Effect of Pathway-specific Polygenic Risk Scores for Alzheimer's Disease on Rate of Change in Cognitive Function and AD-related Biomarkers among Asymptomatic Individuals

Running Title: AD Pathway-specific Polygenic Risk Score

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Abstract

Background: Brain aging and genetic scores for late-onset Alzheimer's disease (LOAD) have been associated with preclinical cognitive decline and biomarker variations. Compared with an overall polygenic risk score (PRS), a pathway-specific polygenic risk score (p-PRS) may be more appropriate in predicting a specific biomarker or cognitive component underlying LOAD pathology earlier in the lifespan.

Objective: In this study, we leveraged 10 years of longitudinal data from the Wisconsin Registry for Alzheimer's Prevention and explored changing patterns in cognition and biomarkers at various age points along six biological pathways among initially cognitively unimpaired individuals.

Methods: PRS and p-PRSs with and without apolipoprotein E (*APOE*) were constructed separately based on the significant genes associated with LOAD in a recent genome-wide association study meta-analysis and compared to *APOE* alone. We used a linear mixed-effects model to assess the association between PRS/p-PRSs and overall/sub-cognitive dimensions among 1,175 individuals. We also applied the model to the outcomes of cerebrospinal fluid biomarkers for beta-amyloid 42 (Aβ42), Aβ42/40 ratio, total tau, and phosphorylated tau based on predetermined hypotheses among 197 individuals. Replication analyses were performed in an independent sample.

Results: We found p-PRSs and the overall PRS can predict preclinical changes in cognition and biomarkers, regardless of the inclusion of *APOE*. The effects of p-PRSs/PRS on rate of change in cognition, beta-amyloid, and tau outcomes are dependent on age and appear earlier in the lifespan when *APOE* is included in these risk scores compared to when *APOE* is excluded.

Conclusion: In addition to *APOE*, the pathway-specific PRSs can predict age-dependent changes in beta-amyloid, tau, and cognition. Once validated, they could be used to identify individuals with an elevated genetic risk of accumulating beta-amyloid and tau, long before the onset of clinical symptoms.

Keywords: ApoE; Alzheimer's Disease; Aging; Cognition; Biomarkers; Longitudinal Studies.

Introduction

Late-onset Alzheimer's disease (LOAD) is an age-dependent neurodegenerative disease that is clinically manifested by a progressive deterioration of cognitive function, memory, and social ability. Abnormal accumulation of proteins such as β-amyloid (Aβ) and tau are two hallmarks that play important roles in LOAD pathology long before the clinical symptoms of neurodegeneration are evident. Under the amyloid hypothesis, an imbalance between Aβ clearance and Aβ production is considered the underlying cause for the initiation of LOAD through the formation of extracellular senile plaques in the brain[1]. Previous studies have provided evidence that neurobiological pathways, such as β-amyloid precursor protein (APP) processing, altered cholesterol metabolism, endocytosis, and tau pathology, are closely linked to Aβ production and clearance[2–8]. Tau, on the other hand, is hypothesized to trigger the progression of LOAD by forming insoluble filaments and accumulating intracellular neurofibrillary tangles of hyperphosphorylated tau in the brain. These accumulations block axonal transport and finally harm the synaptic communications between neurons. In addition to the four pathways affecting Aβ, neurobiological pathways of LOAD that are related to tau accumulation among LOAD patients include immune response and axonal development[2–6,8–11].

Genetics play a major role in neurodegeneration in LOAD. LOAD is highly polygenic, and the heritability estimates from twin studies range from 58% to 79%[12]. The *apolipoprotein E* (*APOE*) gene is the strongest known genetic risk factor for LOAD, with the *APOE* ε4 allele conferring increased risk and the *APOE* ε2 allele conferring a protective effect relative to the *APOE* ε3 allele. A recent meta-analysis of genome-wide association studies (GWAS), which included more than 94,000 individuals with European ancestry, validated 20 previously reported risk loci and discovered five novel, susceptibility single-nucleotide polymorphisms (SNPs)[13]. However,

except for *APOE*, most of the discovered genetic variants only exhibit tiny effects on the risk of LOAD, and therefore the prediction from any single genetic variant is limited. Polygenic risk scores (PRSs), on the other hand, sum the effects of multiple independent genetic risk variants and convert the overall genetic burden to a single score. This score has been utilized under many clinical settings and has been found to serve as a good predictor of disease risk[14,15]. Although an overall PRS that combines all genetic risk variants is more commonly used and may be more powerful in the prediction of the overall cognitive status or LOAD risk, a pathway-specific polygenic risk score (p-PRS) that sums individual SNPs under a specific neurobiological pathway may be more appropriate in predicting a specific biomarker or cognitive component (such as the beta-amyloid 42/40 ratio, phosphorylated tau, or executive function) underlying LOAD pathology[16].

To date, a constellation of studies have been published to examine the prediction performance of p-PRS on LOAD disease risk, cognitive deterioration, and biomarker variation among people with or without LOAD; however, the study findings are mixed. Previous research from our group examined the prediction performance of p-PRSs under three pathways on cognition, Pittsburgh compound B (PiB) amyloid accumulation and cerebrospinal fluid (CSF) Aβ and tau using a prospective cohort of 1,200 asymptomatic individuals[16]. We found that p-PRSs under all three pathways were not predictive of the overall or component cognitive dimensions, whereas p-PRSs in the Aβ and cholesterol pathways were good predictors of variations of PiB amyloid accumulation and CSF Aβ and tau. However, the predictive performance was significantly sacrificed with the exclusion of the *APOE* variants. Another team investigated the effect of p-PRSs under seven pathways on cortical thickness using a longitudinal population cohort of 544 individuals[17]. Promising results were discovered in the APP metabolism, cholesterol

metabolism, and endocytosis pathways when *APOE* was included; however, only the APP metabolism pathway remained predictive after adjustment for the *APOE* variants. A recent study estimated the risk of LOAD among 1,779 Dutch individuals using p-PRSs in five major pathways involved in LOAD[18]. They found that all p-PRSs except for angiogenesis were significantly associated with increased risk of LOAD, regardless of adjustment for the *APOE* variants. Several reasons can explain the discrepant results among the existing AD-related p-PRS analyses, but it is likely mainly because the AD outcomes outlined in the current literature are different and the methods for pathway-gene-variant mapping did not draw from a comprehensive body of literature. In addition, aging is the strongest factor associated with variation in the endophenotypes and cognitive decline, but it was not considered as more than a covariate in the existing literature when assessing the predictive performance of p-PRSs on cognition and LOAD-related biomarkers. A recent study leveraging a 25-year longitudinal cohort of non-demented individuals showed that the overall LOAD genetic risk on cognitive decline is age-dependent during the life course[19].

In the present study, we updated findings from Darst et al. (2017) with five additional years of follow up data from an ongoing longitudinal cohort of cognitively healthy adults enriched for a parental history of AD from the Wisconsin Registry for Alzheimer's Prevention (WRAP) to explore the potential of p-PRSs in the prediction of cognitive deterioration and changes in LOAD-related biomarkers over time. Specifically, after a comprehensive review of the existing literature on the LOAD disease pathways and genetic functions of the single most significant variant from each gene, as identified by the recent GWAS meta-analysis, we constructed weighted PRSs for APP metabolism, cholesterol metabolism, endocytosis, tau pathology, immune response, and axonal development. For each p-PRS, we tested its association with an overall cognitive composite score (Preclinical Alzheimer Cognitive Composite – 3 (PACC-3)), cognitive component

composite scores (Immediate Learning, Delayed Recall, and Executive Function), and biomarkers of Aβ accumulation (CSF Aβ42 and CSF Aβ42/40 ratio), neurodegeneration (CSF total tau (T-tau)), and tau pathology (CSF phosphorylated tau (P-tau)), while taking genetic heterogeneity by age into account. To check the robustness of the results, we further performed a replication analysis using an independent sample of cognitively healthy individuals from the Wisconsin Alzheimer's Disease Research Center (ADRC).

Methods

Study participants

Data leveraged in this study originated from WRAP, an ongoing longitudinal prospective cohort study of middle-aged adults who were cognitively healthy at enrollment and spoke English (N > 1,500). WRAP is enriched for participants with a parental history of clinical AD, increasing the proportion of individuals who will experience AD pathology and cognitive decline during the course of the study. The details of the study design have been described elsewhere[20]. The WRAP study began recruiting participants in 2001 with an initial follow-up after four years and subsequent follow-up every two years. In general, the participants were between 40 and 65 years old at baseline. Siblings of WRAP participants were allowed to enroll. Participants were given an extensive battery of neuropsychological tests at each visit. The maximum number of WRAP visits available at the time of analysis was seven. In the present study, the sample was limited to self-reported non-Hispanic Caucasian participants to match the race and ethnicity of the participants in the GWAS meta-analysis from which the weights for the PRS were drawn. We excluded data from the first wave of WRAP because the cognitive outcome examined in this study cannot be computed

using the neuropsychological tests administered in the first WRAP visit. Data from the seventh visit of WRAP were excluded because data collection in the seventh wave is ongoing and data from this wave were only available for less than 50 participants. Compared to the previous p-PRS study on the LOAD-related outcome using WRAP, the present study includes additional data from two more WRAP visits (approximately four years in calendar length). This study was conducted with the approval of the University of Wisconsin Institutional Review Board, and all subjects provided signed informed consent before participation.

Neuropsychometric assessments

As described above, participants were given a battery of neuropsychological tests for the assessment of cognitive function at each WRAP visit. In the present study, we measured the overall cognitive performance using the PACC-3 score based on work by Donohue and colleagues[21]. Specifically, this composite score is computed by standardizing and averaging performance from three tests that assess the memory and executive function of participants: Rey Auditory Verbal Learning (RAVLT; Trials 1-5), Logical Memory II total score, and Wechsler Adult Intelligence Scale-Revised (WAIS-R) Digit Symbol score[22]. In addition to the overall cognitive performance, we also examined domain-specific cognitive performance for immediate learning, delayed recall, and executive function[23]. The immediate learning domain-specific composite score was derived from the sum of learning trials in RAVLT, Wechsler Memory Scale-Revised (WMS-R) logical memory I total score, and Brief Visuospatial Memory Test-Revised (BVMT-R) immediate recall score. A delayed recall domain-specific composite score is constructed based on the sum of the RAVLT delayed score, WMS-R logical memory delayed recall score, and BVMT-R delayed recall score. The executive function domain-specific composite score is obtained by

standardizing and averaging individual performance from the Trail-Making Test part B (TMT-B) Stroop test (color-word interference, STROOP) and WAIS-R digit symbol total score.

CSF collections, quantification, and analysis

CSF measurements examined in the present study include Aβ42, Aβ42/40 ratio, T-tau, and P-tau. Previous studies have indicated that CSF Aβ42 levels are negatively associated with amyloid burden; however, higher levels of CSF T-tau and P-tau are signals of an increased risk of LOAD[24]. A growing body of evidence has recommended the use of CSF Aβ42/40 ratio as a biomarker to identify early amyloid pathology because CSF Aβ42/40 ratio has greater predictive and diagnostic power in early diagnosis of LOAD compared to the individual biomarker CSF Aβ42 alone[25]. The literature has reported a negative association between levels of CSF Aβ42/40 ratio and LOAD risk[26]. Details and methods for the WRAP CSF processing have been described elsewhere[27]. In brief, 22 mL of CSF were collected through gentle extraction and combined into a 30 mL polypropylene tube. All CSF samples were processed at the Clinical Neurochemistry Laboratory at the Sahlgrenska Academy of the University of Gothenburg in Sweden using the same batch of Roche NeuroToolKit reagents (Roche Diagnostics International Ltd, Rotkreuz, Sitzerland) under strict quality control procedures as previously described.[28].

DNA collection, genotyping, and quality control

Details about genomic data collection have been described elsewhere[16,29]. Briefly, we used the PUREGENE DNA Isolation Kit to extract DNA from whole blood samples, and then we used UV spectrophotometry to quantify DNA concentrations. Of the 23 SNPs included in the analysis, 21

were genotyped in 1,448 individuals using competitive allele-specific PCR-based KASP^{TM} genotyping assays (LGC Genomics, Beverly, MA). Duplicate quality control (QC) samples had 99.9% genotype concordance, and all discordant genotypes were set to missing. The QC was carried out using PLINK v1.07. Individuals with high missingness of alleles (>10%) were removed. A total of 1,415 individuals remained in the sample after QC procedures. All 21 SNPs had call rates >95% and were in Hardy-Weinberg equilibrium (HWE).

Two SNPs (rs12459419 from *CD33* and rs593742 from *ADAM10*) that were not genotyped by the KASP^{TM} assays were extracted from genome-wide genotyping performed using the Illumina Infinium Expanded Multi-Ethnic Genotyping Array (MEGA^{EX}) at the University of Wisconsin Biotechnology Center. Individuals with gender inconsistencies and individuals and SNPs with missingness >5% were excluded. Samples from individuals of genetically-defined European descent were then imputed using the Michigan Imputation Server and the Haplotype Reference Consortium (HRC) reference panel. Variants with a low imputation quality score ($R^2 < 0.8$), with a low minor allele frequency (MAF, MAF < 0.001), or outside of HWE were subsequently removed after imputation. PLINK 2.0 was used to extract the aforementioned two SNPs. A total of 1,198 individuals with data for all 23 SNPs remained after QC.

Mapping variants to pathways

To address the limitations in the traditional pathway-gene-variant mapping method in p-PRS studies that did not draw from a comprehensive body of literature and relieve concerns about the validity (e.g., overweighting or underweighting a particular variant, see Discussion) of the novel approach in the p-PRS construction proposed recently[18], we combined the merits of these two

approaches and designed a new but conservative strategy to map genetic variants to various LOAD pathways. First, we comprehensively browsed pathways explored in the past LOAD-related p-PRSs studies published in peer-reviewed journals between 2017 and early 2020 to determine pathways that had been widely explored[16–18]. After a review of the literature, we included six pathways in the present analysis: APP metabolism, cholesterol metabolism, endocytosis, tau pathology, immune response, and axonal development. Second, we narrowed our focus to the genes that were genome-wide significant, as identified by the most recent and largest International Genomics of Alzheimer's Project (IGAP) GWAS meta-analysis on diagnosed AD[13]. Also included were three genes (*MEF2C*, *NME8,* and *CD33*) that were found to be genome-wide significant by previous GWAS meta-analyses and that were widely mentioned in previous AD review papers and were marginally significant in Kunkle et al. (2019)[30–32]. Third, we extensively browsed recent review papers on LOAD pathology published between 2017 and early 2020, with the number of citations set to higher than 5[2–8]. Then we counted the number of times the genes identified in step 2 presented in any of the specific pathways in the papers we reviewed. A specific gene was finally counted toward one of the pathways identified in step 1 only if more than 50% of the reviewed literature showed evidence that this gene belongs to that particular LOAD pathway. We finally included 22 genes in the main analysis under six pathways: APP metabolism (*CLU, SORL1, ABCA7, PICALM, ADAM10, APOE*), cholesterol metabolism (*CLU, SORL1, ABCA7, APOE*), endocytosis (*SORL1, ABCA7, PICALM, BIN1, CD2AP, PTK2B, FERMT2, SLC24A4, APOE*), tau pathology (*BIN1, FERMT2, CASS4, APOE*), immune response (*CLU, ABCA7, CR1, INPP5D,HLA-DRB1, TREM2, EPHA1, MS4A6A, CD33, MEF2C*), and axonal development (*EPHA1, FERMT2, CASS4, SPI1, NME8*) (Supplemental Figure 1).

Polygenic risk score and pathway-specific polygenic risk scores

For the PRS/p-PRS analyses, genetic variants other than the *APOE* variants were coded additively by counting the number of risk alleles based on IGAP summary statistics. *APOE* was coded according to the odds ratios (ORs) of ε2, ε3, and ε4 genotypes based on rs7412 and rs429358 in the meta-analysis of *APOE* genotype frequencies from AlzGene[33]. Specifically, we constructed an *APOE* score using the ε2/ε2 genotype as the reference (ε2/ε2, OR=1): OR(ε2/ε3) = 1.38, OR(ε3/ε3) = 2, OR(ε2/ε4) = 4.45, OR(ε3/ε4) = 6.78, OR(ε4/ε4) = 25.84[16]. Then, we log-transformed and added the score to the corresponding PRS/p-PRS. Of the 21 genes that were included in the present analysis other than *APOE*, the single most significant variant from each of the 21 genes identified by IGAP GWAS meta-analysis was used in the construction of PRS and p-PRS. PRS and p-PRS were calculated using the formula $PRS_i = \frac{\sum_{n=l}^{k} \ln(OR_n) * C_n}{M}$, where *i* represents the *i*th individual whose PRS is calculated by summing all SNPs *n* in the pathway from the first SNP *l* to last SNP *k*; OR is the odds ratio of the risk allele for SNP *n* from the IGAP GWAS meta-analysis; C is the number of risk alleles for SNP *n* for individual *i*; and M represents the number of non-missing SNPs under each predetermined pathway observed in individual *i*. In addition to the p-PRS, an overall PRS by including all 22 genes was constructed to examine the overall genetic effect by summing SNPs in all pathways of LOAD being investigated on the outcome of interest. A higher PRS/p-PRS indicates a higher genetic risk for LOAD. Since the effect size of *APOE* alone is known to be large, we excluded *APOE* for the pathways that theoretically should include *APOE* to examine the p-PRS on the outcome beyond *APOE* alone. We also tested the independent association between the *APOE* score and the outcome of interest to quantify the effect of *APOE* alone. To facilitate comparison across various pathways, all PRS, p-PRSs, and *APOE* scores were standardized with a mean of 0 and a standard deviation of 1 at baseline.

Statistical analysis

We developed a set of linear mixed effect models fitted with maximum likelihood to examine the genetic association with cognitive outcomes and LOAD-related biomarkers by accounting for within-family and within-individual correlations while allowing for missing data. All analyses were performed using the MIXED procedure implemented in SAS 9.4. Following the previous literature, we included random intercepts for family and study subjects[16,19]. WRAP investigators have reported the nonlinear effect of age on cognitive deterioration, and we therefore included a linear age, quadratic age, and cubic age in the model with cognitive outcomes to achieve better model fit[20]. For the biomarker analysis, we first used spaghetti plots to check the individual trajectory in the change of biomarkers by age and then determine the appropriate functional form of age based on the visualization of individual trajectories. To better model the dynamic relationship between aging, genetic risk, and LOAD-related outcomes, we further included an interaction term between PRS/p-PRS and all age terms to control for the potential age-dependent genetic risk on all outcomes of interest. In addition to the PRS/p-PRSs, age, and interaction between PRS and p-PRSs mentioned above, additional covariates include gender, education, practice effect (only adjusted in cognitive-related outcomes and as quantified by the number of tests completed prior to the current test), and the first five genetic principal components of ancestry[34]. We assessed the performance of each PRS/p-PRS and interaction term between age and PRS/p-PRS using the partial likelihood ratio r^2 (r^2_{LR})[35,36]. No corrections for multiple testing were performed.

Replication analysis

We replicated all analyses performed in the WRAP sample within the Wisconsin ADRC, which began enrolling participants in 2009. Because the Wisconsin ADRC administered a different battery of neuropsychological tests compared to WRAP, we could only replicate our analyses for the overall cognitive performance (PACC-3) and CSF-related outcomes. We replicated our findings using two samples extracted from the Wisconsin ADRC. The first sample is the IMPACT cohort, for which the enrollment criteria (age range of 45-65 at baseline, cognitively intact, and enriched for a parental history of AD) are the most similar and comparable to the WRAP sample. The biggest limitation of the IMPACT sample is that most of the participants are younger than age 65 so the sample may not be old enough for us to observe any effect caused by genetics or aging in late life. To address this limitation, we supplemented the IMPACT sample with the Wisconsin ADRC healthy older controls (HOCs), which includes people who are older than 65 at enrollment and do not meet the National Institute on Aging and Alzheimer's Association (NIA-AA) criteria for mild cognitive impairment (MCI) or the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable AD. We called this combined sample the All Healthy Controls (AHC) sample. All replication analyses in the Wisconsin ADRC were restricted to non-Hispanic Caucasian participants.

The Wisconsin ADRC administered a different battery of neuropsychological tests than WRAP, which resulted in a substantial missingness in the score of Logical Memory II Delayed Recall and Wechsler Adult Intelligence Scale-Revised, Digit Symbol. To make the best use of the current information, we consulted neuropsychologists in the Wisconsin ADRC and created a PACC-3-TMT score to replace PACC-3 in the replication analysis. Specifically, we converted the Craft Story score to an estimated Logical Memory score based on a published crosswalk table and

followed the previous practice of substituting the Digit Symbol score with the points received in the TMT-B test[22,37]. Since the published crosswalk table is only available for converting the Craft Story score to the Logical Memory score for the first five visits, we restricted our replication analysis using only data from the first five Wisconsin ADRC visits. The final PACC-3-TMT score was computed by standardizing and averaging the results from RAVLT, the estimated Logical Memory score, and the TMT-B test. The substantial missingness in the score of the Logical Memory score and Digit symbol makes it difficult to assess the correlation between PACC-3 and PACC-3-TMT in the Wisconsin ADRC; therefore, we used the same method to construct a PACC-3-TMT score in the WRAP and assessed the correlation between PACC-3 and PACC-3-TMT in the WRAP sample.

The Wisconsin ADRC employed the same methods of collection, processing, and quantification for the CSF data as those in WRAP. Details about genomic data collection and QC have been described elsewhere[16,38]. Briefly, the top significant SNPs from 21 genes except for *ADAM10* were genotyped by LGC Genomics (Beverly, MA). No genotypes were removed due to discordant genotypes or low call rates (<95%) or for being outside of HWE. Two individuals with high missingness of alleles (>10%) were excluded from subsequent analyses. Only the APP metabolism pathway-specific PRS and overall PRS were affected by the exclusion of the *ADAM10* gene, but we expect the impact will be small due to the small effect size (β=-0.065) of the top significant SNP from *ADAM10*. The methods of constructing the PRS, p-PRS, and *APOE* scores are the same as those in WRAP analysis.

We leveraged a linear mixed effect model fitted by maximum likelihood to examine the genetic association with the overall cognitive performance and LOAD-related biomarkers by accounting for within-individual correlations and allowing for missing data. All analyses were performed

using SAS 9.4. All other statistical methods in the replication analyses are the same as those described in the WRAP analysis, except for the exclusion of genetic principal components of ancestry as covariates because genome-wide data and, thus, genetic principal components for the full sample are not available in the Wisconsin ADRC genomic dataset.

Results

Descriptive statistics for samples and participants

Table 1 presents the demographic features of WRAP participants included in this study. Briefly, a total of 1,175 individuals with available genetic, cognitive, and demographic data remained in the sample for up to five waves (~8 years) after data cleaning. A subset of 197 WRAP participants had CSF data for up to five waves. Demographic characteristics are comparable between the cognitive and CSF samples. The sample with CSF is slightly older at baseline than the full WRAP sample because WRAP CSF collection began later during the WRAP study. WRAP participants are generally highly educated, female, and enrolled at middle age, and a majority have a parental history of AD. The *APOE* score is not available for five participants in the full sample and one participant in the CSF sample because of missing allele information for either rs7412 and/or rs429358. Following the previous literature, we decided to keep these individuals in the analysis because data are available on other genetic variants we are interested in and the magnitude of missingness is small.

Cognitive outcomes

Figure 1 presents the genetic risk of PRS/p-PRS on the rate of cognitive change by age between 50 and 80 years old in WRAP. An age-dependent association was observed between PRS/p-PRSs and all cognitive outcomes regardless of the inclusion of *APOE*, even though the longitudinal trends vary by pathway and outcome. Specifically, when including the *APOE* variants before the age of 65, we observed a stable, nearly zero, and statistically insignificant association between cognitive outcomes and p-PRSs of all pathways for every cognitive outcome. However, the genetic risk of all LOAD pathways (as quantified by p-PRS) on every cognitive outcome grows exponentially and remains statistically significant after WRAP participants reach the age of 65 years. When *APOE* is excluded, the age-dependent genetic risk for p-PRSs on cognitive outcomes still exists for some pathways but is less obvious (lag effect, slower risk growth rate, and smaller effect size) compared to that of *APOE* alone and when *APOE* is included in these pathways. Specifically, for the immediate learning composite score, only p-PRSs under the endocytosis and APP metabolism pathways are significantly associated with cognitive decline once people reach age 80. For the delayed recall composite score, we observed increased and statistically significant adverse genetic effects for the overall PRS once people reach age 80, as well as for the p-PRSs under the endocytosis, APP metabolism, and cholesterol metabolism pathways once people reach age 75. For the executive function composite score, the overall PRS and p-PRSs under the endocytosis, cholesterol metabolism, and immune response pathways are significantly and adversely associated with cognition once people reach age 75. For the PACC-3 score, the adverse genetic effect starts to occur once WRAP participants reach age 75 for the overall PRS and p-PRSs under the endocytosis, APP metabolism, and cholesterol metabolism pathways. We used a reduced set of WRAP participants who have complete data in all PRS/p-PRSs to compare the performance of each PRS/p-PRS in explaining the amount of variation in the overall and domain-specific

cognitive composite score, as measured by r_{LR}^2 and presented in supplementary table 1. Consistent with Darst (2017), the largest r_{LR}^2 for a single PRS/p-PRS is about 0.2% when *APOE* is included and 0.1% when *APOE* is excluded, which indicates that almost none of the model variance was explained by any of the single PRS/p-PRS. When the interaction between age and PRS/p-PRS was included, an additional 1% of the model variation was explained for all pathways when *APOE* was included, but the additional gain in model variation explained is significantly sacrificed after *APOE* was excluded.

CSF biomarker outcomes

Figure 2 presents the genetic risk of PRS/p-PRS on the rate of biomarker change by age between 50 and 80 years old for WRAP participants. Like cognitive outcomes, an age-dependent association was observed between PRS/p-PRSs and all AD-related biomarkers regardless of the inclusion of *APOE.* Specifically, when *APOE* is included, we observed an adverse effect of p-PRSs under all pathways for Aβ42 beginning at age 60 and increasing linearly with age. The adverse effect of p-PRSs on Aβ42/40 ratio showed a pattern similar to Aβ42 but appeared earlier, at age 55. We also observed an age-dependent genetic risk on T-tau and P-tau for all pathways when *APOE* was included, but the significant adverse effect of p-PRSs appeared a decade later than that on the beta-amyloid outcome. When *APOE* was excluded, the age-dependent genetic risk for p-PRSs on biomarkers still existed for some pathways but was less obvious (lag effect, slower risk growth rate, and smaller effect size) compared to that of *APOE* alone and when *APOE* was included in these pathways. Specifically, we observed a statistically significant adverse effect on Aβ42 for the p-PRSs under APP metabolism and cholesterol metabolism once people reach age 70, and immune response pathway after people reach 75. Similar findings were observed for

Aβ42/40 ratio, but the significant adverse effects of the p-PRSs under APP metabolism, cholesterol metabolism, and immune response appeared about 5~10 years earlier than those predicted for the change of Aβ42, and the overall PRS had a significant adverse effect starting at age 65. Surprisingly, the removal of *APOE* from p-PRSs does not affect the prediction performance on tau for the pathways that should theoretically include *APOE*. For P-tau, after the removal of *APOE*, the adverse effect of p-PRSs appears once people reach age 65 for the APP metabolism and cholesterol metabolism pathway; at age 70 for the overall PRS and p-PRS under the endocytosis pathway; and at age 75 for the immune response p-PRS. The statistically significant adverse effect of the p-PRSs under APP metabolism and cholesterol metabolism on T-tau appeared once people reach age 65, whereas p-PRSs under endocytosis and overall PRS were positively associated with T-tau accumulation once people reach age 75. We used a reduced set of WRAP participants who have complete data in all PRS/p-PRSs to compare the performance of each PRS/p-PRS in explaining the amount of variation in the LOAD-related biomarkers, as measured by r_{LR}^2 and presented in supplementary table 2. When *APOE* is included, single p-PRS/PRS can explain on average, 3~4% variance in Aβ42, 7-8% variance in Aβ42/40, and 1% variance in T-tau/P-tau. Adding an interaction between PRS/p-PRS and age resulted in an additional 3~4%, 1~3%, 1-2% gain in the variance explained for Aβ42, Aβ42/40, and T-tau/P-tau, respectively. For beta-amyloid outcomes, the variance being-explained by the single p-PRS/PRS and the additional gain in model variation explained as a result of the interaction between age and PRS/p-PRSs was significantly sacrificed after *APOE* was excluded. However, the removal of *APOE* from p-PRS/PRS doesn't substantially affect the variance explained by the PRS and interaction term for the tau outcome.

Replication analysis

We used the AHC combined sample from the Wisconsin ADRC to replicate our findings in WRAP. Table 2 details the Wisconsin ADRC participant characteristics for the cognition analysis, the mean baseline age for the AHC cohort is 59.95. The mean education is just over 16 years. About 35% of participants in the AHC cohort are males, and 70% have a family history of AD. The basic characteristics are similar between the WRAP and AHC cohorts, except for the baseline enrollment age. The baseline enrollment age in WRAP is about 5 years younger than the AHC sample because we included a sample of healthy older controls in the AHC sample, with an initial enrollment age higher than 65 years. Similar characteristics were found in the biomarker samples.

For the cognition analysis, the correlation between PACC-3 and PACC-3-TMT is about 0.93 in the WRAP sample. The results from the Wisconsin ADRC are mostly consistent with the WRAP findings in terms of the age-dependent genetic risk variation trend (Supplementary Figure 2). Specifically, the age-dependent genetic effect on the PACC-TMT score and its trend were observed in the AHC cohort, even though the effects are lagged and less obvious than the WRAP findings. When *APOE* is included, the overall PRS and p-PRSs under the endocytosis and tau pathology show a significant adverse effect once people reach age 85. *APOE* is not significantly associated with the rate of cognitive change in any age range. When *APOE* is excluded, the adverse effect of the p-PRSs starts to occur at around age 70 under cholesterol metabolism and 75 for APP metabolism. A reduced set of Wisconsin ADRC participants who have complete data in all PRS/p-PRSs were used to compare the performance of each PRS/p-PRS (supplementary table 3). Similar to the WRAP findings, the largest r_{LR}^2 for a single PRS/p-PRS is about 0.2% in the AHC sample. The largest r_{LR}^2 for PRS/p-PRSs with and without *APOE* increased by 0.4% and 0.7% with the addition of the interaction between PRS/p-PRSs and age, respectively.

The age-dependent genetic risk of PRS/p-PRSs is also observed in the biomarker analysis (Supplementary Figure 3). Specifically, when *APOE* is included, the adverse effect of PRS/p-PRSs on Aβ42 under all pathways starts to appear once people reach age 55 and increases with age. Results for Aβ42/40 ratio are very consistent with the WRAP findings. When *APOE* is included, the significant adverse effect of PRS/p-PRSs of all pathways occurs once people reach age 55 and is similar to the effect of *APOE* alone. Results for T-tau and P-tau are also very similar to the WRAP findings when *APOE* is included. The adverse effect of the PRS/p-PRSs of all the pathways starts to appear once people reach age 65 for both P-tau and T-tau. When *APOE* is excluded, similar to the WRAP findings, only p-PRSs under the APP and cholesterol metabolism pathways start to show a significant adverse effect on Aβ42 once people reach age 65. For Aβ42/40 ratio, when *APOE* is excluded, age-dependent genetic risk was observed for p-PRS starting at age 65 for the cholesterol metabolism pathway, but the effect size is smaller than the *APOE* score. For P-tau, we observed the significant adverse effect for p-PRS after people reach 70 for the axonal development pathway and after people reach 75 for endocytosis, tau pathology, and overall PRS. Significant adverse effects of p-PRSs on T-tau start to occur at age 75 for tau pathology and axon development pathways and at age 80 for endocytosis and the overall PRS. A reduced set of Wisconsin ADRC participants who have complete data in all PRS/p-PRSs were used to compare the performance of each PRS/p-PRS (supplementary table 3). When *APOE* is included, the variance explained by a single PRS is about 3% for Aβ42, 7% for Aβ42/40, 1% for P-tau, and less than 1% for T-tau. The additional interaction between age and p-PRSs contributes to the added variance explained in Aβ42, Aβ42/40, T-tau, and P-tau by about 1%, 2%, 2%, and 1.5%, respectively. When *APOE* is excluded, p-PRSs and age–PRS interaction under all pathways contributed substantially less variance, as shown using Aβ42 and Aβ42/40 ratio, which is

consistent with the WRAP findings. For tau-related outcomes, when *APOE* is excluded, the performance of p-PRSs/PRS and the interaction between p-PRSs/PRS and age under most pathways (except for endocytosis) deteriorated.

Discussion

In the present study, we updated findings from Darst et al. and investigated the potential of pathway-specific PRSs in predicting rate of change in cognitive function and biomarkers of beta-amyloid deposition, neurodegeneration, and tau pathology among asymptomatic individuals in the Wisconsin Registry for Alzheimer's Prevention[16]. With five additional years of data collection, GWAS summary statistics with a larger sample size, our comprehensive variant-pathway mapping method, and the inclusion of an age-interaction effect, we found p-PRSs and the overall PRS can predict preclinical changes in cognition and biomarkers, regardless of the inclusion of *APOE*. The effects of p-PRSs/PRS on rate of change in cognition, beta-amyloid, and tau outcomes are dependent on age and appear earlier in the lifespan when *APOE* is included in these risk scores compared to when *APOE* is excluded. Consistent with Darst et al., *APOE* appears to drive much of the strength of the p-PRSs for APP metabolism, cholesterol metabolism, endocytosis, tau pathology, and the overall PRS on rate of change in cognition and beta-amyloid outcomes when *APOE* is included in these risk scores. However, we did not observe a similar *APOE*-driven effect trend when applying p-PRSs/PRS to predicting tau outcomes.

Results are mixed for current p-PRS studies on LOAD disease risk, cognitive deterioration, and biomarker variation. This is partially due to the discrepancy in the sample characteristics across different studies and outcomes being investigated, but also due to the methodology of attributing

specific genetic variants to its corresponding biological pathway. Most existing studies on p-PRSs have been based entirely on limited or even single-review papers and bioinformatic databases to map a specific genetic variant to a neurobiological pathway. However, the genetic functions of a specific variant have not been consistently defined across the literature, and the functional annotation of genes might differ across various databases being referred. This creates uncertainties in the accuracy of constructing p-PRSs and creates the possibility that the same pathway various studies explored might not be comparable and a specific pathway might not comprehensively reflect the underlying biological mechanism that it intends to represent. A recent study on p-PRSs proposed a novel approach to construct p-PRSs by including a multiplicative factor that represents the degree of involvement of a given genetic variant in the preselected pathways in calculating p-PRSs to allow for uncertainty in gene and pathway assignment[18]. Even though this approach overcomes some limitations that the traditional p-PRSs studies may encounter when constructing p-PRSs, the accuracy of the p-PRSs under this approach (overweight or underweight of a particular variant) and to what extent the resulting p-PRSs reflect the underlying biological mechanism are still unknown. In our study, we combined the merits of these two approaches and proposed a conservative but comprehensive variant-pathway mapping method via only matching variants and corresponding pathways for which we have good confidence of an "actually existing" biological relationship, after widely referring to the literature published within recent years.

Late on-set Alzheimer's disease is an age-dependent brain disorder. Although genetics play a large role in the development and expression of LOAD, the complex relationships in the etiology between age, *APOE*, and non-*APOE* p-PRSs/PRS are not generally considered. Our study shows the risk of p-PRSs/PRS on rate of change in cognition, beta-amyloid, and tau are age dependent in both WRAP and the Wisconsin ADRC, regardless of including *APOE*. However, the adverse

effects of p-PRSs/PRS appear earlier in the lifespan when *APOE* is included in these risk scores compared to when *APOE* is excluded, with the exception of tau outcomes. In addition, including the interaction between p-PRSs/PRS and age in the model led to the model explaining additional variance. Our findings are consistent with a recent study that leveraged ADNI and UKBB samples and concluded both *APOE* and PRS predicted AD risk and presented age-dependent effects, but the effects of *APOE* were stronger in younger groups (age <80)[39]. Zimmerman et al. examined the age-dependent genetic effect of *APOE* and PRS in UKBB. In support of our findings, they reported that an AD PRS modified the association between age and cognition, that *APOE* ε4 allele carriers experienced earlier cognitive decline than non-carriers did, and that models using the PRS that excluded *APOE* ε4 had attenuated and later modification of age associations compared to when *APOE* was included in the PRS[40]. Our study also demonstrated a pattern in the timing of the earliest detectable genetic effect on rate of change in beta-amyloid (age ~55), tau (age ~65), and cognition (age ~65-70) that aligns with findings from Hanseeuw et al.[41] This pattern also explained that the reason for observing significant associations between p-PRSs/PRS and beta-amyloid outcomes in Darst et al., but not for tau and cognition outcomes is that beta-amyloid accumulation occurs at a younger age than variation in tau and cognition, and the sample leveraged in Darst et al. was too young to detect polygenic effects on tau and cognition.

Pathway-specific PRS could also predict earlier changes in AD-related outcomes than the overall PRS could, especially for beta-amyloid outcomes and when *APOE* is excluded from the risk score. Our results demonstrate when *APOE* is excluded, p-PRSs for APP and cholesterol pathway can predict changes in Aβ42, which is about 15 years earlier than the overall PRS, and this finding was replicated in the Wisconsin ADRC. Even though p-PRSs for APP and cholesterol metabolism pathways also show potential for predicting earlier changes in Aβ42/40 ratio compared to the

overall PRS, only the finding for the cholesterol metabolism pathway was replicated in the Wisconsin ADRC. Our results also show p-PRSs under certain pathways can predict adverse change in tau and cognition outcomes earlier than the overall PRS in WRAP can, but these findings were not fully replicated in the Wisconsin ADRC, which may warrant further investigation as longitudinal data focusing on the preclinical stage of AD with a larger sample size become available.

One finding – that the tau pathology PRS is not predictive of tau outcomes after the exclusion of *APOE* in WRAP, but is predictive of both P-tau and T-tau in the Wisconsin ADRC - may require further investigation once we have a larger sample size and longer follow-up time. There are two possible explanations for the discrepancies in the effect of the tau pathology PRS between WRAP and the Wisconsin ADRC. First, the genes known to be related to the tau pathology pathway may not be well established and may not fully reflect the biological pathway of tau since only three SNPs in addition to *APOE* were included in the tau pathology pathway and all these SNPs overlapped with the SNPs included in the other disease-related pathways (Supplemental Figure 1). Second, the AHC sample that was extracted from the Wisconsin ADRC includes a sample of older healthy controls (enrollment age >= 65) and tau-levels are higher in the older age groups[42].

The present study has limitations. First, although we tend to match genetic variants and biological pathways for which we have good confidence of an "actually existing" variant-pathway relationship, our variant-pathway mapping method is conservative and may not comprehensively reflect the genetic role in a specific disease pathway. Our results' accuracy is subject to the knowledge of biological function of genes and pathways at the time of performing this study. It would be crucial to modify the variant-pathway mapping as additional knowledge becomes available. Second, we only considered the significant variants as identified from the most recent

IGAP case-control GWAS-meta-analyses as the weight to construct pathway-specific PRSs; however, a larger panel of SNPs from the recent genome-wide association study by proxy (GWAX) and a combined study of GWAS and GWAX may provide additional insights into the variant-pathway mapping[43,44]. This was not considered in the current study. Third, results from the AHC sample extracted from the Wisconsin ADRC are not absolutely comparable with the WRAP findings because the AHC sample was constructed based on two separate cohorts with different characteristics (e.g., age).. Additional replication analyses may be carried out once the data for an older IMPACT cohort (more like WRAP) become available.

In conclusion, in addition to *APOE*, the pathway-specific PRSs can predict age dependent changes in beta-amyloid, tau, and cognition. Once validated, they could be used to identify individuals with an elevated genetic risk of accumulating beta-amyloid and tau, long before the onset of clinical symptoms. This information could be useful for selection of high risk participants for clinical trials and, as effective therapeutic targets further develop, pPRSs could be used to determine an individual's risk for accumulating amyloid and the predicted age of onset so that resources could be used effectively in screening individuals for amyloid accumulation with more expensive and invasive, but accurate tests. This idea is being explored in other diseases, such as breast cancer.

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Conflicts of interest:

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Passage Bio, Pinteon Therapeutics, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant or at advisory boards for Abcam, Axon, Biogen, Lilly, MagQu, Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. GK and NW are full-time employees of Roche DiagnosticsGmbH. IS is a full-time employee and shareholder of Roche Diagnostics International Ltd.

Reference

[1] Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353–356.

[2] Karch CM, Goate AM (2015) Alzheimer's disease risk genes and mechanisms of disease pathogenesis. *Biol Psychiatry* **77**, 43–51.

[3] Guerreiro R, Hardy J (2014) Genetics of Alzheimer's Disease. *Neurotherapeutics* **11**, 732–737.

[4] Muralidar S, Ambi SV, Sekaran S, Thirumalai D, Palaniappan B (2020) Role of tau protein in Alzheimer's disease: The prime pathological player. *Int J Biol Macromol* **163**, 1599–1617.

[5] Calabrò M, Rinaldi C, Santoro G, Crisafulli C (2020) The biological pathways of Alzheimer disease: a review. *AIMS Neurosci* **8**, 86–132.

[6] Medeiros R, Baglietto-Vargas D, LaFerla FM (2011) The role of tau in Alzheimer's disease and related disorders. *CNS Neurosci Ther* **17**, 514–524.

[7] Ando K, Houben S, Homa M, de Fisenne M-A, Potier M-C, Erneux C, Brion J-P, Leroy K (2020) Alzheimer's Disease: Tau Pathology and Dysfunction of Endocytosis. *Front Mol Neurosci* **13**, 583755.

[8] Hardy J, Bogdanovic N, Winblad B, Portelius E, Andreasen N, Cedazo-Minguez A, Zetterberg H (2014) Pathways to Alzheimer's disease. *J Intern Med* **275**, 296–303.

[9] Serrano-Pozo A, Das S, Hyman BT (2021) APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches. *Lancet Neurol* **20**, 68–80.

[10] Kanaan NM, Pigino GF, Brady ST, Lazarov O, Binder LI, Morfini GA (2013) Axonal degeneration in Alzheimer's disease: When signaling abnormalities meet the axonal transport system. *Exp Neurol* **246**, 44–53.

[11] Van Cauwenberghe C, Van Broeckhoven C, Sleegers K (2016) The genetic landscape of Alzheimer disease: clinical implications and perspectives. *Genet Med Off J Am Coll Med Genet* **18**, 421–430.

[12] Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S, Fiske A, Pedersen NL (2006) Role of genes and environments for explaining Alzheimer disease. *Arch Gen Psychiatry* **63**, 168–174.

[13] Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, Boland A, Vronskaya M, van der Lee SJ, Amlie-Wolf A, Bellenguez C, Frizatti A, Chouraki V, Martin ER, Sleegers K, Badarinarayan N, Jakobsdottir J, Hamilton-Nelson KL, Moreno-Grau S, Olaso R, Raybould R, Chen Y, Kuzma AB, Hiltunen M, Morgan T, Ahmad S, Vardarajan BN, Epelbaum J, Hoffmann P, Boada M, Beecham GW, Garnier J-G, Harold D, Fitzpatrick AL, Valladares O, Moutet M-L, Gerrish A, Smith AV, Qu L, Bacq D, Denning N, Jian X, Zhao Y, Del Zompo M, Fox NC, Choi S-H, Mateo I, Hughes JT, Adams HH, Malamon J, Sanchez-Garcia F, Patel Y, Brody JA, Dombroski BA, Naranjo MCD, Daniilidou M, Eiriksdottir G, Mukherjee S, Wallon D, Uphill J, Aspelund T, Cantwell LB, Garzia F, Galimberti D, Hofer E, Butkiewicz M, Fin B, Scarpini E, Sarnowski C, Bush WS, Meslage S, Kornhuber J, White CC, Song Y, Barber RC, Engelborghs S, Sordon S, Voijnovic D, Adams PM, Vandenberghe R, Mayhaus M, Cupples LA, Albert MS, De Deyn PP, Gu W, Himali JJ, Beekly D, Squassina A, Hartmann AM, Orellana A, Blacker D, Rodriguez-Rodriguez E, Lovestone S, Garcia ME, Doody RS, Munoz-Fernadez C, Sussams R, Lin H, Fairchild TJ, Benito YA, Holmes C, Karamujić-Čomić H, Frosch MP, Thonberg H, Maier W, Roshchupkin G, Ghetti B, Giedraitis V, Kawalia A, Li S, Huebinger RM, Kilander L, Moebus S, Hernández I, Kamboh MI, Brundin R, Turton J, Yang Q, Katz MJ, Concari L, Lord J, Beiser AS, Keene CD, Helisalmi S, Kloszewska I, Kukull WA, Koivisto AM, Lynch A, Tarraga L, Larson EB, Haapasalo A, Lawlor B, Mosley TH, Lipton RB, Solfrizzi V, Gill M, Longstreth WT, Montine TJ, Frisardi V, Diez-Fairen M, Rivadeneira F, Petersen RC, Deramecourt V, Alvarez I, Salani F, Ciaramella A, Boerwinkle E, Reiman EM, Fievet N, Rotter JI, Reisch JS, Hanon O, Cupidi C, Andre Uitterlinden AG, Royall DR, Dufouil C, Maletta RG, de Rojas I, Sano M, Brice A, Cecchetti R, George-Hyslop PS, Ritchie K, Tsolaki M, Tsuang DW, Dubois B, Craig D, Wu C-K, Soininen H, Avramidou D, Albin RL, Fratiglioni L, Germanou A, Apostolova LG, Keller L, Koutroumani M, Arnold SE, Panza F, Gkatzima O, Asthana

S, Hannequin D, Whitehead P, Atwood CS, Caffarra P, Hampel H, Quintela I, Carracedo Á, Lannfelt L, Rubinsztein DC, Barnes LL, Pasquier F, Frölich L, Barral S, McGuinness B, Beach TG, Johnston JA, Becker JT, Passmore P, Bigio EH, Schott JM, Bird TD, Warren JD, Boeve BF, Lupton MK, Bowen JD, Proitsi P, Boxer A, Powell JF, Burke JR, Kauwe JSK, Burns JM, Mancuso M, Buxbaum JD, Bonuccelli U, Cairns NJ, McQuillin A, Cao C, Livingston G, Carlson CS, Bass NJ, Carlsson CM, Hardy J, Carney RM, Bras J, Carrasquillo MM, Guerreiro R, Allen M, Chui HC, Fisher E, Masullo C, Crocco EA, DeCarli C, Bisceglio G, Dick M, Ma L, Duara R, Graff-Radford NR, Evans DA, Hodges A, Faber KM, Scherer M, Fallon KB, Riemenschneider M, Fardo DW, Heun R, Farlow MR, Kölsch H, Ferris S, Leber M, Foroud TM, Heuser I, Galasko DR, Giegling I, Gearing M, Hüll M, Geschwind DH, Gilbert JR, Morris J, Green RC, Mayo K, Growdon JH, Feulner T, Hamilton RL, Harrell LE, Drichel D, Honig LS, Cushion TD, Huentelman MJ, Hollingworth P, Hulette CM, Hyman BT, Marshall R, Jarvik GP, Meggy A, Abner E, Menzies GE, Jin L-W, Leonenko G, Real LM, Jun GR, Baldwin CT, Grozeva D, Karydas A, Russo G, Kaye JA, Kim R, Jessen F, Kowall NW, Vellas B, Kramer JH, Vardy E, LaFerla FM, Jöckel K-H, Lah JJ, Dichgans M, Leverenz JB, Mann D, Levey AI, Pickering-Brown S, Lieberman AP, Klopp N, Lunetta KL, Wichmann H-E, Lyketsos CG, Morgan K, Marson DC, Brown K, Martiniuk F, Medway C, Mash DC, Nöthen MM, Masliah E, Hooper NM, McCormick WC, Daniele A, McCurry SM, Bayer A, McDavid AN, Gallacher J, McKee AC, van den Bussche H, Mesulam M, Brayne C, Miller BL, Riedel-Heller S, Miller CA, Miller JW, Al-Chalabi A, Morris JC, Shaw CE, Myers AJ, Wiltfang J, O'Bryant S, Olichney JM, Alvarez V, Parisi JE, Singleton AB, Paulson HL, Collinge J, Perry WR, Mead S, Peskind E, Cribbs DH, Rossor M, Pierce A, Ryan NS, Poon WW, Nacmias B, Potter H, Sorbi S, Quinn JF, Sacchinelli E, Raj A, Spalletta G, Raskind M, Caltagirone C, Bossù P, Orfei MD, Reisberg B, Clarke R, Reitz C, Smith AD, Ringman JM, Warden D, Roberson ED, Wilcock G, Rogaeva E, Bruni AC, Rosen HJ, Gallo M, Rosenberg RN, Ben-Shlomo Y, Sager MA, Mecocci P, Saykin AJ, Pastor P, Cuccaro ML, Vance JM, Schneider JA, Schneider LS, Slifer S, Seeley WW, Smith AG, Sonnen JA, Spina S, Stern RA, Swerdlow RH, Tang M, Tanzi RE, Trojanowski JQ, Troncoso JC,

Van Deerlin VM, Van Eldik LJ, Vinters HV, Vonsattel JP, Weintraub S, Welsh-Bohmer KA, Wilhelmsen KC, Williamson J, Wingo TS, Woltjer RL, Wright CB, Yu C-E, Yu L, Saba Y, Pilotto A, Bullido MJ, Peters O, Crane PK, Bennett D, Bosco P, Coto E, Boccardi V, De Jager PL, Lleo A, Warner N, Lopez OL, Ingelsson M, Deloukas P, Cruchaga C, Graff C, Gwilliam R, Fornage M, Goate AM, Sanchez-Juan P, Kehoe PG, Amin N, Ertekin-Taner N, Berr C, Debette S, Love S, Launer LJ, Younkin SG, Dartigues J-F, Corcoran C, Ikram MA, Dickson DW, Nicolas G, Campion D, Tschanz J, Schmidt H, Hakonarson H, Clarimon J, Munger R, Schmidt R, Farrer LA, Van Broeckhoven C, C. O'Donovan M, DeStefano AL, Jones L, Haines JL, Deleuze J-F, Owen MJ, Gudnason V, Mayeux R, Escott-Price V, Psaty BM, Ramirez A, Wang L-S, Ruiz A, van Duijn CM, Holmans PA, Seshadri S, Williams J, Amouyel P, Schellenberg GD, Lambert J-C, Pericak-Vance MA (2019) Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. *Nat Genet* **51**, 414–430.

[14] Sm P, Nr W, Jl S, Pm V, Mc O, Pf S, P S (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* **460**,.

[15] Sims R, Hill M, Williams J (2020) The multiplex model of the genetics of Alzheimer's disease. *Nat Neurosci* **23**, 311–322.

[16] Darst BF, Koscik RL, Racine AM, Oh JM, Krause RA, Carlsson CM, Zetterberg H, Blennow K, Christian BT, Bendlin BB, Okonkwo OC, Hogan KJ, Hermann BP, Sager MA, Asthana S, Johnson SC, Engelman CD (2017) Pathway-specific polygenic risk scores as predictors of β-amyloid deposition and cognitive function in a sample at increased risk for Alzheimer's disease. *J Alzheimers Dis JAD* **55**, 473–484.

[17] Caspers S, Röckner ME, Jockwitz C, Bittner N, Teumer A, Herms S, Hoffmann P, Nöthen MM, Moebus S, Amunts K, Cichon S, Mühleisen TW (2020) Pathway-Specific Genetic Risk for Alzheimer's Disease Differentiates Regional Patterns of Cortical Atrophy in Older Adults. *Cereb Cortex* **30**, 801–811.

[18] Tesi N, van der Lee SJ, Hulsman M, Jansen IE, Stringa N, van Schoor NM, Scheltens P, van der Flier WM, Huisman M, Reinders MJT, Holstege H (2020) Immune response and endocytosis pathways are associated with the resilience against Alzheimer's disease. *Transl Psychiatry* **10**, 1–12.

[19] Kauppi K, Rönnlund M, Nordin Adolfsson A, Pudas S, Adolfsson R (2020) Effects of polygenic risk for Alzheimer's disease on rate of cognitive decline in normal aging. *Transl Psychiatry* **10**, 1–8.

[20] Johnson SC, Koscik RL, Jonaitis EM, Clark LR, Mueller KD, Berman SE, Bendlin BB, Engelman CD, Okonkwo OC, Hogan KJ, Asthana S, Carlsson CM, Hermann BP, Sager MA (2018) The Wisconsin Registry for Alzheimer's Prevention: A review of findings and current directions. *Alzheimers Dement Amst Neth* **10**, 130–142.

[21] Donohue MC, Sun C-K, Raman R, Insel PS, Aisen PS (2017) Cross-validation of optimized composites for preclinical Alzheimer's disease. *Alzheimers Dement Transl Res Clin Interv* **3**, 123–129.

[22] Jonaitis EM, Koscik RL, Clark LR, Ma Y, Betthauser TJ, Berman SE, Allison SL, Mueller KD, Hermann BP, Van Hulle CA, Christian BT, Bendlin BB, Blennow K, Zetterberg H, Carlsson CM, Asthana S, Johnson SC (2019) Measuring longitudinal cognition: Individual tests versus composites. *Alzheimers Dement Amst Neth* **11**, 74–84.

[23] Clark LR, Racine AM, Koscik RL, Okonkwo OC, Engelman CD, Carlsson CM, Asthana S, Bendlin BB, Chappell R, Nicholas CR, Rowley HA, Oh JM, Hermann BP, Sager MA, Christian BT, Johnson SC (2016) Beta-amyloid and cognitive decline in late middle age: Findings from the WRAP study. *Alzheimers Dement J Alzheimers Assoc* **12**, 805–814.

[24] Blennow K, Hampel H, Weiner M, Zetterberg H (2010) Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol* **6**, 131–144.

[25] Hansson O, Lehmann S, Otto M, Zetterberg H, Lewczuk P (2019) Advantages and disadvantages of the use of the CSF Amyloid β (Aβ) 42/40 ratio in the diagnosis of Alzheimer's Disease. *Alzheimers Res Ther* **11**, 34.

[26] Lee JC, Kim SJ, Hong S, Kim Y (2019) Diagnosis of Alzheimer's disease utilizing amyloid and tau as fluid biomarkers. *Exp Mol Med* **51**, 1–10.

[27] Dong R, Denier-Fields DN, Lu Q, Suridjan I, Kollmorgen G, Wild N, Betthauser TJ, Carlsson CM, Asthana S, Johnson SC, Zetterberg H, Blennow K, Engelman CD (2022) Principal components from untargeted cerebrospinal fluid metabolomics associated with Alzheimer's disease biomarkers. *Neurobiol Aging* **117**, 12–23.

[28] Van Hulle C, Jonaitis EM, Betthauser TJ, Batrla R, Wild N, Kollmorgen G, Andreasson U, Okonkwo O, Bendlin BB, Asthana S, Carlsson CM, Johnson SC, Zetterberg H, Blennow K (2021) An examination of a novel multipanel of CSF biomarkers in the Alzheimer's disease clinical and pathological continuum. *Alzheimers Dement* **17**, 431–445.

[29] Darst BF, Lu Q, Johnson SC, Engelman CD (2019) Integrated analysis of genomics, longitudinal metabolomics, and Alzheimer's risk factors among 1,111 cohort participants. *Genet Epidemiol* **43**, 657–674.

[30] Estus S, Shaw BC, Devanney N, Katsumata Y, Press EE, Fardo DW (2019) Evaluation of CD33 as a Genetic Risk Factor for Alzheimer's Disease. *Acta Neuropathol (Berl)* **138**, 187–199.

[31] Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, DeStafano AL, Bis JC, Beecham GW, Grenier-Boley B, Russo G, Thorton-Wells TA, Jones N, Smith AV, Chouraki V, Thomas C, Ikram MA, Zelenika D, Vardarajan BN, Kamatani Y, Lin CF, Gerrish A, Schmidt H, Kunkle B, Dunstan ML, Ruiz A, Bihoreau MT, Choi SH, Reitz C, Pasquier F, Cruchaga C, Craig D, Amin N, Berr C, Lopez OL, De Jager PL, Deramecourt V, Johnston JA, Evans D, Lovestone S, Letenneur L, Morón FJ, Rubinsztein DC, Eiriksdottir G, Sleegers K, Goate AM, Fiévet N, Huentelman MW, Gill M, Brown K, Kamboh MI, Keller L, Barberger-Gateau P, McGuiness B, Larson EB, Green R, Myers AJ, Dufouil C, Todd S, Wallon D, Love S, Rogaeva E, Gallacher J, St George-Hyslop P, Clarimon J, Lleo A, Bayer A, Tsuang DW, Yu L, Tsolaki M, Bossù P, Spalletta G, Proitsi P, Collinge J, Sorbi S, Sanchez-Garcia F, Fox NC, Hardy J, Deniz Naranjo MC, Bosco P, Clarke R, Brayne C, Galimberti D, Mancuso M, Matthews F, European Alzheimer's Disease

Initiative (EADI), Genetic and Environmental Risk in Alzheimer's Disease, Alzheimer's Disease Genetic Consortium, Cohorts for Heart and Aging Research in Genomic Epidemiology, Moebus S, Mecocci P, Del Zompo M, Maier W, Hampel H, Pilotto A, Bullido M, Panza F, Caffarra P, Nacmias B, Gilbert JR, Mayhaus M, Lannefelt L, Hakonarson H, Pichler S, Carrasquillo MM, Ingelsson M, Beekly D, Alvarez V, Zou F, Valladares O, Younkin SG, Coto E, Hamilton-Nelson KL, Gu W, Razquin C, Pastor P, Mateo I, Owen MJ, Faber KM, Jonsson PV, Combarros O, O'Donovan MC, Cantwell LB, Soininen H, Blacker D, Mead S, Mosley TH, Bennett DA, Harris TB, Fratiglioni L, Holmes C, de Bruijn RF, Passmore P, Montine TJ, Bettens K, Rotter JI, Brice A, Morgan K, Foroud TM, Kukull WA, Hannequin D, Powell JF, Nalls MA, Ritchie K, Lunetta KL, Kauwe JS, Boerwinkle E, Riemenschneider M, Boada M, Hiltuenen M, Martin ER, Schmidt R, Rujescu D, Wang LS, Dartigues JF, Mayeux R, Tzourio C, Hofman A, Nöthen MM, Graff C, Psaty BM, Jones L, Haines JL, Holmans PA, Lathrop M, Pericak-Vance MA, Launer LJ, Farrer LA, van Duijn CM, Van Broeckhoven C, Moskvina V, Seshadri S, Williams J, Schellenberg GD, Amouyel P (2013) Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* **45**, 1452–1458.

[32] Zhao L (2019) CD33 in Alzheimer's Disease - Biology, Pathogenesis, and Therapeutics: A Mini-Review. *Gerontology* **65**, 323–331.

[33] AlzGene.

[34] Jonaitis EM, Koscik RL, La Rue A, Johnson SC, Hermann BP, Sager MA (2015) Aging, Practice Effects, and Genetic Risk in the Wisconsin Registry for Alzheimer's Prevention. *Clin Neuropsychol* **29**, 426–441.

[35] Magee L (1990) R2 Measures Based on Wald and Likelihood Ratio Joint Significance Tests. *Am Stat* **44**, 250–253.

[36] Kramer M (2005) R^2 STATISTICS FOR MIXED MODELS. *Conf Appl Stat Agric*.

[37] Monsell SE, Dodge HH, Zhou X-H, Bu Y, Besser LM, Mock C, Hawes SE, Kukull WA, Weintraub S (2016) Results from the NACC Uniform Data Set neuropsychological battery Crosswalk Study

Running head: Neuropsychological Battery Crosswalk Study Results. *Alzheimer Dis Assoc Disord* **30**, 134–139.

[38] Panyard DJ, Kim KM, Darst BF, Deming YK, Zhong X, Wu Y, Kang H, Carlsson CM, Johnson SC, Asthana S, Engelman CD, Lu Q (2021) Cerebrospinal fluid metabolomics identifies 19 brain-related phenotype associations. *Commun Biol* **4**, 1–11.

[39] Bellou E, Baker E, Leonenko G, Bracher-Smith M, Daunt P, Menzies G, Williams J, Escott-Price V (2020) Age-dependent effect of APOE and polygenic component on Alzheimer's disease. *Neurobiol Aging* **93**, 69–77.

[40] Zimmerman SC, Brenowitz WD, Calmasini C, Ackley SF, Graff RE, Asiimwe SB, Staffaroni AM, Hoffmann TJ, Glymour MM (2022) Association of Genetic Variants Linked to Late-Onset Alzheimer Disease With Cognitive Test Performance by Midlife. *JAMA Netw Open* **5**, e225491.

[41] Hanseeuw BJ, Betensky RA, Jacobs HIL, Schultz AP, Sepulcre J, Becker JA, Cosio DMO, Farrell M, Quiroz YT, Mormino EC, Buckley RF, Papp KV, Amariglio RA, Dewachter I, Ivanoiu A, Huijbers W, Hedden T, Marshall GA, Chhatwal JP, Rentz DM, Sperling RA, Johnson K (2019) Association of Amyloid and Tau With Cognition in Preclinical Alzheimer Disease. *JAMA Neurol* **76**, 915–924.

[42] Chiu M-J, Fan L-Y, Chen T-F, Chen Y-F, Chieh J-J, Horng H-E (2017) Plasma Tau Levels in Cognitively Normal Middle-Aged and Older Adults. *Front Aging Neurosci* **9**,.

[43] Jansen IE, Savage JE, Watanabe K, Bryois J, Williams DM, Steinberg S, Sealock J, Karlsson IK, Hägg S, Athanasiu L, Voyle N, Proitsi P, Witoelar A, Stringer S, Aarsland D, Almdahl IS, Andersen F, Bergh S, Bettella F, Bjornsson S, Brækhus A, Bråthen G, de Leeuw C, Desikan RS, Djurovic S, Dumitrescu L, Fladby T, Hohman TJ, Jonsson PV, Kiddle SJ, Rongve A, Saltvedt I, Sando SB, Selbæk G, Shoai M, Skene NG, Snaedal J, Stordal E, Ulstein ID, Wang Y, White LR, Hardy J, Hjerling-Leffler J, Sullivan PF, van der Flier WM, Dobson R, Davis LK, Stefansson H, Stefansson K, Pedersen NL, Ripke S, Andreassen OA, Posthuma D (2019) Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nat Genet* **51**, 404–413.

[44] de Rojas I, Moreno-Grau S, Tesi N, Grenier-Boley B, Andrade V, Jansen IE, Pedersen NL, Stringa N, Zettergren A, Hernández I, Montrreal L, Antúnez C, Antonell A, Tankard RM, Bis JC, Sims R, Bellenguez C, Quintela I, González-Perez A, Calero M, Franco-Macías E, Macías J, Blesa R, Cervera-Carles L, Menéndez-González M, Frank-García A, Royo JL, Moreno F, Huerto Vilas R, Baquero M, Diez-Fairen M, Lage C, García-Madrona S, García-González P, Alarcón-Martín E, Valero S, Sotolongo-Grau O, Ullgren A, Naj AC, Lemstra AW, Benaque A, Pérez-Cordón A, Benussi A, Rábano A, Padovani A, Squassina A, de Mendonça A, Arias Pastor A, Kok AAL, Meggy A, Pastor AB, Espinosa A, Corma-Gómez A, Martín Montes A, Sanabria Á, DeStefano AL, Schneider A, Haapasalo A, Kinhult Ståhlbom A, Tybjærg-Hansen A, Hartmann AM, Spottke A, Corbatón-Anchuelo A, Rongve A, Borroni B, Arosio B, Nacmias B, Nordestgaard BG, Kunkle BW, Charbonnier C, Abdelnour C, Masullo C, Martínez Rodríguez C, Muñoz-Fernandez C, Dufouil C, Graff C, Ferreira CB, Chillotti C, Reynolds CA, Fenoglio C, Van Broeckhoven C, Clark C, Pisanu C, Satizabal CL, Holmes C, Buiza-Rueda D, Aarsland D, Rujescu D, Alcolea D, Galimberti D, Wallon D, Seripa D, Grünblatt E, Dardiotis E, Düzel E, Scarpini E, Conti E, Rubino E, Gelpi E, Rodriguez-Rodriguez E, Duron E, Boerwinkle E, Ferri E, Tagliavini F, Küçükali F, Pasquier F, Sanchez-Garcia F, Mangialasche F, Jessen F, Nicolas G, Selbæk G, Ortega G, Chêne G, Hadjigeorgiou G, Rossi G, Spalletta G, Giaccone G, Grande G, Binetti G, Papenberg G, Hampel H, Bailly H, Zetterberg H, Soininen H, Karlsson IK, Alvarez I, Appollonio I, Giegling I, Skoog I, Saltvedt I, Rainero I, Rosas Allende I, Hort J, Diehl-Schmid J, Van Dongen J, Vidal J-S, Lehtisalo J, Wiltfang J, Thomassen JQ, Kornhuber J, Haines JL, Vogelgsang J, Pineda JA, Fortea J, Popp J, Deckert J, Buerger K, Morgan K, Fließbach K, Sleegers K, Molina-Porcel L, Kilander L, Weinhold L, Farrer LA, Wang L-S, Kleineidam L, Farotti L, Parnetti L, Tremolizzo L, Hausner L, Benussi L, Froelich L, Ikram MA, Deniz-Naranjo MC, Tsolaki M, Rosende-Roca M, Löwenmark M, Hulsman M, Spallazzi M, Pericak-Vance MA, Esiri M, Bernal Sánchez-Arjona M, Dalmasso MC, Martínez-Larrad MT, Arcaro M, Nöthen MM, Fernández-Fuertes M, Dichgans M, Ingelsson M, Herrmann MJ, Scherer M, Vyhnalek M, Kosmidis MH, Yannakoulia M, Schmid M, Ewers M, Heneka MT,

Wagner M, Scamosci M, Kivipelto M, Hiltunen M, Zulaica M, Alegret M, Fornage M, Roberto N, van Schoor NM, Seidu NM, Banaj N, Armstrong NJ, Scarmeas N, Scherbaum N, Goldhardt O, Hanon O, Peters O, Skrobot OA, Quenez O, Lerch O, Bossù P, Caffarra P, Dionigi Rossi P, Sakka P, Hoffmann P, Holmans PA, Fischer P, Riederer P, Yang Q, Marshall R, Kalaria RN, Mayeux R, Vandenberghe R, Cecchetti R, Ghidoni R, Frikke-Schmidt R, Sorbi S, Hägg S, Engelborghs S, Helisalmi S, Botne Sando S, Kern S, Archetti S, Boschi S, Fostinelli S, Gil S, Mendoza S, Mead S, Ciccone S, Djurovic S, Heilmann-Heimbach S, Riedel-Heller S, Kuulasmaa T, del Ser T, Lebouvier T, Polak T, Ngandu T, Grimmer T, Bessi V, Escott-Price V, Giedraitis V, Deramecourt V, Maier W, Jian X, Pijnenburg YAL, Kehoe PG, Garcia-Ribas G, Sánchez-Juan P, Pastor P, Pérez-Tur J, Piñol-Ripoll G, Lopez de Munain A, García-Alberca JM, Bullido MJ, Álvarez V, Lleó A, Real LM, Mir P, Medina M, Scheltens P, Holstege H, Marquié M, Sáez ME, Carracedo Á, Amouyel P, Schellenberg GD, Williams J, Seshadri S, van Duijn CM, Mather KA, Sánchez-Valle R, Serrano-Ríos M, Orellana A, Tárraga L, Blennow K, Huisman M, Andreassen OA, Posthuma D, Clarimón J, Boada M, van der Flier WM, Ramirez A, Lambert J-C, van der Lee SJ, Ruiz A (2021) Common variants in Alzheimer's disease and risk stratification by polygenic risk scores. *Nat Commun* **12**, 3417.

Data, tables, and figures

Table 1. Participant Characteristics for WRAP

Variable	**Full sample (N=1,175)**	**Sample with CSF (N=197)**
Baseline Age	54.16 (6.53)	61.98 (6.64)
Education (years)	15.81 (2.24)	16.16 (2.14)
Gender (male)	353 (30%)	69 (35%)
Family history of AD	858 (73%)	142 (72%)
Max visits		
1	41 (4%)	70 (36%)
2	85 (7%)	42 (21%)
3	182 (15%)	60 (30%)
4	369 (31%)	23 (12%)
5	498 (42%)	2 (1%)
***APOE* genotypes**		
e2/e2	4 (0.3%)	0 (0)
e2/e3	96 (8%)	21 (11%)
e3/e3	619 (53%)	107 (54%)
e2/e4	39 (3%)	6 (3%)
e3/e4	369 (31%)	56 (28%)
e4/e4	43 (4%)	6 (3%)
CSF Aβ42 (pg/mL)	N/A	899.31 (391.24)
CSF Aβ42/40	N/A	0.06 (0.02)
CSF T-tau (pg/mL)	N/A	208.82 (69.89)
CSF P-tau (pg/mL)	N/A	18.34 (6.71)

Mean (SD); n (%)

Table 2. Participant characteristics for the Wisconsin ADRC

	Wisconsin ADRC AHC	
Variable	**PACC3-TMT (N=427)**	**CSF Biomarker (N=259)**
Baseline Age	59.95 (8.26)	60.53 (8.08)
Education (years)	16.35 (2.46)	16.20 (2.39)
Gender (male)	149 (35%)	80 (31%)
Family history of AD	299 (70%)	192 (74%)
Max visits		
1	31 (7%)	203 (78%)
2	56 (13%)	34 (13%)
3	60 (14%)	6 (2%)
4	34 (8%)	11 (4%)
5	246 (58%)	3 (1%)
6		1 (0.4%)
7		1 (0.4%)
***APOE* genotype**		
e2/e2	1 (0.2%)	1 (0.4%)
e2/e3	49 (11%)	30 (12%)
e3/e3	219 (51%)	132 (51%)
e2/e4	14 (3%)	7 (3%)
e3/e4	124 (29%)	76 (29%)
e4/e4	20 (5%)	13 (5%)
CSF Aβ42 (pg/mL)	N/A	957.40 (381.79)
CSF Aβ42/40	N/A	0.07 (0.01)
CSF T-tau (pg/mL)	N/A	194.47 (73.58)
CSF P-tau (pg/mL)	N/A	17.00 (6.91)

Mean (SD); n (%)

Supplementary table 1. Partial R-squared for each model in WRAP with cognitive outcomes. Based on a reduced subset of WRAP participants who had all PRSs

	APP		Immune		Cholesterol		Endocytosis		Tau		Axon		APOE		Overall	
Outcome	***PRS***	***Interaction***	***PRS***	***Interaction***	***PRS***	***Interaction***	***PRS***	***Interaction***	***PRS***	***Interaction***	***PRS***	***Interaction***	***APOE Score***	***Interaction***	***PRS***	***Interaction***
APOE included																
Delayed Recall	**0.002**	0.009	/	/	**0.002**	0.009	0.001	0.010	0.001	0.008	/	/	0.001	0.008	0.001	0.009
Executive Function	**0.001**	0.007	/	/	**0.001**	0.007	0.000	0.008	0.000	0.007	/	/	**0.001**	0.006	0.000	0.007
Immediate Learning	**0.002**	0.011	/	/	**0.002**	0.010	0.001	0.011	0.001	0.010	/	/	**0.002**	0.010	0.001	0.010
PACC3	**0.002**	0.010	/	/	**0.002**	0.010	**0.002**	0.011	0.001	0.010	/	/	**0.002**	0.010	0.001	0.011
APOE excluded																
Delayed Recall	0.000	0.002	0.000	0.000	**0.001**	0.001	0.000	0.005	0.000	0.001	**0.001**	0.000	/	/	0.000	0.001
Executive Function	0.000	0.001	0.000	0.001	0.000	0.001	0.000	0.005	0.000	0.002	0.000	0.000	/	/	0.000	0.002
Immediate Learning	0.000	0.001	0.000	0.001	**0.001**	0.000	0.000	0.002	0.000	0.000	**0.001**	0.000	/	/	0.000	0.001
PACC3	0.000	0.001	0.000	0.001	**0.001**	0.000	0.000	0.004	0.000	0.001	**0.001**	0.001	/	/	0.000	0.003

Bolded value explains the most percentage of variance in outcome for single PRS; Underscored value explains the most percentage of variance in outcome for interaction terms.

APP metabolism pathway: CLU, SOR1, ABCA7, PICALM, ADAM10, APOE

Cholesterol metabolism pathway: CLU, SORL1, ABCA7, APOE

Endocytosis pathway: SORL1, ABCA7, PICALM, BIN1, CD2AP, PTK2B, FERMT2, SLC24A4, APOE

Tau pathway: BIN1, FERMT2, CASS4, APOE

Immune response: CLU, ABCA7, CR1, INPP5D, HLA-DRB1, TREM2, EPHA1, MS4A6A, CD33, MEF2C

Axonal development: EPHA1, FERMT2, CASS4, SPI1, NME8

Supplementary table 2. Partial R-squared for each model in WRAP with biomarker outcome. Based on a reduced subset of WRAP participants who had all PRSs

Outcome	APP		Immune		Cholesterol		Endocytosis		Tau		Axon		APOE		Overall	
	PRS	Interaction	PRS	Interaction	PRS	Interaction	PRS	Interaction	PRS	Interaction	PRS	Interaction	APOE Score	Interaction	PRS	Interaction
APOE included																
Aβ42	0.039	0.035	/	/	**0.041**	0.035	0.036	0.032	0.035	0.029	/	/	0.039	0.031	0.029	0.037
Aβ42/40	**0.079**	0.024	/	/	0.078	0.022	0.073	0.018	0.066	0.016	/	/	0.071	0.019	0.072	0.022
P TAU	0.009	0.020	/	/	0.008	0.016	0.008	0.019	0.006	0.011	/	/	0.005	0.012	**0.010**	0.021
T_TAU	**0.009**	0.017	/	/	0.007	0.014	0.007	0.018	0.005	0.013	/	/	0.005	0.012	**0.009**	0.021
APOE excluded																
Aβ42	0.001	0.007	0.000	0.01	**0.004**	0.006	0.000	0.003	0.000	0.000	0.000	0.000	/	/	0.000	0.010
Aβ42/40	0.013	0.011	0.01	0.007	**0.015**	0.006	0.005	0.001	0.000	0.002	0.001	0.001	/	/	0.008	0.007
P_TAU	**0.013**	0.036	0.003	0.017	0.010	0.014	0.006	0.019	0.001	0.004	0.002	0.001	/	/	0.008	0.022
T_TAU	**0.012**	0.027	0.003	0.015	0.008	0.009	0.004	0.017	0.000	0.005	0.002	0.001	/	/	0.006	0.021

Bolded value explains the most percentage of variance in outcome for single PRS; Underscored value explains the most percentage of variance in outcome for interaction terms.

APP metabolism pathway: CLU, SOR1, ABCA7, PICALM, ADAM10, APOE

Cholesterol metabolism pathway: CLU, SORL1, ABCA7, APOE

Endocytosis pathway: SORL1, ABCA7, PICALM, BIN1, CD2AP, PTK2B, FERMT2, SLC24A4, APOE

Tau pathway: BIN1, FERMT2, CASS4, APOE

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Axonal development: EPHA1, FERMT2, CASS4, SPI1, NME8

Supplementary table 3. Partial R-squared for each model in the Wisconsin ADRC with biomarker outcome and cognitive outcome. Based on a reduced subset of Wisconsin ADRC participants who had all PRSs

Outcome	APP		Immune		Cholesterol		Endocytosis		Tau		Axon		APOE		Overall	
	PRS	**Interaction**	**PRS**	**Interaction**	**PRS**	**Interaction**	**PRS**	**Interaction**	**PRS**	**Interaction**	**PRS**	**Interaction**	**Score**	**Interaction**	**PRS**	**Interaction**
APOE included																
PACC3_TMT	0.001	0.003	/	/	0.001	0.003	0.001	0.004	0.001	0.004	/	/	0.001	0.003	0.000	0.003
Aβ42	0.031	0.010	/	/	**0.033**	0.010	0.031	0.004	0.024	0.003	/	/	0.027	0.004	0.028	0.010
Aβ42/40	0.075	0.023	/	/	**0.079**	0.023	0.066	0.022	0.066	0.021	/	/	0.071	0.019	0.065	0.026
P_TAU	0.009	0.020	/	/	**0.010**	0.022	0.007	0.023	**0.010**	0.022	/	/	**0.010**	0.020	**0.010**	0.027
T_TAU	0.004	0.015	/	/	0.004	0.017	0.003	0.016	**0.005**	0.016	/	/	**0.005**	0.016	0.004	**0.019**
APOE excluded																
PACC3_TMT	0.000	0.005	**0.002**	0.001	0.000	0.007	0.000	0.003	0.000	0.003	**0.002**	0.001	/	/	**0.002**	0.001
Aβ42	0.005	0.007	0.000	0.002	**0.009**	0.010	0.003	0.000	0.002	0.001	0.000	0.001	/	/	0.000	0.000
Aβ42/40	0.001	0.001	0.000	0.005	**0.006**	0.007	0.000	0.000	0.002	0.002	0.001	0.001	/	/	0.000	0.002
P_TAU	0.001	0.010	0.002	0.007	0.000	0.000	**0.002**	0.029	0.000	**0.030**	**0.002**	0.017	/	/	0.000	0.016
T_TAU	0.004	0.010	0.000	0.006	0.001	0.000	**0.005**	0.035	0.000	0.029	0.001	0.013	/	/	0.001	0.017

Bolded value explains the most percentage of variance in outcome for single PRS; Underscored value explains the most percentage of variance in outcome for interaction terms.

APP metabolism pathway: CLU, SOR1, ABCA7, PICALM, APOE

Cholesterol metabolism pathway: CLU, SORL1, ABCA7, APOE

Endocytosis pathway: SORL1, ABCA7, PICALM, BIN1, CD2AP, PTK2B, FERMT2, SLC24A4, APOE

Tau pathway: BIN1, FERMT2, CASS4, APOE

Immune response: CLU, ABCA7, CR1, INPP5D, HLA-DRB1, TREM2, EPHA1, MS4A6A, CD33, MEF2C

Axonal development: EPHA1, FERMT2, CASS4, SPI1, NME

WRAP main analysis figures

Figure 1. Effect of Pathway-specific PRSs on Cognition Change at different Age points with 95% Confidence Interval in WRAP

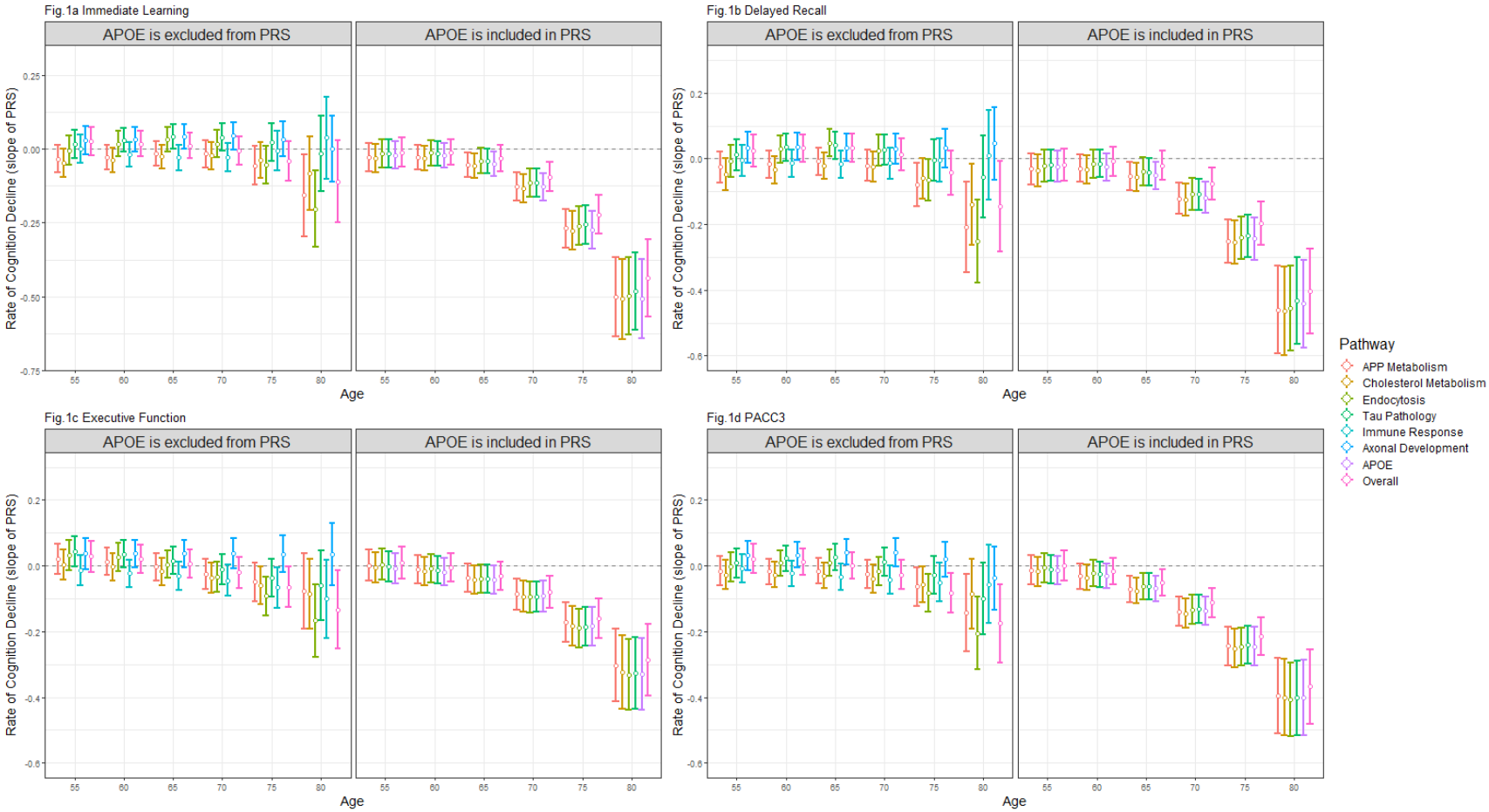


Figure 1 presents the effects of p-PRSs/PRS on immediate learning (Figure 1a), delayed recall (Figure 1b), executive function (Figure 1c), and PACC3 (Figure 1d) at various age points in WRAP with 95% confidence intervals. Within each figure, the left panel depicts the effects of p-PRSs/PRS, excluding *APOE* score, and the results of p-PRSs/PRS including *APOE* score are shown in the right panel. *APOE* is not theoretically affecting immune response and axonal development pathways, so the effects of p-PRSs/PRS of these two pathways are only shown in the left panel. All association analyses are performed using the linear mixed effect model and adjusted for within-individual and within-family correlation. In addition to p-PRSs/PRS, age (cubic), and their interactions, additional covariates include gender, education years, practice effect, and the first five genetic principal components of ancestry.

Figure 2. Effect of Pathway-specific PRSs on Biomarker Change at different Age points with 95% Confidence Interval in WRAP

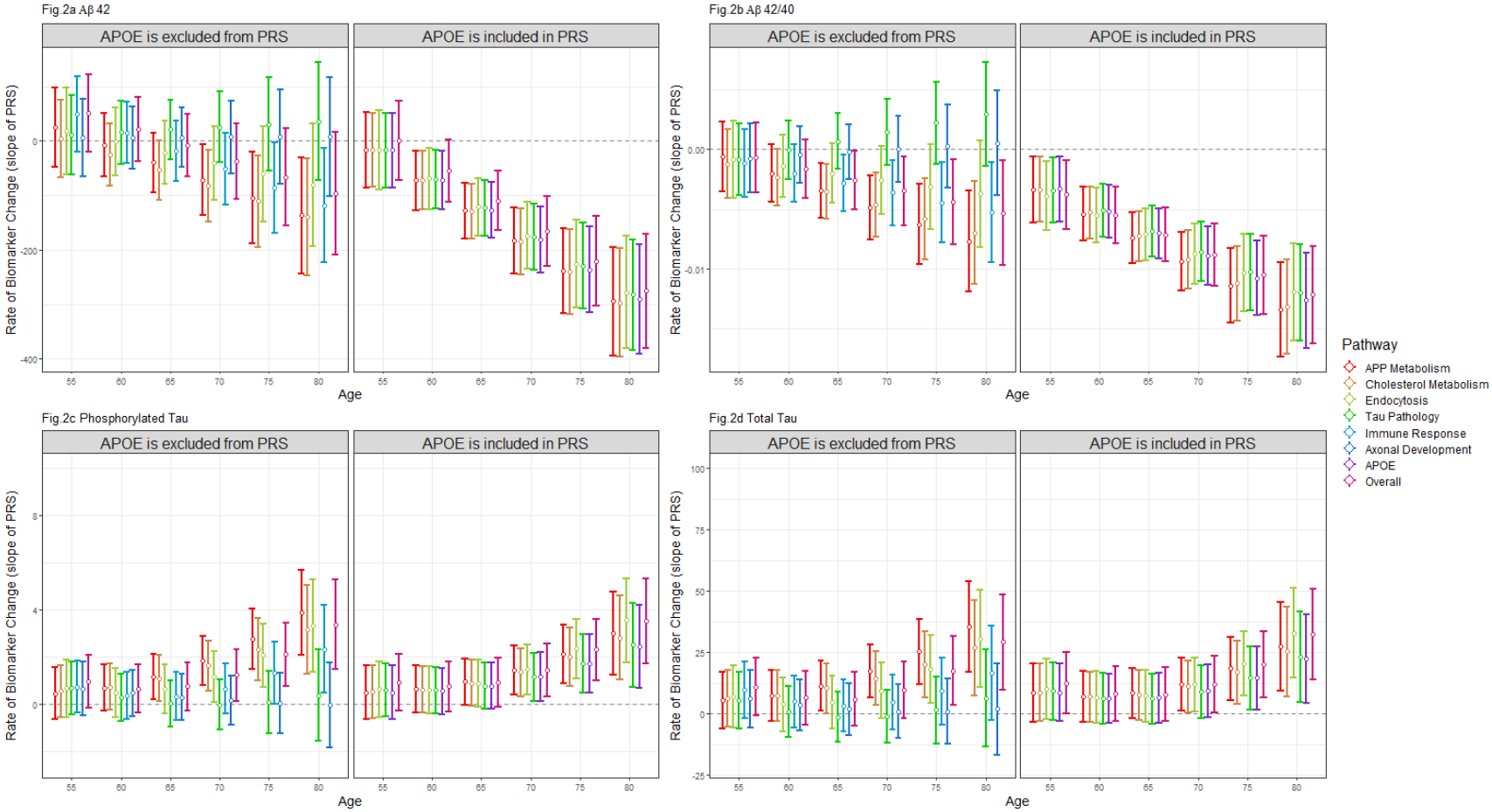
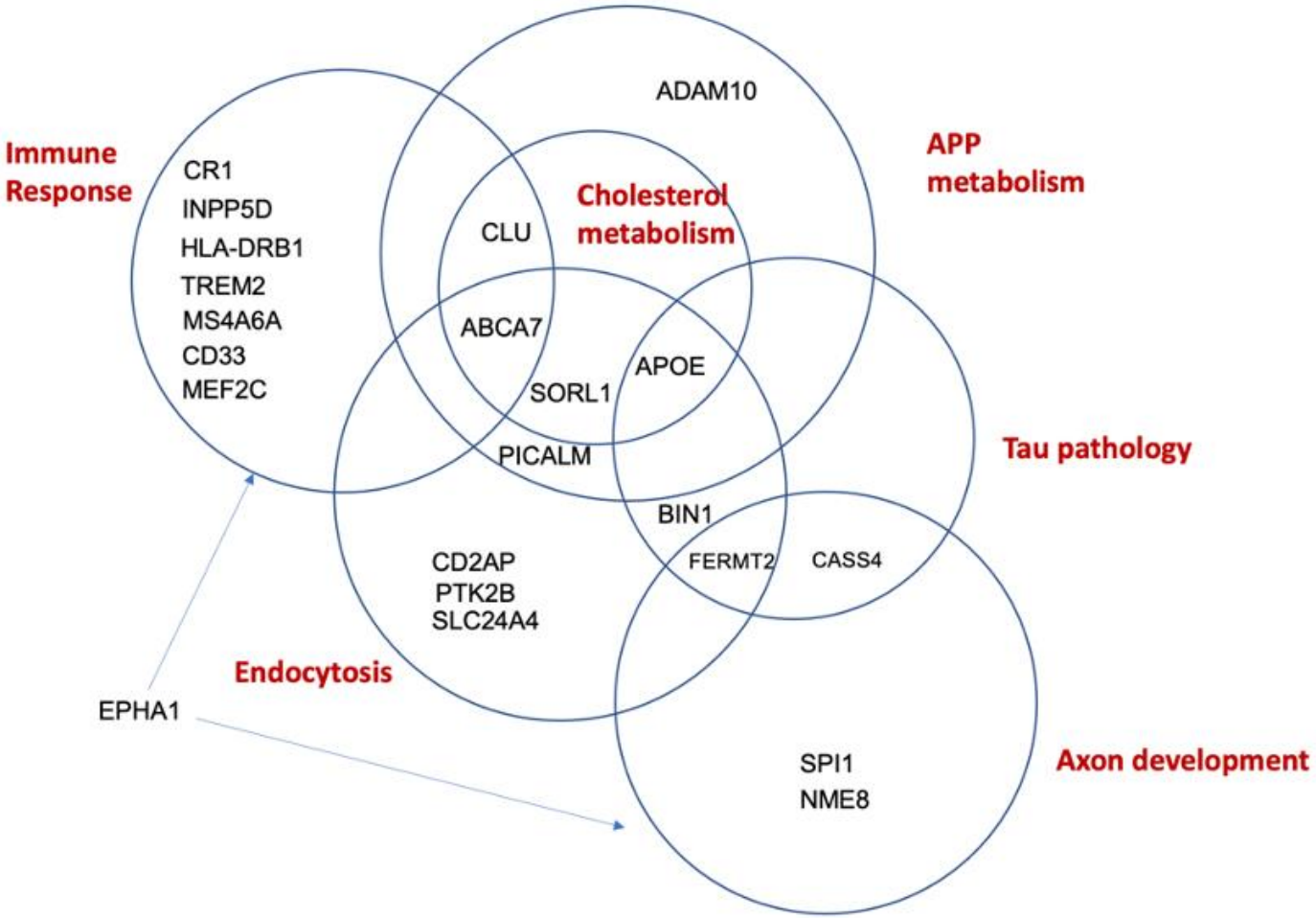


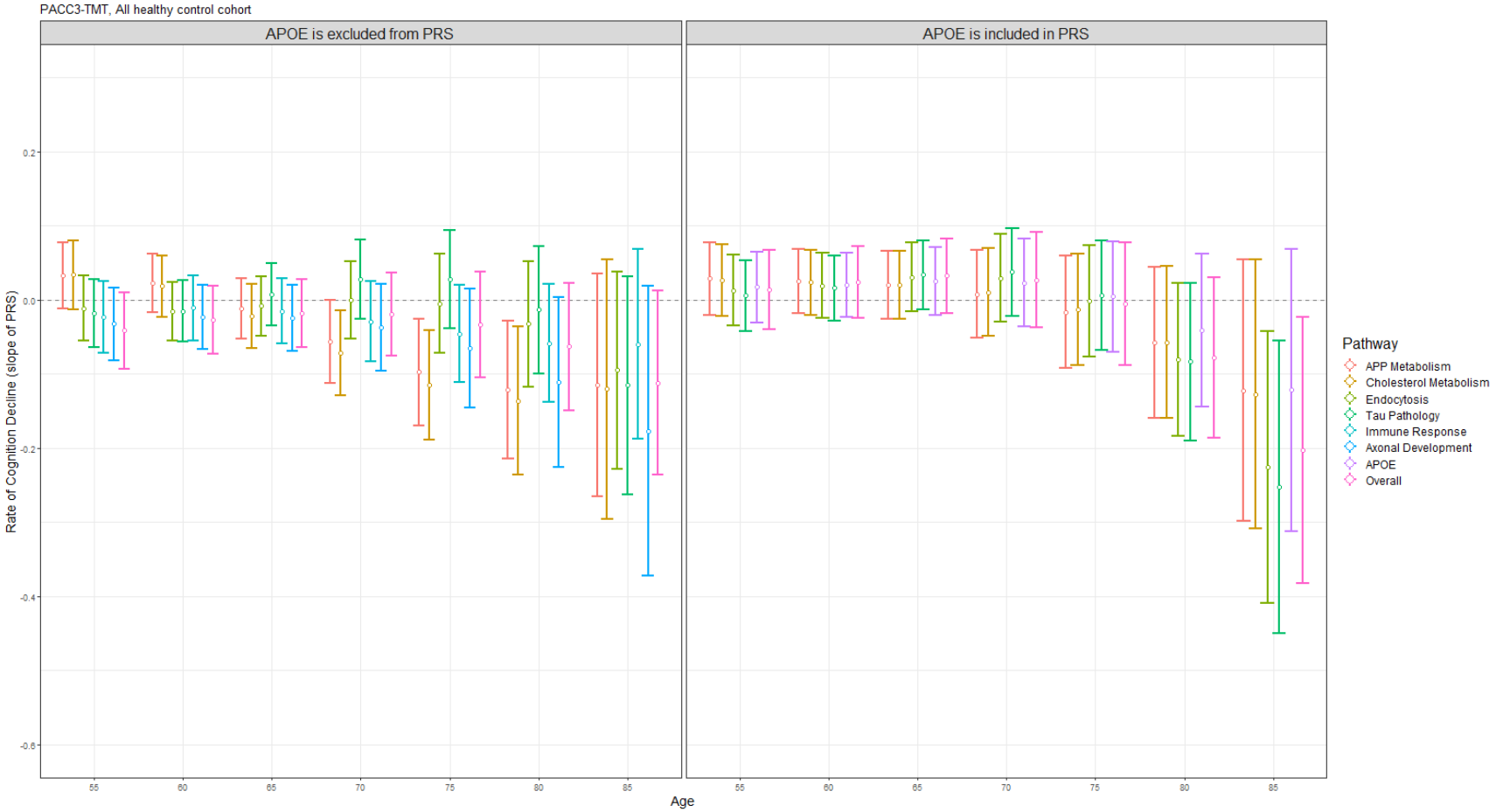
Figure 2 presents the effects of p-PRSs/PRS on beta-amyloid 42 (Figure 2a), beta-amyloid 42/40 ratio (Figure 2b), phosphorylated tau (Figure 2c), and total tau (Figure 2d) at various age points in WRAP with 95% confidence interval. Within each figure, the left panel depicts the effects of p-PRSs/PRS, excluding *APOE* score, and the results of p-PRSs/PRS including *APOE* score are shown in the right panel. *APOE* is not theoretically affecting immune response and axonal development pathways, so the effects of p-PRSs/PRS of these two pathways are only shown in the left panel. All association analyses are performed using the linear mixed effect model and adjusted for within-individual and within-family correlation. Spaghetti plots determine the functional form of age for all biomarker analyses. In addition to p-PRSs/PRS, age, and their interactions, additional covariates include gender, education years, and the first five genetic principal components of ancestry.

Supplementary Figures

Supplementary figure 1. Venn diagram of the gene-pathway mapping

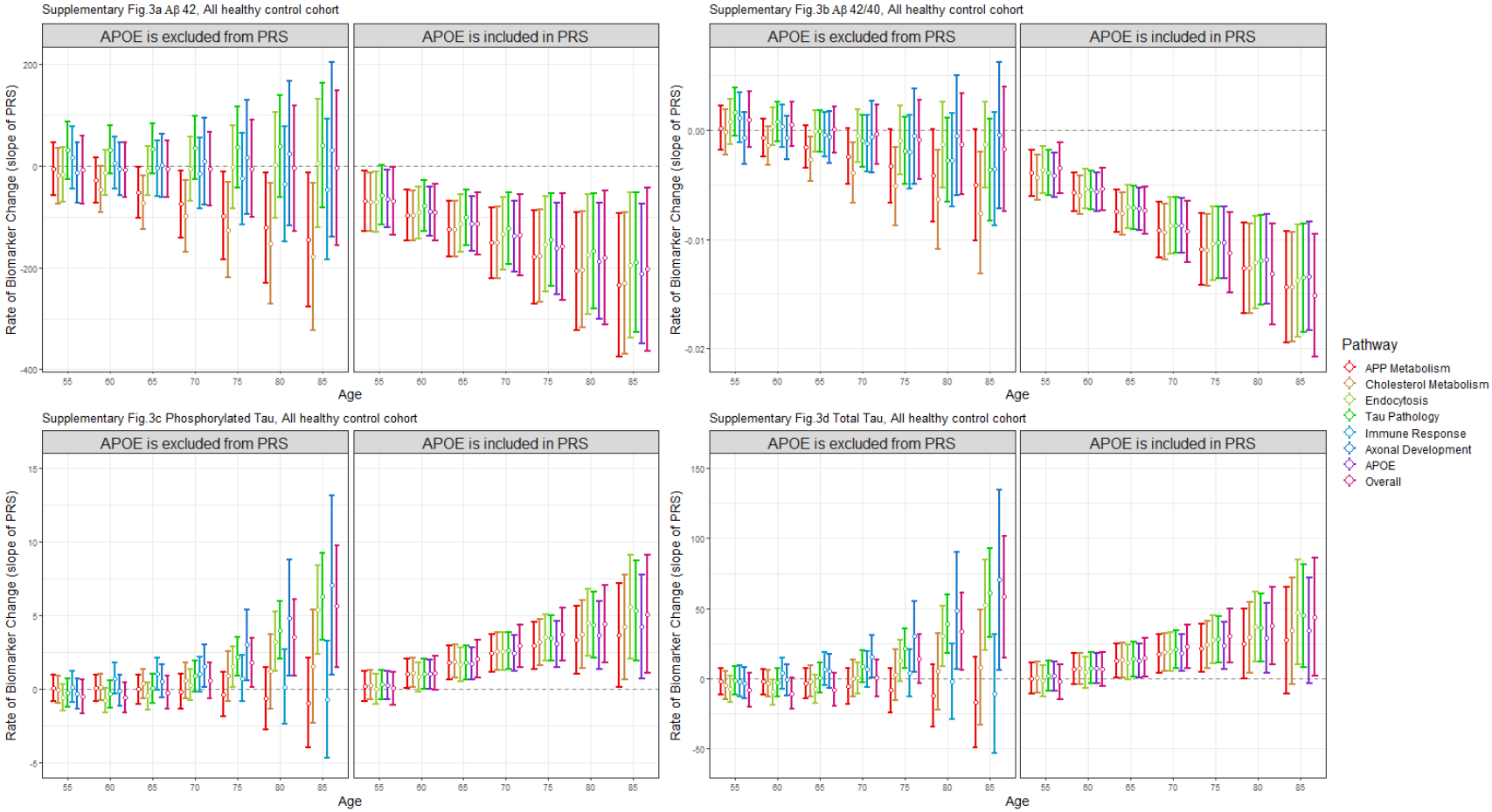


Supplementary Figure 2. Effect of Pathway-specific PRSs on Cognition Change at different Age points with 95% Confidence Interval in Wisconsin ADRC



Supplementary Figure 2 presents the effects of p-PRSs/PRS on the PACC3-TMT score at various age points in the AHC sample extracted from the Wisconsin ADRC with a 95% confidence interval. Within each figure, the left panel depicts the effects of p-PRSs/PRS, excluding *APOE* score, and the results of p-PRSs/PRS including *APOE* score are shown in the right panel. *APOE* is not theoretically affecting immune response and axonal development pathways, so the effects of p-PRSs/PRS of these two pathways are only shown in the left panel. All association analyses are performed using the linear mixed effect model and adjusted for within-individual. In addition to p-PRSs/PRS, age (cubic), and their interactions, additional covariates include gender, education years, and practice effect.

Supplementary Figure 3. Effect of Pathway-specific PRSs on Biomarker Change at different Age points with 95% Confidence Interval in Wisconsin ADRC



Supplementary Figure 3 presents the effects of p-PRSs/PRS on beta-amyloid 42 (Figure 3a), beta-amyloid 42/40 ratio (Figure 3b), phosphorylated tau (Figure 3c), and total tau (Figure 3d) at various age points in the AHC sample extracted from the Wisconsin ADRC with a 95% confidence interval. Within each figure, the left panel depicts the effects of p-PRSs/PRS, excluding *APOE* score, and the results of p-PRSs/PRS including *APOE* score are shown in the right panel. *APOE* is not theoretically affecting immune response and axonal development pathways, so the effects of p-PRSs/PRS of these two pathways are only shown in the left panel. All association analyses are performed using the linear mixed effect model and adjusted for within-individual correlation. The functional form of age for all biomarker analyses is determined by spaghetti plots. In addition to p-PRSs/PRS, age, and their interactions, additional covariates include gender, and education years.