44×10^{-4}
Cholesterol Transport	0.11	0.082	0.164	0.39	0.392	0.322
Hematopoietic cell lineage†	0.09	0.084	0.269	-	-	-
Hemostasis	-	-	-	-0.08	0.524	0.872
Clathrin/AP2 Adaptor complex	0.12	0.082	0.128	0.72	0.423	0.089
Protein folding†	0.10	0.083	0.218	-	-	-

Note: Multiple testing correction by Bonferroni 0.05/ (5 phenotypes x 11 risk scores); $P < 9.09 \times 10^{-4}$ was considered significant

Abbreviations: GRS1 ~ Combined genetic risk score, SNP ~ Single nucleotide polymorphism, β ~ Regression coefficient, SE ~ Standard error.

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

† Only one SNP available in excluding *APOE* pathway based GRS2

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Table 4: Results for association of risk scores ~~GRS~~ with MRI phenotypes

	Including *APOE*									Excluding *APOE*								
SNP cluster *	**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	0.448	-0.001	0.016	0.929	0.002	0.007	0.806	0.059	0.037	0.114	-0.009	0.038	0.810	-0.006	0.016	0.724
Immune response	-	-	-	-	-	-	-	-	-	0.149	0.062	**0.016**	-0.024	0.062	0.706	-0.010	0.026	0.692
Endocytosis	-	-	-	-	-	-	-	-	-	0.071	0.062	0.254	-0.046	0.063	0.462	0.004	0.026	0.865
Cholesterol Transport	0.005	0.017	0.785	0.001	0.017	0.964	0.004	0.007	0.574	0.063	0.080	0.434	0.013	0.080	0.875	0.023	0.033	0.497
Hematopoietic cell lineage†	0.002	0.017	0.901	0.001	0.017	0.976	0.004	0.007	0.556	-	-	-	-	-	-	-	-	-
Hemostasis	-	-	-	-	-	-	-	-	-	0.228	0.108	0.034	-0.077	0.109	0.479	-0.009	0.045	0.835
Clathrin/AP2 Adaptor complex	0.011	0.017	0.507	-0.002	0.017	0.924	0.003	0.007	0.658	0.258	0.088	**3.55×10^{-3}**	-0.077	0.109	0.479	-0.009	0.045	0.835
Protein folding†	0.007	0.017	0.700	-0.001	0.017	0.970	0.004	0.007	0.619	-	-	-	-	-	-	-	-	-

Note: Multiple testing correction by Bonferroni 0.05/ (5 phenotypes x 11 risk scores); $P < 9.09 \times 10^{-4}$ was considered significant

Abbreviations: GRS1 ~ Combined genetic risk score, MRI ~ Magnetic resonance imaging, SNP ~ Single nucleotide polymorphism, β ~ Regression coefficient, SE ~ Standard error, *P* ~ P-value

* Linear regression model with MRI phenotype as outcome and risk score ~~GRS~~ as predictor, adjusted for age at MRI scan, sex in RS (N=4527)

† Only one SNP available in excluding *APOE* pathway based GRS2

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