Genetic Resilience to Amyloid Related Cognitive Decline

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Abstract

Preclinical Alzheimer's disease (AD) is characterized by amyloid deposition in the absence of overt clinical impairment. There is substantial heterogeneity in the long-term clinical outcomes among amyloid-positive individuals, yet limited work has focused on identifying molecular factors driving resilience from amyloid-related cognitive impairment. We apply a recently developed predicted gene expression analysis (PrediXcan) to identify genes that modify the association between baseline amyloid deposition and longitudinal cognitive changes. Participants free of clinical AD (n=631) were selected from the AD Neuroimaging Initiative (ADNI) who had a baseline positron emission tomography measure of amyloid deposition (quantified as a standard uptake value ratio), longitudinal neuropsychological data, and genetic data. PrediXcan was used to impute gene expression levels across 15 heart and brain tissues. Mixed effect regression models assessed the interaction between predicted gene expression levels and amyloid deposition on longitudinal cognitive outcomes. The predicted gene expression levels for two genes in the coronary artery (*CNTLN, PROK1*) and two genes in the atrial appendage (*PRSS50, PROK1*) interacted with amyloid deposition on episodic memory performance. The predicted gene expression levels for two additional genes (*TMC4* in the basal ganglia and *HMBS* in the aorta) interacted with amyloid deposition on executive function performance. Post-hoc analyses provide additional validation of the *HMBS* and *PROK1* effects across two independent subsets of ADNI using two additional metrics of amyloid deposition. These results highlight a subset of unique candidate genes of resilience and provide evidence that cell-cycle regulation, angiogenesis, and heme biosynthesis likely play a role in AD progression.

1. Introduction

The neuropathological cascade in Alzheimer's disease (AD) includes a long preclinical period in which neuropathologies emerge in the absence of cognitive impairment. The

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6. Compliance with Ethical Standards The authors report no conflicts of interest. Informed consent was obtained from all participants included in the study.

predominant models of AD biomarker progression suggest that amyloid plaque deposition occurs earliest and ultimately drives downstream neurofibrillary tangle formation, neurodegeneration, and cognitive decline (Jack et al., 2013; Jack et al., 2010). Amyloid plaques, whether measured at autopsy or *in vivo* using cerebrospinal fluid (CSF) or positron emission tomography (PET) biomarkers, are present in approximately 30% of cognitively normal older adults, highlighting the substantial heterogeneity in clinical progression of AD that exists among amyloid-positive individuals (Mormino, Betensky, Hedden, Schultz, Amariglio, et al., 2014; Rahimi & Kovacs, 2014).

The identification of factors that promote resilience among amyloid-positive individuals may provide alternative targets for clinical intervention. High cognitive reserve, for example, has been shown to be particularly protective against cognitive decline among amyloid-positive individuals (Rentz et al., 2010). Certain genetic factors, such as the presence of the *APOE* ε4 allele (Lim et al., 2013; Mormino, Betensky, Hedden, Schultz, Ward, et al., 2014), and proteomic factors, such as CSF levels of vascular endothelial growth factor (Hohman, Bell, & Jefferson, 2015), have been shown to strengthen the association between amyloid and cognition. Yet, to our knowledge, a comprehensive analysis of genomic modifiers of amyloid-related cognitive decline has not been performed. One methodological approach would be to perform a simple genome-wide association analysis (GWAS) to test the interaction of millions of single nucleotide polymorphisms (SNPs) with amyloid on downstream cognitive change. However, statistical power is a major concern for any such analysis given the relatively limited number of studies with both baseline AD biomarker data and longitudinal cognitive data. Additionally, the interpretation of significant SNP hits becomes extremely challenging without additional relevant functional data.

This manuscript leverages PrediXcan, a recently developed predicted gene expression technique (Gamazon et al., 2015) that can be applied in a gene interaction analysis. This technique ameliorates concerns about statistical power and functional interpretation and is well positioned to provide novel insights into potential candidate pathways of resilience. In this study, we first impute levels of gene expression across 15 tissues relevant to AD from the heart and brain and then identify genes that modify the association between amyloid and cognitive decline. We chose to focus on gene expression in heart tissues as well as brain tissues because there is growing evidence that vascular risk factors and subclinical deficits in cardiac function are associated with poor brain aging and AD (Jefferson, Beiser, et al., 2015; Jefferson et al., 2010; Jefferson, Hohman, et al., 2015). This application of the PrediXcan approach provides a focused analysis of 13,000 genetic models, all of which have a straight-forward functional interpretation. Our hypothesis is that tissue-specific, genetically-determined expression in the heart and brain will modify the association between amyloid and change in cognition.

2. Materials and Methods

Participants were drawn from the Alzheimer's Disease Neuroimaging Initiative database (ADNI; adni.loni.usc.edu) launched in 2003 as a public-private partnership. The original ADNI study enrolled approximately 800 participants, aged 55–90 years, excluding serious neurological disease, other than AD, and history of brain lesion, head trauma, or

psychoactive medication use (for full inclusion/exclusion criteria see http://www.adni-info.org). Written informed consent was obtained from all participants at each site, and analysis of ADNI's publically available database was approved by our local Institutional Review Board prior to data analysis.

2.1 Participants

We accessed publicly available participant data from ADNI on 5/1/2016, and ADNI enrollment criteria are outlined in the ADNI protocol (http://www.adniinfo.org/Scientists/AboutADNI.aspx). For the present analyses, we included all participants with a diagnosis of normal cognition or mild cognitive impairment at baseline who also had a PET measurement of amyloid deposition, genomic data, and longitudinal cognitive data, yielding 631 participants for analyses. We chose to focus on individuals free of clinical AD because we are interested in identifying factors that promote resilience to the onset of clinical AD.

2.2 PET Image Processing

Amyloid deposition was quantified using an ^{18}F-AV-45 tracer as previously described (Landau et al., 2012). The mean standardized uptake value ratio (SUVR) was calculated across the cingulate (anterior and posterior regions), frontal, temporal (middle and lateral regions), and lateral parietal (precuneus and supramarginal gyrus) cortices, and divided by the reference region (whole cerebellum).

2.3 Neuropsychological Composites

The ADNI neuropsychological protocol, including calculation of episodic memory and executive function composite measures, has been reported previously (Crane et al., 2012; Gibbons et al., 2012). We leveraged memory (ADNI-MEM) and executive function (ADNI-EF) composite scores in the present analyses. ADNI-MEM included a composite z-score based on item-level data from Rey Auditory Verbal Learning Test, AD Assessment Scale-Cognitive Test, Mini-Mental State Examination, and Logical Memory I and II. ADNI-EF included item-level data from Trail Making Test Parts A and B, Digit Span Backward, Digit Symbol, Animal Fluency, Vegetable Fluency, and Clock Drawing Test.

2.4 Tissue-Specific Predicted Gene Expression

Genotyping was performed using the Illumina HumanOmni 2.5 BeadChip (Illumina, Inc., San Diego, CA). Quality control was performed using PLINK (http://pngu.mgh.harvard.edu/purcell/plink; Purcell et al., 2007) and included applying a 98% threshold for genotyping efficiency and a minimum minor allele frequency of 0.01. Participants were excluded if they had a call rate <98%, if there was a reported versus genetic sex inconsistency, or if relatedness to another sample was established. Population structure was analyzed using the fastStructure software package (Raj, Stephens, & Pritchard, 2014).

Tissue-specific gene expression profiles were quantified using the PrediXcan procedure (Gamazon et al., 2015). PrediXcan uses reference transcriptomic data to impute tissue-specific gene expression profiles. Scripts are freely available online (https://github.com/hakyimlab/PrediXcan/tree/master/Software). For the present analysis, we focused on all heart and brain tissues available through the Genotype-Tissue Expression (GTEx) Project

given the importance of both heart (Jefferson, Hohman, et al., 2015; Jefferson et al., 2010; Jefferson, Beiser, et al., 2015) and brain in AD. Specifically, we considered predicted gene expression level estimates for anterior cingulate gyrus, aorta, tibial artery, atrial appendage, basal ganglia, cerebellum, coronary artery, cortex, frontal cortex, hippocampus, hypothalamus, left ventricle (heart), nucleus acumbens, putamen, and whole blood. Gene expression profiles were imputed based on the previously published multi-SNP equations identified using elastic net (all databases are available through the PrediXcan webpage). We restricted imputation to genes with R^2 0.15 in elastic net prediction models based on previously completed power simulations in the seminal PrediXcan manuscript (Gamazon et al., 2015). A list of all genes evaluated and the imputation quality of each is presented in Supplementary Table 1.

2.5 Statistical Analyses

All statistical analyses were performed in R (RStudio version 0.99.485; https://www.rstudio.com/; R version 3.0.1). Participant demographic and clinical characteristics were summarized and compared across baseline diagnostic categories (normal cognition, mild cognitive impairment) using independent sample t-tests for continuous variables and Pearson's χ^2 for categorical variables. Genetic modifiers of the association between amyloid and change in cognition were evaluated using mixed-effects regression models with either composite memory performance or composite executive function performance set as the quantitative outcome. Fixed effects included baseline age, sex, years of education, mean SUVR, predicted gene expression, and a 3-way interaction for age × mean SUVR × predicted gene expression (including all lower order 2-way interactions). Random effects included the intercept and age. Correction for multiple comparisons was performed using the Bonferroni procedure based on the total number of unique gene-tissue combinations evaluated (13,300 total tests, corrected $\alpha = 3.76 \times 10^{-6}$).

Post-hoc analyses were performed for all significant hits to assess additional confounding factors. First, analyses were rerun covarying for the number of *APOE* ε4 alleles to assess whether *APOE* variation explained the observed effects. Second, sex-stratified analyses were performed to evaluate sex-specific effects. Third, analyses including the first 5 principal components from population structure analysis were included to adjust for potential population stratification effects. We also reran all analyses restricting to the White participants based on self-reported race and ethnicity to further ensure such stratification effects were not driving our result. Finally, additional validation analyses (see Supplemental Materials) were also performed in independent subsamples of the ADNI dataset in which we replaced the original amyloid metric with amyloid measured using Pittsburgh compound B (PiB) or measured in CSF.

3. Results

Demographic characteristics are presented in Table 1. Participants were followed for an average of 2.34 years (range: 0–4 years) after the baseline visit. Expected diagnostic differences were observed in amyloid load, memory performance, and executive function performance.

As expected, we observed a significant association between baseline amyloid deposition and longitudinal age-related change in both memory (t(2384)=−2.29, p=0.022) and executive function performance (t(2369)=−2.36, p=0.018).

In the longitudinal memory analyses, we observed four gene interaction effects. Two effects were with predicted gene expression levels in the atrial appendage: Protease, Serine 50 (*PRSS50*; t(1865)=5.02, p=5.6×10⁻⁷; Figure 1) and *PROK1* (t(1865)=−4.67, p=3.3×10⁻⁶). Lower *PROK1* expression was associated with an attenuated effect, while lower *PRSS50* predicted expression was associated with an enhanced effect of baseline amyloid deposition on longitudinal decline in memory. The remaining two effects were with predicted expression levels in coronary artery tissue: centlein (*CNTLN*; t(1865)=−4.81, p=1.6×10⁻⁶) and prokineticin 1 (*PROK1*; t(1865)=−4.80, p=1.7×10⁻⁶). In both cases, lower predicted expression was associated with an attenuated effect of baseline amyloid deposition on longitudinal change in memory.

In longitudinal executive function analyses, two significant interactions were observed, including transmembrane channel-like 4 (*TMC4*) in the basal ganglia (t(1853)=−4.79, p=1.8×10⁻⁶) and hydroxymethylbilane synthase (*HMBS*) in the aorta (t(1853)=4.73, p=2.4×10⁻⁶; Figure 2). Lower expression of *TMC4* and higher expression of *HMBS* were associated with an attenuated effect of baseline amyloid deposition on longitudinal change in executive function. All associations evaluated are presented in Supplementary Table 2.

In post-hoc analyses, all six observed effects remained statistically significant when covarying for the number of *APOE* ε4 alleles (p-values<5×10⁻⁵) and when including a 3-way *APOE* ε4 × age × amyloid interaction in the model (p-values<5×10⁻⁵). In sex-stratified analyses, a sex difference was observed in the longitudinal memory analysis whereby a significant interaction between *PROK1* (in both the atrial appendage and the coronary artery) and amyloid was observed among females (p-values<1.5×10⁻⁵) but not among males (p-values>0.10). We did not observe a sex difference in raw predicted expression levels of *PROK1* in either tissue. No other sex differences were observed among the identified genetic effects. In analyses covarying for population stratification (i.e., the first five principal components), our main findings remained unchanged (p-values<3.8×10⁻⁶). When restricting analyses to White participants, the magnitude of effects dropped slightly, likely due to the reduction in power, but all interactions remained significant (p-values<5×10⁻⁵).

In post-hoc validation analyses, although somewhat under powered when restricting the analyses to non-overlapping subsets of the cohort, we did observe additional evidence of an interaction between predicted *PROK1* expression in the atrial appendage and amyloid on longitudinal memory function (Supplemental Table 3, p-values<0.05) and between predicted *HMBS* expression in the aorta and amyloid on longitudinal executive function (Supplemental Table 3, p-values<0.05) in both PiB and CSF Aβ-42 analyses.

4. Discussion

The present manuscript evaluated genetic resilience to amyloid-related cognitive impairment using a predicted gene expression methodology (Gamazon et al., 2015). Our results suggest

that genetically-determined expression of five novel candidate genes in heart and brain tissue attenuate the downstream effects of amyloid deposition. Importantly, these results also provide a proof of concept for the application of predicted expression methodology in the context of genetic resilience analyses in AD.

It is quite interesting that all four significant gene interactions on memory were from imputed expression levels in heart tissue (the coronary artery and atrial appendage). While the association between peripheral vascular disease and AD is well established (Beeri et al., 2006; Jefferson, Hohman, et al., 2015; Jefferson et al., 2010; Jefferson, Beiser, et al., 2015), the mechanisms underlying such associations remain elusive. Our findings suggest that the genetic architecture of the heart may indeed be relevant to individual susceptibility to amyloid-related cognitive decline. The three genes identified, *PRSS50, CNTLN*, and *PROK1* are promising candidates. Unfortunately, we were only able to provide partial validation of these associations in small independent subsamples of the ADNI cohort, highlighting the need for future replication in larger datasets. The serine protease *PRSS50* has not been implicated in AD previously. Methylation differences in *PRSS50* have been noted in age-related macular degeneration (Oliver et al., 2015), and *PRSS50* has been shown to induce apoptosis when knocked down in cancer cells (Zhou et al., 2010), suggesting a potential role in neurodegeneration. The highest expression of *PRSS50* is observed in the testis, thyroid, and pituitary. Very low levels of expression are observed in the atrial appendage (Lonsdale et al., 2013), and it remains unclear how expression of *PRSS50* in the heart may relate to amyloid-related cognitive decline.

CNTLN codes a centrosomal protein that shows cell-cycle-dependent localization within centrosomes (Makino et al., 2008). Centrosomes are critical for microtubule organization and genetic stability (Makino et al., 2008) and play a crucial role in neuronal development (Kuijpers & Hoogenraad, 2011). Microtubule disorganization is a mark of the abnormal phosphorylation of tau, providing a possible connection between centrosome and tau pathology (e.g., Alonso, Zaidi, Grundke-Iqbal, & Iqbal, 1994). There is also evidence that both the amyloid precursor protein and presenlin 1 are part of a signaling cascade at the centrosome that regulates the cell cycle (Nizzari et al., 2007). Although cell cycle alterations appear to be an important aspect of the AD cascade (Moh et al., 2011), additional work is needed to clarify whether such amyloid-related alterations in cell cycle activity in the heart may mediate downstream neurofibrillary changes in the brain. The present results suggest that genetic expression of *CNTLN* in the heart is relevant to amyloid-related cognitive deterioration. It is also interesting to note that intronic variants in *CNTLN* have been associated with left ventricular hypertrophy, a cardiac condition that has also been associated with age-related cognitive decline (Jefferson et al., 2015).

PROK1 is another interesting candidate gene. *PROK1* is most commonly expressed in steroidogenic glands (LeCouter, Lin, & Ferrara, 2002), although it is also expressed in astrocytes and neurons (Zhang et al., 2014), and has a tissue-specific role in angiogenesis (LeCouter et al., 2002). *PROK1* levels are significantly reduced in embryonic neuronal cell cultures when treated with Aβ-42 (Romito-DiGiacomo, Menegay, Cicero, & Herrup, 2007), suggesting a direct association between amyloid and *PROK1*. Unfortunately, we were not

able to impute *PROK1* in any brain tissues in the present analysis, making it difficult to speculate on the role of *PROK1* in relation to plaques and tangles.

PROK1 is also called endocrine-gland-derived vascular endothelial growth factor (EG-VEGF) as it is indistinguishable from VEGF. While the present study indicates a detrimental effect of predicted expression of arterial *PROK1* on longitudinal memory, previous work has demonstrated a neuroprotective effect of CSF VEGF levels in modifying the association between AD biomarkers and longitudinal change in cognition, particularly in interacting with CSF Aβ-42 on longitudinal memory (Hohman et al., 2015). This discrepancy may be due, in part, to the fact that there is mixed epidemiological evidence of risk and resilience when measuring VEGF. High levels of serum VEGF have been associated with an increased risk of stroke (Pikula et al., 2013) and more frequent cerebral microbleeds among individuals with Alzheimer's disease (Zhang et al., 2016), while CSF VEGF levels have been reported to be higher (Tarkowski et al., 2002), lower (Guo, Alexopoulos, & Perneczky, 2013), and equivalent (Blasko et al., 2006) among individuals with AD compared to controls. Perhaps VEGF-related genes like *PROK1* will provide clues to help unravel the complex associations among VEGF, cerebrovascular disease, and AD. Future work validating the interaction observed here and investigating gene-gene interactions previously reported between *PROK1* and *VEGF* (Su, Lin, Chen, & Kuo, 2014) may help move the field forward.

There also appears to be a sex difference in the effect of *PROK1* on amyloid-related cognitive decline. In post hoc analyses the interaction between *PROK1* and amyloid was restricted to females. This sex-specific effect is particularly interesting given the known sex differences in the association between AD biomarkers and longitudinal change in cognition (Koran, Wagener, & Hohman, *In Press*) and the fact that *PROK1* is hormonally mediated under certain circumstances (Brouillet et al., 2012). Importantly, the present finding of a sex difference re-emphasizes the need to evaluate AD risk and resilience in a sex-specific manner (Koran et al., *In Press*).

In executive function analyses, we identified an interaction between amyloid and *HMBS* expressed in the aorta. Importantly, we also observed additional evidence of such an interaction in our validation analyses across two independent subsamples of the ADNI cohort using two additional methods for measuring amyloid levels, providing additional confidence in the validity of the observed effect. *HMBS* codes a protein required for the biosynthesis of heme, and it has been shown to be down-regulated in AD, particularly among homozygous carriers of the *APOE* ε4 allele (Dwyer, Smith, Richardson, Perry, & Zhu, 2009). However, the *HMBS* effect remained statistically significant even after adjusting for *APOE*. While past work has highlighted the possible role of cerebral heme biosynthesis in AD, our results within the aorta suggest that peripheral heme may also be relevant to clinical progression in AD that may reflect central heme processes.

The second identified interacting gene was *TMC4* expressed in the basal ganglia. Validation results provided mixed evidence of such an interaction in independent subsamples of the dataset using additional metrics of amyloid levels, suggesting a need for future replication analyses in larger datasets. The basal ganglia is a plausible candidate region given its known

role in executive function (e.g., Cummings, 1993; Graybiel, 2000; Monchi, Petrides, Strafella, Worsley, & Doyon, 2006) and the known association between basal ganglia damage and executive function deficits (Elliott, 2003). *TMC4* is another interesting candidate gene, although its proximity to the *APOE* locus leaves open the possibility of a confounding effect of *APOE*. However, in post-hoc sensitivity analyses, the *TMC4* interaction remained statistically significant when adjusting for both *APOE* ε4 status and the interaction between *APOE* ε4 and amyloid, suggesting a unique contribution of *TMC4* on AD progression. *TMC4* is a paralog of the *TMC* gene family associated with hearing loss (Kurima, Yang, Sorber, & Griffith, 2003), but it has not been implicated in AD previously.

The present results highlight the potential of predicted gene expression analyses to help further delineate the genetic architecture of AD. In neurodegenerative diseases, it is often difficult to take a full organism approach and consider peripheral changes that drive disease risk and progression. Our results add to a growing literature on the relevance of heart health on AD progression and further emphasize the need to consider pathways in which non-brain genomic alterations may have upstream effects that impact AD. That said, additional work is needed to validate the present results and improve predicted gene expression analyses for AD and other diseases of aging.

The present expression imputation models were based on data from a well-characterized sample from the GTEx database. However, the brain sample in particular included a large proportion of individuals over age 60, leaving open the possibility that some individuals may have been in the preclinical phases of AD. The ADNI sample also has strengths and limitations. The availability of AD biomarker data, genetic data, and comprehensive neuropsychological data, including documented AD status made this an ideal cohort for analyzing genetic modifiers of AD biomarker effects. However, the ADNI cohort is predominately comprised of highly-educated, non-Hispanic White individuals, limiting generalizability to other cohorts. Additionally, the present analysis had a relatively short follow-up period (2.3 years) and a relatively small sample size, suggesting that we may have lacked the statistical power needed to identify additional loci. Additional effort is needed to harmonize amyloid, cognitive, and genetic data from a variety of sources to increase statistical power and identify new pathways of resilience. Future work is also needed to extend these models to more diverse cohorts and to understand how and to what degree the inclusion of individuals with early preclinical AD changes may alter the PrediXcan prediction models.

In conclusion, predicted levels of gene expression in the coronary artery, aorta, atrial appendage, and basal ganglia are relevant to amyloid-related cognitive decline. Our findings highlight a few novel candidate genes of resilience while providing additional evidence that the cell cycle, angiogenesis, and heme biosynthesis play a role in AD progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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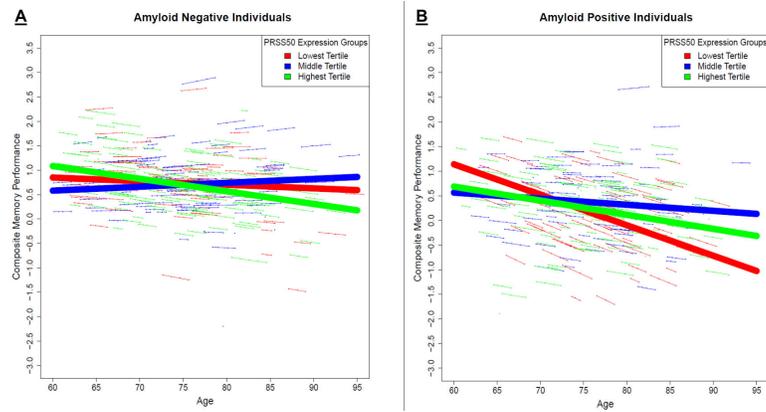
Figure 1. Predicted Levels of *PRSS50* in the Atrial Appendage Interact with Amyloid Levels in the Brain on Age-Related Change in Memory Performance

Age in years is along the x-axis, composite memory performance is along the y-axis. Individual lines represent fitted trajectories from the mixed-effects regression model. Amyloid positivity and *PRSS50* expression groups were for illustration purposes only. Amyloid positivity was defined based on the established cut-point of 1.11 and *PRSS50* expression was divided into tertiles. Panel A includes amyloid negative individuals and panel B includes amyloid positive individuals.
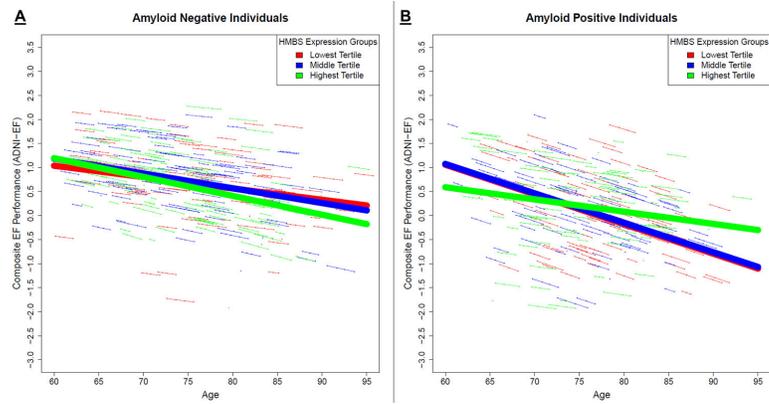
Figure 2. Predicted Levels of *HMBS* in the Aorta Interact with Amyloid Levels in the Brain on Age-Related Change in Executive Function

Age in years is along the x-axis, composite executive function performance is along the y-axis. Individual lines represent fitted trajectories from the mixed-effects regression model. Amyloid positivity and *HMBS* Expression Groups were for illustration purposes only. Amyloid positive was defined based on the established cut-point of 1.11 and *HMBS* expression was divided into tertiles. Panel A includes amyloid negative individuals and panel B includes amyloid positive individuals.

Table 1

Sample Characteristics

	Baseline Clinical Diagnosis[#]		
	Normal Control	Mild Cognitive Impairment	Statistical Test
Sample Size, n	233	398	
APOE, % ε4 Carriers	27	44	$\chi^2(1)=17.61$, p<0.001
Sex, % Female	53	42	$\chi^2(1)=6.30$, p=0.012
Baseline Age, years	76±6	74±7	$t(551.7)=-4.58$, p<0.001
Baseline Education, years	16±3	16±3	$t(551.7)=-1.80$, p=0.07
Mean SUVR	1.12±0.19	1.21±0.22	$t(551.7)=5.75$, p<0.001
Memory Performance	0.88±0.54	0.24±0.65	$t(551.7)=-13.19$, p<0.001
Executive Function Performance	0.78±0.73	0.22±0.87	$t(551.7)=-8.58$, p<0.001

[#]Diagnostic groups were defined according to the ADNI protocol. <u>Normal Control</u> participants had a Mini-Mental State Examination (MMSE) score between 24 and 30, a Clinical Dementia Rating (CDR) score of 0, and were not depressed (Geriatric Depression Scale score <6). <u>Mild Cognitive Impairment</u> participants had an MMSE score between 24 and 30, objective memory impairment, subjective memory impairment, and a CDR score of 0.5.