Pathway specific polygenic risk scores as predictors of beta-amyloid deposition and cognitive function in a sample at increased risk for Alzheimer's disease

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ABSTRACT

Polygenic risk scores (PRSs) have been used to combine the effects of variants with small effects identified by genome-wide association studies. We explore the potential for using pathway specific PRSs as predictors of early changes in Alzheimer's disease (AD)-related biomarkers. Participants were from the Wisconsin Registry for Alzheimer's Prevention, a longitudinal study of initially asymptomatic adults enriched for a parental history of AD. Using the genes associated with AD in the International Genomics of Alzheimer's Project's meta-analysis, we identified clusters of genes that grouped into pathways involved in Aβ deposition: Aβ clearance and cholesterol metabolism. Weighted pathway specific and overall PRSs were developed and compared to *APOE* alone. Mixed models were used to assess whether each PRS was associated with cognition in 1,200 individuals, cerebral Aβ deposition measured using amyloid ligand (Pittsburgh compound B) positron emission imaging (PET) in 168 individuals, and cerebrospinal fluid (CSF) Aβ levels in 111 individuals. We found that the Cholesterol Metabolism PRS tended to be either a similar or stronger predictor than *APOE*, while both the Cholesterol and Aβ Clearance PRSs were stronger predictors than the Overall PRS. Thus, for investigations of disease-related traits, limiting a PRS to variants known to contribute to a biological pathway of the particular trait may be a more informative approach than including variants known to contribute to the disease more broadly. In the future, pathway specific PRSs could be used to prioritize screening for Aβ accumulation.

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INTRODUCTION

Alzheimer's disease (AD) is clinically characterized by a decline in cognitive function. However, β-amyloid (Aβ) deposits as plaques and hyperphosphorylation and aggregation of tau into tangles are believed to have accumulated in the brain of an AD patient long before cognitive decline is evident[1]. This accumulation causes blocked neural signaling and nutrient transport, respectively, and is thought to lead to the characteristic neurodegeneration of AD. It has been hypothesized that an imbalance between Aβ production and Aβ clearance leads to Aβ accumulation (REF). Aβ clearance rates have been shown to be reduced in late onset AD (LOAD) patients while Aβ production rates appear to be normal[2]. Changes in cholesterol metabolism is another characteristic of AD and may be closely linked to Aβ production and Aβ clearance[3]. Studying these risk factors could help explain the underlying genetic mechanisms that contribute to the onset of AD.

In a meta-analysis of genome-wide association studies (GWAS) that included over 74,000 individuals with and without LOAD, 11 novel susceptibility single nucleotide polymorphisms (SNPs) were identified, as well as 8 that had previously been found to be associated with LOAD[4]. While this study confirmed that many genetic factors contribute to the risk of LOAD, these variants each have fairly small effect sizes (Supplementary Table 1). Polygenic risk scores (PRSs) utilize allele-counting methods to combine the effects of many individual SNPs and have been found to produce good predictors[5, 6]. While an overall PRS that combines the effects of all LOAD associated SNPs may be powerful for predicting cognitive

function and decline due to underlying LOAD pathology, a pathway specific PRS[7] may be more powerful for LOAD biomarkers, such as Aβ deposition.

Using a longitudinal sample of cognitively healthy adults enriched for a parental history of LOAD from the Wisconsin Registry for Alzheimer's Prevention (WRAP), we explore the potential for using pathway specific PRSs as predictors of early changes in AD-related biomarkers. In particular, we created weighted PRSs for Aβ Clearance and Cholesterol Metabolism, in addition to an Overall PRS, using the single most significant variant from each gene identified to be associated with LOAD in the International Genomics of Alzheimer's Project's (IGAP) meta-analysis. These PRSs were assessed against cerebrospinal fluid (CSF) levels of Aβ42 (reflecting Aβ deposition in the brain) and tau protein (reflecting neurodegeneration) for relevant PRSs, [C-11]Pittsburgh compound B (PiB) positron emission tomography (PET), and cognition. This unique study design could have the potential to identify individuals at increased genetic risk for AD pathology before the onset of clinical symptoms. This could be particularly useful as therapies to target particular pathways become available.

MATERIALS AND METHODS

Participants

Study participants were from WRAP, a longitudinal study of initially asymptomatic middle-aged adults enriched for a parental history of LOAD. A positive parental history was defined as having one or both parents with either autopsy-confirmed or probable AD as defined by NINCDS-ADRDA research criteria[8]. Baseline recruitment began in 2001 with initial follow up after 4 years and subsequent ongoing follow up every 2 years. Further details of the study design and methods used have been previously described[9-11].

The present analyses were limited to non-Hispanic Caucasian participants due to sample size limitations of other racial groups. Participants were excluded if they reported having diseases or comorbidities that might be expected to influence cognitive test performance (e.g., multiple sclerosis, Parkinson's disease, stroke, epilepsy/seizures, or meningitis) or developed AD on or before the second visit.

This study was conducted with the approval of the University of Wisconsin Institutional Review Board and all subjects provided signed informed consent before participation.

DNA Collection, Genotyping, and Quality Control

DNA was extracted from whole blood samples using the PUREGENE® DNA Isolation Kit (Gentra Systems, Inc., Minneapolis, MN). DNA concentrations were quantified using UV spectrophotometry (DU® 530 Spectrophotometer, Beckman Coulter, Fullerton, CA).

A total of 37 SNPs in 1,448 individuals were genotyped by LGC Genomics (Beverly, MA) using competitive allele-specific PCR based KASP™ genotyping assays. Duplicate quality control (QC) samples from 102 individuals were placed randomly throughout each of the 96-well plates. The genotype concordance rate was 99.89%. All discordant genotypes were set to missing. To assess the potential for sample mislabeling, we checked concordance against a previous round of genotyping that included three overlapping variants (rs7412 and rs429358 in apolipoprotein E (*APOE*), and rs6656401 in *CR1*) in 1,152 individuals. Six genotypes were discordant in six different individuals, who were removed from further analyses.

Further QC was conducted using PLINK v1.07[12]. We removed 27 individuals due to high missingness (>10%), 0 SNPs due to low call rates (<95%), and 4 SNPs due to being monomorphic in our sample (2 SNPs from an African-American GWAS meta-analysis[13]:

rs145848414 (an intergenic SNP on chromosome 5) and rs6973770 in *EPHA1-AS1*, as well as rs4819 in *PLD3*, and rs63750847 in *APP*). Hardy-Weinberg equilibrium (HWE) was assessed among a subset of 1,120 unrelated individuals using a Bonferroni adjusted p-value threshold of .05/33=.0015. One SNP failed HWE (p=.00104) but was kept due to the marginal difference from the p-value threshold to allow for comparison to existing literature (rs28834970 in *PTK2B*). This left a total of 1,415 individuals and 33 SNPs after the completion of all QC procedures. The present analyses used 21 of these SNPs: the 19 IGAP SNPs (Supplementary Table 1) and the 2 *APOE* SNPs (Table 1).

Polygenic Risk Scores

Pathway specific PRSs were determined according to existing literature and included Aβ Clearance (*PICALM*, *CLU*, *CR1*, and *APOE*)[14, 15] and Cholesterol Metabolism (*ABCA7*, *CLU*, and *APOE*)[3, 16]. An Overall PRS (including all of the IGAP genes, as listed in Supplementary Table 1, plus *APOE*) and an *APOE* alone risk score were also created for comparison. PRSs were calculated for each participant using the following equation:, where *i* represents the individual, whose score is calculated by summing over all SNPs, *n*, in the pathway ranging from *l* to *k*; *OR* is the odds ratio of SNP *n*; and *C* is the individual's count of risk alleles for SNP *n*. If the minor allele was protective, the inverse of the OR was used instead as follows: . With the exception of *APOE*, SNP ORs used in this analysis were taken from the IGAP meta-analysis and are indicated in Table 1. Risk due to *APOE* was not calculated additively, but instead according to the OR of the ε2/ε3/ε4 genotype formed by SNPs rs429358 and rs7412, as indicated in AlzGene[17]. Since *APOE* is known to have a large effect size, PRSs were also calculated excluding *APOE* to determine the effect of the PRS beyond that of *APOE* alone.

Neuropsychometric Assessments

The WRAP cognitive test battery consists of standardized widely used clinical neuropsychological tests, which were selected to provide a comprehensive estimate of cognitive abilities with an emphasis on abilities most likely to be affected in early-stage AD. Factor analysis was conducted to reduce the number of outcome measures to a smaller number of reliable cognitive factors and obtain weights used to combine the measures within each factor[18]. The following 6 cognitive factor scores were used in the present analysis: Immediate Memory, Verbal Learning and Memory, Speed and Flexibility, Visual Learning and Memory, Story Recall, and Working Memory. Tests comprising each of these factors are described elsewhere[19]. The resulting weighted factor scores were then standardized (~N [0,1]) into z-scores, using means and standard deviations obtained from the whole baseline sample.

PiB PET Imaging

[C-11]Pittsburgh compound B (PiB) positron emission tomography (PET) radiochemical synthesis, acquisition parameters and generation of distribution volume ratio (DVR) maps were detailed previously[20]. Briefly, after a 70 minute dynamic [C-11]PiB PET acquisition, PET data were reconstructed using a filtered back-projection algorithm (DIFT) and were corrected for random events, attenuation of annihilation radiation, deadtime, scanner normalization, and scatter radiation and were realigned and coregistered in SPM12. The data were then transformed into voxel-wise DVR maps of [C-11]PiB binding using the time activity curve in the gray matter of the cerebellum as the reference region.

To reduce the number of statistical tests, a summary measure of amyloid burden was calculated by averaging the means of amyloid binding within 8 bilateral regions of interest, as previously described[21].

CSF quantification

CSF was collected via lumbar puncture (LP) in the morning after a 12-hour fast using a Sprotte 25- or 24-gauge spinal needle at L3/4 or L4/5 using gentle extraction into polypropylene syringes. CSF (22 mL) was then combined, gently mixed, and centrifuged at 2000g for 10 minutes. Supernatants were frozen in 0.5 mL aliquots in polypropylene tubes and stored at −80°C.

CSF $A\beta_{42}$, total tau (T-tau), and phosphorylated tau (p-tau) were quantified with sandwich ELISAs (INNOTEST β-amyloid1-42, hTAU-Ag, and Phospho-Tau[181P], respectively; Fujirebio Europe, Ghent, Belgium). For the $A\beta_{42}/A\beta_{40}$ ratio, CSF levels of $A\beta_{42}$ and $A\beta_{40}$ (a less amyloidogenic Aβ fragment as compared to $A\beta_{42}$) were quantified by electrochemiluminescence (ECL) using an Aβ triplex assay (MSD Human Aβ peptide Ultra-Sensitive Kit, Meso Scale Discovery, Gaithersburg, MD). All measurements were performed in one round of analyses using one batch of reagents by board-certified laboratory technicians who were blind to the clinical characteristics of participants. Intra-assay coefficients of variation were below 10%.

Statistical Analyses

Each individual SNP and PRS was included in a linear mixed effects regression model to test for association with the 6 cognitive factor outcomes and Aβ deposition, adjusting for age and

gender. Since genetic effects often vary by age, models were also stratified by the median age of the given subset of participants (i.e., full sample, CSF, and PiB). For individuals with multiple observations, age at each observation was considered instead of categorizing an individual by their age at a particular time point. Random effects for the intercept, participant, and family were included to account for dependency within and between participant's observations and family members. The variance structure used for each outcome was determined using unconditional growth models that included a fixed effect of time. Variance structures tested included unstructured, compound symmetry, and variance components, and that with the lowest Akaike Information Criterion (AIC) was used for the corresponding outcome. Unconditional growth models were similarly used to compare the inclusion of a random effect for time for each outcome. In all models, age was used as the measure of time, as time between visits could vary slightly within and between individuals.

RESULTS

Participants

A total of 1,200 participants met study criteria and had cognitive data available for up to five visits. A subset of 111 and 168 of these participants had CSF and PiB data, respectively, for up to two visits. At baseline, the full set of participants were 53.6 years of age on average, fairly well educated, predominantly female, and most had a parental history of AD. As expected, this sample had a higher frequency of the *APOE* ε4 allele and lower frequency of the ε2 allele than the general population. These characteristics were fairly consistent within the two subsets of participants, although the mean age was about 7 years older in both of them when compared to

the full set of participants, since these studies were ancillary to and began later than the main WRAP study. Participant characteristics are described further in Table 1.

The distribution of each PRS and the *APOE* risk score are described for the full sample in Supplementary Table 2. As expected, the mean PRSs were lower when *APOE* was excluded.

Cognitive Outcomes

There were fewer associations between single SNPs and cognitive outcomes than expected by chance and of the three significant effects, two were not in the expected direction based on the IGAP results (Supplementary Table 3). Effects of the associations between the PRSs and cognitive outcomes were almost always in the direction expected, suggesting that a higher Aβ Clearance, Cholesterol, and Overall PRS trend towards lower cognition (although most of these relationships were not statistically significant; Table 2). The two associations that met statistical significance were in the pathway specific PRSs with the outcome of Working Memory (p=.03, for both), which was also the only statistically significant outcome for *APOE* (p=.04). The model fit of the Aβ Clearance and Cholesterol PRSs compared to the model fit of *APOE* alone was better for each outcome, as measured by AIC, although the Overall PRS had the best model fit (results not shown). Upon removing *APOE* from the PRSs, the Aβ Clearance and Overall PRS associations became insignificant. However, a statistically significant relationship between higher Cholesterol PRS and lower Visual Learning and Memory scores appeared (p=.0498), which was not present while *APOE* was included in the PRSs. An association between the Overall PRS and Speed & Flexibility also appeared (p=.03), however, the effect was not in the expected direction (Table 2).

Aβ Outcomes

The majority of associations between single SNPs and Aβ outcomes were insignificant, but 6 out of the 10 significant effects were in the expected direction based on IGAP results (Supplementary Table 4). All effects of the associations between the PRSs and Aβ outcomes were in the direction expected, although many of these relationships were not statistically significant (Table 3). A higher Aβ Clearance, Cholesterol, and Overall PRS were statistically associated with lower levels of $A\beta_{42}$, a smaller $A\beta_{42}/A\beta_{40}$ ratio, and higher PiB, all indicators of Aβ deposition. A higher Cholesterol and Overall PRS were also statistically associated with higher P-tau/$A\beta_{42}$ and T-Tau/$A\beta_{42}$ ratios, indicating higher risk for AD. The pathway specific PRSs (Aβ Clearance and Cholesterol) were either equal or better predictors than *APOE* alone, while the significance of the Overall PRS relative to *APOE* alone was less consistent. *APOE* alone had a better model fit than the PRSs for P-tau/$A\beta_{42}$, T-Tau/$A\beta_{42}$, $A\beta_{42}/A\beta_{40}$, and PiB, while the Overall PRS had a better model fit for $A\beta_{42}$, T-tau, and P-tau than *APOE* alone and the pathway specific PRSs (results not shown). Upon removing *APOE* from the PRSs, the strength of the associations was greatly attenuated, but the effects remained in the expected direction and the associations between the Cholesterol PRS and $A\beta_{42}/A\beta_{40}$, as well as T-tau/$A\beta_{42}$ were still statistically significant (p=.02 and p=.048, respectively) (Table 3).

Stratification by Age

The median age used to stratify analyses using cognitive outcomes was 58.00, while it was 62.56 for CSF outcomes and 62.48 for PiB, as the latter two sets of outcomes contained different subsets of participants from the complete sample.

As expected, *APOE* had the strongest effect in younger participants for most outcomes (Supplementary Table 5). This was most notable in Working Memory, $A\beta_{42}$, T-tau/$A\beta_{42}$, $A\beta_{42}/A\beta_{40}$, and PiB, where younger participants with a higher *APOE* risk score had significantly lower Working Memory scores, levels of $A\beta_{42}$, and $A\beta_{42}/A\beta_{40}$, and higher levels of PiB and T-tau/$A\beta_{42}$ ratio, all risk factors for AD. The Aβ Clearance and Overall PRSs showed this stronger effect in younger participants, but upon removing *APOE*, differences between the age groups were not notable (results not shown). The Cholesterol PRS had a similar stronger effect in younger participants (Supplementary Table 6), however, upon removing *APOE*, the CSF and PiB outcomes showed the opposite effect, in which the PRS had a stronger effect in older individuals. This was particularly evident for the P-tau/$A\beta_{42}$ (p=.03), T-tau/$A\beta_{42}$ (p=.03), and $A\beta_{42}/A\beta_{40}$ (p=.03) outcomes (Table 4). We further investigated these findings with an interaction term for age and the Cholesterol PRS excluding *APOE* and found that this interaction was significant for P-tau/$A\beta_{42}$ (p=.03), marginally significant for T-tau/$A\beta_{42}$ (p=.07), and insignificant for $A\beta_{42}/A\beta_{40}$ (p=.14; results not shown).

DISCUSSION

In this investigation, we assessed the potential for pathway specific PRSs to predict AD-related outcomes, including cognitive function and biomarkers of Aβ deposition (amyloid PET and CSF $A\beta_{42}$), neurodegeneration (CSF T-tau) and tau pathology (CSF p-tau). Our findings suggest that the pathway specific PRS for Cholesterol Metabolism tended to perform either similarly or better than the *APOE* risk score alone, while both pathway specific PRSs (Aβ Clearance and Cholesterol) performed better than the Overall PRS. Thus, for investigations of disease-related traits or biomarkers, limiting a PRS to variants that are known to contribute to a

biological pathway of the particular trait/biomarker may be a more informative approach than including variants known to contribute to the disease more broadly. Consistent with other studies, we also found that the PRSs were typically much better predictors of the outcomes than were single SNPs[22, 23].

As expected, *APOE* was found to have a stronger effect in younger age for both cognitive outcomes and biomarkers of Aβ deposition. However, the opposite effect was seen for the Cholesterol PRS after removing *APOE*, such that it had a stronger effect in older age. This is a particularly interesting finding as it could suggest that the remaining variants in the Cholesterol PRS may interact with long-term accumulation of environmental risk factors to influence brain pathology later in life. More research is necessary to better understand the biological mechanisms driving this observation.

The minimal statistically significant findings observed when assessing cognitive function is similar to the findings of another study that concluded that a PRS based on all IGAP genes, including *APOE*, was not associated with cognitive function after testing for associations with a battery of neuropsychological tests in older non-demented individuals[24]. Two other studies did find associations between a PRS using some IGAP genes and cognitive function, however, upon removing *APOE* from the PRS, associations were no longer significant[25, 26]. Even when *APOE* was included in our analyses, the Overall PRS was not statistically associated with any of the cognitive outcomes. However, our cognitive outcomes were factor scores combining several tests, while the other studies used individual test scores. Further, while 9 of the 11 genes included in the Verhaaren, et al. PRS and 8 of 10 genes in the Vivot, et al. PRS were IGAP genes, unlike our analysis, the variants used from each of these genes were not the most significant variants found in the IGAP meta-analysis. And lastly, because our sample had a

younger mean age than these studies, it is possible that our sample may not have experienced sufficient cognitive decline to detect the effect of *APOE* on cognition. It is notable that our most consistent findings were in Working Memory, as we recently found that Working Memory has the highest heritability of the cognitive factor scores assessed here[19]. This could be because PRS analyses have been reported to have better power to detect associations when the traits have high heritability[27]. A much larger sample size may be needed to detect associations in cognitive traits with lower heritability.

The observed association between the Overall PRS and CSF $A\beta_{42}$ has been seen in other studies investigating the IGAP variants[22, 23]. Similar to our study, Sleegers, et al. found that upon removing *APOE* from the Overall PRS, this association was no longer significant. However, Martiskainen et al. found that this significance held upon removing *APOE*. Possible reasons for this difference could be due to the different SNPs included in the Martiskainen, et al. PRS, which included only 12 of the IGAP meta-analysis variants and the top 10 variants from AlzGene, whereas our Overall PRS and that of Sleegers, et al. included all of the IGAP variants. Given that our study is based on a preclinical sample of participants, the lack of associations with T-tau and P-tau is consistent with other findings suggesting that these are later markers of AD, whereas $A\beta_{42}$ is an earlier marker[28].

The associations were stronger for Aβ deposition than for cognition, a finding that draw attention to the AD pathological cascade, which suggests that Aβ biomarkers become abnormal long before cognition during the development of AD[1]. Although our cohort may not be old enough to be experiencing detectable cognitive decline, we are able to detect differences in Aβ deposition using the pathway specific PRSs. The current diagnostic criteria for AD primarily concerns impairments in cognition, however, mounting evidence suggests that measurement of

Aβ deposition could be used to diagnose AD at a much earlier stage than cognitive function is able to[28-30]. Since measuring Aβ deposition can be expensive and require access to a PET facility (amyloid PET) and experience in performing lumbar puncture that also is regarded as an invasive procedure (CSF Aβ), and proxy measures of Aβ based on peripheral blood proteins are not quite able to definitively identify abnormal Aβ levels[31], a pathway specific PRS, such as the one developed here, could be used to determine who has a higher genetic risk of having elevated Aβ deposits and thus should receive screening for Aβ accumulation. The ability to identify these individuals will be of particular importance if effective treatments for AD become available.

Due to the limited sample size of participants with CSF and PiB data, it is possible that this study did not have sufficient power to detect some associations, particularly in our stratified analyses. It will be important to replicate associations with Aβ deposition using a larger sample size in order to validate our findings regarding pathway specific PRSs. The accuracy of our results is, in part, limited by current knowledge regarding the biological function of genes, which guided the development of the pathway specific PRSs used in this study. It is likely that in the future, we may learn that additional variants contribute to cholesterol metabolism and Aβ clearance. As our knowledge base improves, other important biological pathways involved with the development of AD should be investigated, such as Aβ production, tau pathology, and immune system response. Public repositories, such as Gene Ontology[32], could be useful tools to facilitate the creation of pathway specific PRSs.

Pathway specific PRSs may be more predictive of disease-related traits or biomarkers than PRSs including all known disease variants. In particular, the Cholesterol Metabolism and Aβ Clearance PRSs appear to be more strongly associated with cognition and Aβ deposition than

an Overall PRS and *APOE* alone. These PRSs could be useful in facilitating preventative, diagnostic, and treatment decisions, especially as more knowledge is gained on the genetic variants involved in specific biological pathways of AD and as therapies to target particular pathways become available.

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