The association between a polygenic Alzheimer score and cortical thickness in clinically normal subjects[a]

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Abstract

Late-onset Alzheimer's disease (AD) is 50-70% heritable with complex genetic underpinnings. In addition to Apoliprotein E (APOE) ε4, the major genetic risk factor, recent genome-wide association studies (GWAS) have identified a growing list of sequence variations associated with the disease. Building on a prior large-scale AD GWAS, we used a recently developed analytic method to compute a polygenic score that involves up to twenty-six independent common sequence variants and is associated with AD dementia, above and beyond APOE. We then examined the associations between the polygenic score and MRI derived thickness measurements across AD-vulnerable cortex in clinically normal (CN) human subjects (N=104). AD-specific cortical thickness was correlated with the polygenic risk score, even after controlling for APOE genotype and CSF levels of β-amyloid ($A\beta_{1-42}$). Furthermore, the association remained significant in CN subjects with levels of CSF $A\beta_{1-42}$ in the normal range and in APOE ε3 homozygotes. The observation that genetic risk variants are associated with thickness across AD-vulnerable ROIs in clinically normal older individuals, suggests that the combination of polygenic risk profile, neuroimaging, and CSF biomarkers may hold synergistic potential to aid in the prediction of future cognitive decline.

Keywords: Alzheimer's disease, Polygenic score, Imaging genetics

Late-onset Alzheimer's disease (AD) is a complex disease with heritability estimates of 50-70% (Gatz M *et al*. 1997; Pedersen N *et al*. 2004). While the ε4 allele of Apoliprotein E (APOE) is the best-established genetic risk factor (Saunders A *et al*. 1993; Strittmatter W *et al.* 1993), recent genome-wide association studies (GWAS) have identified novel common DNA variants associated with AD (Harold D *et al*. 2009; Lambert J *et al*. 2009; Seshadri S *et al*. 2010; Hollingworth P *et al*. 2011; Naj AC *et al*. 2011), and a growing number of studies have pointed to further candidate loci (Waring S and R Rosenberg 2008; Bertram L and R Tanzi 2009). An open question is whether these newly identified genetic risk variants are associated with markers of neurodegeneration and, if so, how early in the progression of the disease.

Clinical AD is preceded by a long, asymptomatic phase, which is characterized by the aberrant processing and accumulation of amyloid and tau variants in the brain (Grundke-Iqbal I *et al*. 1986; Price JL and JC Morris 1999; Selkoe D 2004; Chiti F and C Dobson 2006). Current *in vivo* markers of amyloid and tau pathology include cerebrospinal fluid (CSF) concentrations of $A\beta_{1\text{-}42}$, phosphorylated tau (p-tau) and total tau (t-tau) (Buerger K *et al*. 2006; Shaw L *et al*. 2009), and amyloid-binding positron emission tomography (PET) tracers, e.g., Pittsburgh Compound B (PIB) (Klunk W *et al*. 2004). Super-threshold levels of amyloid and/or tau burden in clinically normal (CN) individuals have been associated with a heightened risk for future dementia (Meyer GD *et al*. 2010; Villemagne VL *et al*. 2011). Thus preclinical AD research has increasingly relied on stratifying CN subjects based on PIB PET and/or CSF measurements (Pike KE *et al*. 2007; Villemagne V e*t al*. 2008).

The prevailing AD model (Jack Jr C *et al*. 2010) predicts that amyloid pathology reaches a plateau before atrophy in vulnerable brain regions (e.g. the medial temporal lobe and association cortices) can be detected *in vivo*, e.g., via magnetic resonance imaging (MRI) (Fox N, P Freeborough *et al*. 1996; Jack Jr C *et al*. 1997; Dickerson B *et al*. 2009; Frisoni G *et al*. 2010). Although increased rates of atrophy have been shown to precede the clinical diagnosis of familial AD (Fox N, E Warrington *et al*. 1996; Ridha B *et al*. 2006), accelerated atrophy is often assumed to coincide with cognitive decline during the MCI or dementia phases of late-onset AD (Fox N *et al*. 1999; Jack Jr C *et al.* 1999).

Several neuroimaging studies have demonstrated APOE-associated functional, metabolic and structural variation in CN individuals (Reiman E *et al*. 1998; Small G *et al*. 2000; Reiman E *et al*. 2004; Wishart H *et al*. 2006; Filippini N *et al*. 2009; Sheline Y *et al*. 2010). However, how newly identified genetic risk variants influence structural variation and how they relate to the effects of APOE remains largely unknown. In the present study, we built on a prior GWAS and computed a polygenic score (Purcell S *et al*. 2009) that is associated with clinical AD above and beyond APOE (Biffi A *et al*. 2010). We then examined the association between the polygenic score and structural MRI-based thickness measurements within *a priori* AD-vulnerable cortical regions across the heteromodal association cortex and medial temporal lobe in CN subjects. Given the known significant heterogeneity in the risk of clinical progression among normal older individuals, even after stratification by APOE genotype and amyloid burden, we hypothesized that the polygenic score would provide additional explanation to the remaining variance in cortical thickness.

Materials and Methods

ADNI Data

Our analyses focused on the Alzheimer's Disease Neuroimaging Initiative (ADNI) subjects who were clinically diagnosed as normal (zero clinical dementia rating and no objective memory loss) with available CSF measurements of tau and $A\beta_{1\text{-}42}$ concentration (N = 116). We removed 12 individuals from this sample because they either failed the genotype quality control tests or were of non-European descent, as determined based on genome-wide data, resulting in a sample of 104 CN for further analyses. In the first part of our analysis, in order to assess the clinical value of the polygenic score, we compared the CN group with the ADNI AD subjects with CSF samples (N=100). Table 1 provides summary statistics for the analysis sample. The CN and AD groups did not significantly differ in age, gender and education (all $P > 0.1$). As expected, the groups differed on measures of disease severity: Mini-Mental State Examination (MMSE) and Clinical Dementia Rating Sum of Boxes (CDR-SB) ($P < 0.001$). CDR-SB is a refined version of the global Clinical Dementia Rating (CDR) and provides increased sensitivity in tracking

disease progression within and across various stages of disease severity (O'Bryant SE *et al.* 2008).

-- Table 1 about here--

An independent portion of the ADNI sample (called the discovery sample) in which CSF data were not available was utilized to construct the polygenic score (N=197).

Genotype Processing

We merged individual-level genotype data from the ADNI database (ADNI) into a single data set containing genome-wide SNP information for 810 individuals (female ratio 41.85%). We performed the following genotype quality control (QC) using PLINK version 1.07 (Purcell S *et al*. 2007). First, we removed individuals with missing genotype rates of greater than 0.05. Subjects were additionally removed if they were outliers based on nearest neighbor-based clustering using identity-by-state (IBS) measures. Next, we applied SNP-level filtering, removing SNPs with a minor allele frequency (MAF) less than 0.01, missing rates greater than 0.05, and significant departure from Hardy-Weinberg Equilibrium (HWE) ($p < 1E-6$). SNPs were further removed if genotype missingness was significantly different between cases and controls ($p < 1E-6$) or if there was non-random missingness based on the PLINK haplotype test. To control for population stratification, we conducted a multi dimensional scaling (MDS) analysis on the ADNI data with the HapMap phase 3 reference dataset (Thorisson GA *et al*. 2005; Price A *et al.* 2006). Based on clustering with 988 HapMap subjects using the first two MDS components, we retained 745 ADNI individuals with European ancestry (female ratio of 40.81%). The first three MDS components were highly correlated with the ADNI case/control data, and thus were included as covariates in all remaining analyses. Total genotyping rates for final QC'ed ADNI genotype data was 99.72% for 548,340 SNPs. Ungenotyped SNPs were imputed using Beagle v.3.3.1 (Browning BL and SR Browning 2009) with HapMap phase 3 CEU reference data. Further analysis focused on a total of 986,993 autosomal SNPs with imputation quality scores not less than 0.8.

Computation of Polygenic Score

To compute the polygenic AD-risk score, we used the "score" utility in PLINK (Purcell S

et al. 2009) and the detailed GWAS results reported in the supplemental material of (Harold D *et al.* 2009). First, we obtained the list of 761 SNPs that showed nominal association with AD ($P \leq 1E\text{-}3$) in (Harold D *et al.* 2009) (3,941 cases and 7,848 controls). To account for only independent association signals from the list of AD-susceptibility SNPs, we conducted linkage disequilibrium (LD) based clumping, implemented in PLINK and selected the index SNPs with the most significant association p-value from each clumped association region based on the (Harold D *et al.* 2009) GWAS. The polygenic score of ADNI subjects was then calculated as the sum of the number of susceptibility alleles of the index SNPs, weighted by the logarithm of the corresponding odds ratios (ORs). Of note, for imputed SNPs in the ADNI data, expected allele counts (i.e., dosage data) were used in the scoring process in order to reflect imputation uncertainty.

The SNPs that make up the score were determined by identifying those exceeding a certain statistical threshold in the (Harold D *et al.* 2009) GWAS. In general, a more stringent threshold yields a score consisting of a smaller number of SNPs with a higher fraction of them being truly associated with AD. On the other hand, an increasing number of truly associated SNPs (which can be obtained by relaxing the statistical threshold) will increase the effect size of the score and thus improve our statistical power for detecting associations with disease correlates on a limited sample size. Our initial goal was therefore to conduct a discovery analysis and identify a good threshold that would yield a score with as many truly associated SNPs as possible. Follow-up analyses were then conducted to demonstrate the utility and clinical relevance of this score on an independent analysis sample.

The discovery analysis to determine the statistical threshold for establishing the list of SNPs that would contribute to the polygenic score, was performed on a portion of the ADNI data (discovery sample; CN and AD patients with no CSF, N=197) that was independent from the analysis sample that consisted of the ADNI data with CSF measurements. We evaluated 5 different threshold values: 1E-3, 1E-4, 1E-5, 1E-6, and 1E-7, yielding five different polygenic scores. These scores were entered into a logistic regression (CN versus AD) and correlated with MMSE and CDR-SB in the ADNI

discovery sample (see Supplementary Figure 1). 1E-5 yielded the highest correlation values and strongest association with AD diagnosis (P-value = 1.47E-7), and was therefore used to compute the polygenic score in the remaining analyses on the independent, analysis sample. The list of SNPs contributing to this polygenic score and corresponding GWAS ORs are listed in Supplementary Table 1. The closest genes for these SNPs and a list of studies that report these genes as promising associations with AD are included in Supplementary Table 2.

MRI Processing

We used FreeSurfer version 4.5 to process all MRI scans automatically (Freesurfer). We first utilized the independent, cross-sectional OASIS dataset (Marcus D et al. 2007) consisting of 94 participants to generate an exploratory map of cortical thickness differences between older controls (N=47, 58% female, mean age±standard deviation: 78±5.6 years) and CDR 0.5 individuals clinically classified as incipient AD (N=47, 63% female, 76.4±4.7 years). Based on the resulting statistical maps, we delineated seven regions of interest (ROIs) on the average cortical surface template that demonstrated the greatest magnitude of bilateral cortical thinning in incipient AD participants relative to older controls. These regions include the *entorhinal cortex, temporopolar cortex, lateral temporal cortex, inferior parietal cortex, inferior parietal sulcus, posterior cingulate cortex,* and *inferior frontal cortex*. These ROIs and corresponding statistical maps have been published elsewhere (Sabuncu MR *et al*. 2011) and are made publicly available with FreeSurfer. We further used FreeSurfer's longitudinal stream to process a set of serial MRIs (baseline and month 12) from each study participant; this stream yields accurate and unbiased estimates of subtle changes over time (Reuter M and B Fischl 2011).

We transferred the OASIS-derived AD-vulnerable ROIs from the surface template onto the individual ADNI subjects' cortical representations via surface-based registration (Fischl B, M Sereno and A Dale 1999; Fischl B, M Sereno, R Tootell *et al*. 1999; Fischl B *et al*. 2001; Segonne F *et al.* 2007). We computed average thickness values (across the cortical ROIs in both hemispheres) and used these measurements in all our analyses. Average thickness measurements from the automatically delineated cortical gyral ROIs

(parcels covering the whole cortex) were used for a supplementary analysis (Desikan R *et al.* 2006).

Statistical Analysis

All statistical analyses were conducted using the general linear model (GLM) framework implemented in Matlab. Age, sex and education (years) were included as covariates in all analyses. To control for APOE genotype, we included both ε2 and ε4 allele counts as covariates[c]. As effect size, we reported partial correlations (ρ) between the polygenic score and outcome of interest (such as cortical thickness) and linear coefficients (β) for the effect of APOE allele counts on the outcome.

Results

Polygenic score predicts dementia above and beyond APOE

The polygenic score was significantly associated with CDR-SB ($\rho = 0.40$, $P < 0.001$), MMSE ($\rho = -0.34$, $P < 0.001$), and AD diagnosis (1 standard deviation increase in the polygenic score was associated with an increase of 1.06 in the log-odds of AD diagnosis, $P < 0.001$) in the CN and AD combined analysis sample (N=204). To explore non-APOE contributions, we excluded the APOE-linkage region 19q13 from the polygenic score computation and controlled for APOE genotype by including APOE ε4 and ε2 allele counts as covariates. The association between the non-APOE polygenic score and CDR-SB ($\rho = 0.20$, $P < 0.005$), MMSE ($\rho = -0.14$, $P < 0.05$), and AD diagnosis (1 standard deviation increase in the non-APOE polygenic score was associated with an increase of 0.44 in the log-odds of AD diagnosis, $P<0.01$) remained significant. Figure 1 shows the average polygenic score values in the CN and AD groups. Supplementary Table 1 lists the individual SNPs included in the polygenic score and corresponding associations in the GWAS (Harold D *et al.* 2009) and ADNI samples.

[c] The ADNI sample we analyzed included 17 subjects with the APOE e2/e4 combination. Only 2 of these were CN and 1 was included in the main analysis (i.e., had a CSF sample). Excluding this subject from all our analyses did not alter our main results.

These results demonstrated the clinical relevance of the polygenic score using the CN and AD combined analysis sample. All remaining analyses focused on the CN analysis sample.

--Figure 1 about here--

Polygenic score is correlated with AD-specific cortical thickness in CN subjects

In CN subjects, the average thickness in AD-vulnerable cortex was significantly associated with the polygenic score ($\rho = -0.195$, $P < 0.05$). Supplementary Table 3 lists SNP-level associations with AD-specific cortical thickness, whereas Table 2 lists the partial correlations between the thickness measurements from individual ROIs and the polygenic risk score.

--Table 2 about here --

We further conducted a supplementary analysis across the entire cortex, where we correlated the average thickness within each cortical ROI of the Desikan atlas (Desikan R *et al.* 2006) and the polygenic score (see Supplementary Figure 2). Isthmus of the cingulate, a region that overlaps with the manually defined AD-vulnerable posterior cingulate ROI, exhibited a statistically significant association ($P<0.05$, Bonferroni corrected), while inferior temporal and orbito-frontal regions, which are known to be targeted in early AD, exhibited a suggestive association. However most other cortical regions exhibited *no* statistically significant association between thickness and polygenic risk. For example, across two regions that are known to show little, if any, atrophy during early AD (primary motor and sensory cortices) the partial correlation was +0.10 ($P=0.31$, uncorrected. Note that we expect the true correlation to be negative). Furthermore, the average cortical thickness across the two hemispheres was not associated with the polygenic score in CN subjects ($\rho = -0.006$, $P = 0.95$, uncorrected).

The association between AD-specific cortical thickness and polygenic score is above and beyond APOE

Next, we examined APOE and non-APOE contributions to the association between AD-specific cortical thickness and the polygenic score. APOE ε2 allele was significantly

associated with AD-specific cortical thickness in a load-dependent manner ($\beta = 0.09$, $P = 0.03$), but the ε4 allele was not ($P = 0.83$). The non-APOE polygenic score (that excludes 19q13) was significantly correlated with the thickness measurements in CN subjects ($\rho = -0.26$, $P<0.01$; including APOE as covariate). In APOE ε3 homozygote CN subjects (N=61, 50.8% female), the association between the non-APOE polygenic score and AD-specific cortical thickness remained significant ($\rho = -0.28$, $P<0.05$).

CSF $A\beta_{1-42}$ does not fully explain the association between cortical thickness and polygenic score

Out of the three CSF measurements (CSF $A\beta_{1-42}$, p-tau and t-tau), only $A\beta_{1-42}$ was significantly associated with the polygenic score in the CN group ($\rho = -0.45$, $P < 10^{-5}$). This association remained significant ($\rho = -0.29$, $P < 0.05$) even among those CN subjects with CSF $A\beta_{1-42}$ levels greater than 192 pg/ml, the threshold previously implicated in predicting progression from mild cognitive impairment to AD dementia, (Shaw L *et al.* 2009) (N=64, 48.4% female). Henceforth, we refer to this group as CN with "sub-threshold amyloid burden," since high CSF $A\beta_{1-42}$ is an indication of low amyloid deposition in the brain (Fagan A *et al*. 2006).

To examine whether CSF $A\beta_{1-42}$ explained the correlation between cortical thickness and the polygenic score, we first conducted an analysis where we included CSF $A\beta_{1-42}$ as a covariate. In the entire CN group, AD-specific cortical thickness remained significantly correlated with the non-APOE polygenic score ($\rho = -0.27$, $P<0.01$), whereas the association with APOE ε2 weakened to trend-level ($\beta = 0.06$, $P = 0.08$). In CN individuals with sub-threshold amyloid burden, the association between AD-vulnerable cortical thickness and the non-APOE polygenic score remained significant ($\rho = -0.29$, $P < 0.05$; including CSF $A\beta_{1-42}$ as a covariate) (see Figure 2).

--Figure 2 about here--

The association between AD-specific cortical thickness and polygenic score in CN subjects is possibly driven by a neurodegenerative effect

Our analyses so far demonstrated polygenic AD-related variation in thickness measurements across the vulnerable cortex in CN individuals, even among those without evidence of abnormal brain Aβ deposition. There are at least two possible explanations for this effect: (1) the polygenic risk is associated with cortical thinning via a modulation of the neurodegenerative mechanisms, or (2) the polygenic risk reflects variation in "brain reserve", i.e., the amount of brain tissue available before neurodegeneration begins.

We conducted an exploratory analysis to assess which one of the two scenarios provides a better explanation for the association between polygenic score and AD-specific cortical thickness we observe in our data. We divided the CN group with sub-threshold amyloid burden into two subgroups based on the non-APOE polygenic score. Those with a score greater than the average score were classified as "high risk" (N=17), while the remaining subjects were considered "low risk" (N=46). We then examined the relationship between AD-specific cortical thickness and subject age in these two groups (see Figure 3). A stepwise regression (with sex, education, and the first three principal components from the population substructure analysis as control variables) revealed that the slopes of these two groups were statistically significantly different ($P<0.05$), yet their offsets were not. These results remained robust after discarding the two outlier subjects who were younger than 70 years (see Supplementary Figure 3). These results provided preliminary support for the "variable neurodegeneration" hypothesis and not the "brain reserve" hypothesis, which would predict different offsets for the two groups.

--Figure 3 about here—

Finally, to test the variable neurodegeneration hypothesis directly, we conducted a preliminary analysis on longitudinal (annual) thinning measurements in the CN subjects who received a month 12 MRI scan (N=91, 46% female, 75.2±5.0 years). Average thinning measurements were computed as baseline thickness minus month 12 thickness divided by the time difference, and the processing was done with FreeSurfer's longitudinal stream. The partial correlation between the annual thinning rate and the non-APOE polygenic score was 0.18, which was significant at a trend level ($P=0.07$).

Discussion

The clinical associations of the polygenic score

Late-onset AD is a polygenic disease, with APOE contributing most to the underlying genetic susceptibility. Large-scale case-control studies are expanding the list of genetic variations associated with AD (Waring S and R Rosenberg 2008; Bertram L and R Tanzi 2009; Harold D *et al.* 2009; Lambert J *et al.* 2009; Seshadri S *et al.* 2010; Hollingworth P *et al.* 2011; Naj AC *et al.* 2011). While these newly identified genetic loci may provide valuable insights into the mechanisms that lead to AD (van Es M and L van den Berg 2009) and thus offer targets for intervention, their immediate clinical value is under debate (Pedersen N 2010; Seshadri S *et al.* 2010). Furthermore, the details of the associations between these genetic variants and markers of AD remain unknown.

In this study, we demonstrated that aggregating data across many genetic markers, each suspected to be associated with the disease with a small effect size, yields a score that is associated significantly with clinically defined AD dementia. This association is complementary to APOE genotype status, with a partial correlation between CDR-SB and the polygenic score of 0.20 ($N = 204$, $P < 0.05$) after the effect of APOE genotype was removed. The polygenic score offers a way to investigate AD-related variation in biomarker measurements across healthy individuals, similar to previous studies using APOE (Small G *et al.* 2000; Reiman E *et al.* 2004; Filippini N *et al.* 2009; Fleisher A *et al*. 2009; Sheline Y *et al.* 2010).

Evidence of AD-like neurodegeneration in clinically normal individuals

Structural MRI allows for the detection of macroscopic tissue atrophy associated with neurodegeneration in AD (Jack Jr C *et al.* 1997; Whitwell J *et al*. 2007; Dickerson B *et al.* 2009; Frisoni G *et al.* 2010). Robust markers of AD based on structural MRI include hippocampal volume (Laakso M *et al*. 1995; Jack Jr C *et al.* 1999; Fischl B *et al*. 2002) and thickness of AD-vulnerable cortex (Dickerson B *et al.* 2009; Desikan R *et al*. 2010). Recent evidence has suggested that cortical thickness in AD-vulnerable regions may be a sensitive marker for AD during the preclinical phase (Sabuncu MR *et al.* 2011).

It is often assumed that macroscopically detectable neurodegeneration in AD is preceded by a sequence of molecular events that involve the aberrant accumulation of the proteins Aβ and tau, e.g. (Selkoe D 2004). Although there is some evidence that AD-specific atrophy patterns are detectable prior to cognitive impairment (Csernansky J *et al*. 2005; Jagust W *et al*. 2006; Becker J.A. *et al*. 2011; Sabuncu MR *et al.* 2011), most empirical observations suggest that atrophy more closely tracks with clinical decline (Jack Jr C *et al*. 2005; Savva G *et al*. 2009; Frisoni G *et al.* 2010). In the present study, evidence for an association between thickness in vulnerable cortical regions and genetic risk to AD was found in CN individuals with sub-threshold amyloid burden. Our preliminary analysis suggests that this association is possibly due to a genetic modulation of neurodegeneration, which may be driven by amyloid and/or other factors. This observation agrees with results that demonstrate AD-associated accelerated atrophy rates before the onset of cognitive impairment (Mori E *et al.* 2002; Chen K *et al.* 2007; Schott JM *et al.* 2010). However, we need to emphasize that our interpretation is only preliminary and needs to be further elucidated in future longitudinal studies.

CSF $A\beta_{1\text{-}42}$ measurements were not sufficient to explain the correlation between AD-specific cortical thickness and polygenic score in CN individuals. This result may be indicative of a disassociation between AD-like patterns of cortical thinning and fibrillar amyloid, yet, the correlation between sub-threshold levels of amyloid burden (as measured by CSF $A\beta_{1\text{-}42}$) and the polygenic score is consistent with amyloid-mediated mechanisms of neurodegeneration. Another potential mechanism is mitochondrial dysfunction that may precede AD histopathology (Yao J et al. 2009; Valla J et al.). Although we found evidence of a polygenic association with cortical thickness in AD-vulnerable regions, some of these genes may confer more general vulnerability to oxidative stress, inflammation, microvascular damage, or other processes associated with aging and neurodegenerative diseases. Conversely, some of these genes may reflect varying levels of resilience to neuronal damage due to synaptic density or plasticity mechanisms. Finally, an alternative possibility that we cannot rule out is that the genetic association is in fact due to developmental, and not degenerative, effects – a hypothesis that can be tested in a young and healthy cohort.

The SNP-level associations (Supplementary Table 3) indicate that outside of 19q13, rs7561528 is the SNP that is driving most of the observed effect. This SNP was recently confirmed to be associated with AD and is located close to BIN1 (the bridging integrator 1 gene), which is expressed abundantly in brain tissue (Wechsler-Reya R *et al*. 1997). Amongst the several roles of BIN1 are the promotion of caspase-independent apoptosis, neuronal membrane organization and clathrin-mediated synaptic vessel formation (Wigge P *et al*. 1997), which is disrupted by Aβ (Kelly BL and A Ferreira 2007). BIN1 has also been associated with schizophrenia (Carrasquillo MM *et al*. 2009).

Anatomical ROI-level analyses (Table 2) demonstrate that the associations between thickness measurements and polygenic AD score, while in the same direction, vary in effect size across different regions. This variation might reflect the spatial extent and/or magnitude of vulnerability in the different regions during the early phase of the disease. Intriguingly the posterior cingulate cortex, a region that is particularly vulnerable to early amyloid deposition (Mintun M *et al.* 2006*)* and hypometablism in very early AD (Minoshima S *et al*. 1997), demonstrates the strongest association.

Caveats

The general philosophy behind our MRI and genetic association methodology is to build on prior, independent studies for determining *a priori* anatomic and genomic regions the analysis can focus on. We then chose to aggregate signal across these regions in examining associations. The strength of this approach, which has been employed in neuroimaging (Kloppel S *et al*. 2008; Wolk D and B Dickerson 2010; Sabuncu MR *et al.* 2011) and genetics (Purcell S *et al.* 2009), is that statistical power can be boosted substantially and previously undetectable effects may be revealed. There are, however, two major weaknesses of this approach. First, the analysis does not allow for inferences outside of the *a priori* anatomic and genomic regions. Second, our results provide limited insights into the precise relationship between individual anatomic regions and genomic loci. A detailed characterization will require follow-up studies on independent data sets.

The *a priori* anatomic regions were derived from the OASIS sample and have been used in a prior study (Sabuncu MR *et al.* 2011). We restricted our analyses to these ROIs to

maximize statistical power and minimize bias. In the prior study, we found that AD-specific cortical thickness was a more sensitive biomarker than hippocampal volume in asymptomatic individuals and more closely tracked CSF $A\beta_{1-42}$. Therefore, we opted to focus on cortical thickness as a structural MRI-derived marker in the present study. We further note that there may be systematic discrepancies between the OASIS and ADNI samples that may make these ROIs suboptimal. For example, gender proportion is an obvious difference between the two datasets. One way we attempted to control for such discrepancies was to include relevant covariates in all our analyses.

Although the lack of correlation between the polygenic score, and whole-brain average cortical thickness or thickness in control regions such as the primary motor cortex would suggest otherwise, we cannot fully exclude the possibility that the reported associations are not AD-specific but due to a more general mechanism. One piece of evidence in favor of this argument is the minimal correlation between thickness in the entorhinal cortex, an ROI that exhibits neurodegeneration in early AD (Gomez-Isla T *et al*. 1996; Du A *et al*. 2003), and the polygenic score in CN subjects. On the other hand, the only region to exhibit an association that was strong enough to survive the correction for multiple comparisons included the posterior cingulate, a region that seems to play a central role in early AD.

The list of SNPs examined in constructing the polygenic score was derived from a single recent GWAS (Harold D *et al.* 2009), because the complete list of nominally associated SNPs and corresponding odds ratios were reported in that study. The polygenic score was derived based on analyzing each candidate SNP independently and aggregating the data in an additive model(Purcell S *et al.* 2009). Therefore, potential gene-gene interactions (epistasis) were not explicitly considered. Future research should explore more sophisticated multi-variate models that will allow for the characterization of epistasis(Cordell HJ 2009); and should incorporate additional SNPs discovered by other studies, e.g. (Lambert J *et al.* 2009; Seshadri S *et al.* 2010; Hollingworth P *et al.* 2011; Naj AC *et al.* 2011).

A significant weakness of the present study is due its cross-sectional nature. The characterization of Alzheimer-related variation in clinically normal individuals was

achieved by examining across subject variation of a genetic risk score. Therefore the accuracy of the presented interpretations hinges on how predictive, at the individual-level, this score is of future clinical decline toward AD. For example, it is entirely possible that many of the studied CN subjects with "sub-threshold amyloid burden," may not eventually progress to AD, nullifying any preclinical interpretations of the presented results. Future longitudinal studies that follow healthy individuals with genetic susceptibility and/or biomarker evidence suggestive of early AD pathology are required to elucidate the precise links between genetic susceptibility, neurodegenerative/neuroprotective effects, and brain reserve. An interesting direction of research that may shed light on these links would be the further characterization of the presented effects in a younger (e.g., middle-aged) cohort.

Finally, it is important to note that our discussion of the variation in clinically normal individuals strongly depends on our definition of "clinically normal". In the ADNI study, CNs were selected on the basis of the clinical dementia rating (CDR = 0) and MMSE, which had to be between 24-30. Additionally, CNs had to be non-MCI and thus did not suffer from an objective memory loss, as measured by education-adjusted scores on Wechsler Memory Scale-Revised (WMS-R) (Wechsler D 1987). Further criteria for the categorization of CN were: (i) no active neurological or psychiatric disorders; (ii) some subjects may have had ongoing medical problems, yet the illnesses or their treatments did not interfere with cognitive function; (iii) normal neurological exam; and (iv) were independently functioning community dwellers. Converging evidence suggests that a combination of biomarker abnormalities, and subtle cognitive decline in episodic memory and non-memory domains may portend the future onset of MCI and/or AD (Sperling RA et al. 2011), suggesting that more sensitive cognitive measures may be useful in characterizing preclinical AD-associated behavioral variation. Once such tests are established, future studies will examine the relationships between these test scores and imaging, genetic and CSF markers of AD, and determine the combination of markers that best predicts the likelihood of future decline.

Conclusions

We presented a polygenic score that is associated with clinical dementia, above and beyond APOE genotype and age, the two major risk factors for AD. The strength of the polygenic score is that it aggregates evidence from multiple, weakly associated genomic loci. Similar to the use of APOE in CN subjects (Wishart H *et al.* 2006; Morris J *et al*. 2010; Sheline Y *et al.* 2010), we employed the polygenic score to make inferences about AD biomarkers in clinically normal individuals, a subset of whom may be in the preclinical stages of AD.

The polygenic score was correlated with markers of amyloid but not tau in CN subjects. This is in agreement with the temporal characterization of these biomarkers (Jack Jr C *et al.* 2010) and extends a recent result of a similar APOE ε4/ε2 effect to other loci (Morris J *et al.* 2010).

We further demonstrated an APOE-independent association between AD-specific cortical thickness and the polygenic score in CN subjects, which also remained in the group of individuals with sub-threshold levels of amyloid burden. Our results suggest that CSF biomarkers, structural MRI and genotype data may provide complementary information about AD-like neurodegenerative processes in asymptomatic individuals. Future longitudinal studies will determine whether this combination of markers will prove useful in more accurately predicting prospective cognitive decline and progression to clinical stages of AD.

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Figure Legends

Figure 1. Mean polygenic scores (z-score normalized across the entire analysis sample) in CN (N=104) and AD (N=100) groups. Error bars show standard error of the mean. (a) The polygenic score based on all 26 LD-independent SNPs, (b) excluding 19q13, the APOE-linkage region.

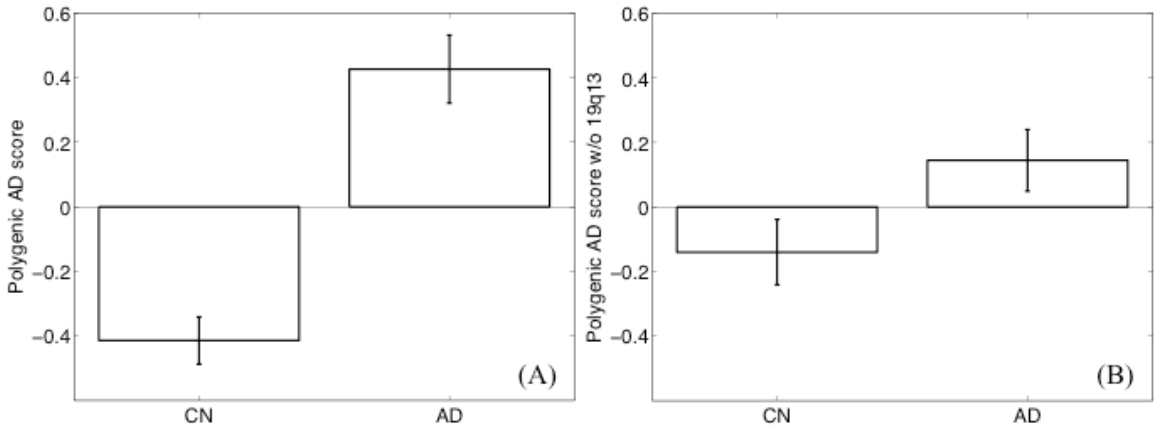


Figure 2. Thickness across AD-vulnerable cortex versus Alzheimer-associated non-APOE polygenic score in CN subjects with sub-threshold levels of amyloid burden (N=64, $\rho = -0.29$, $P < 0.05$).

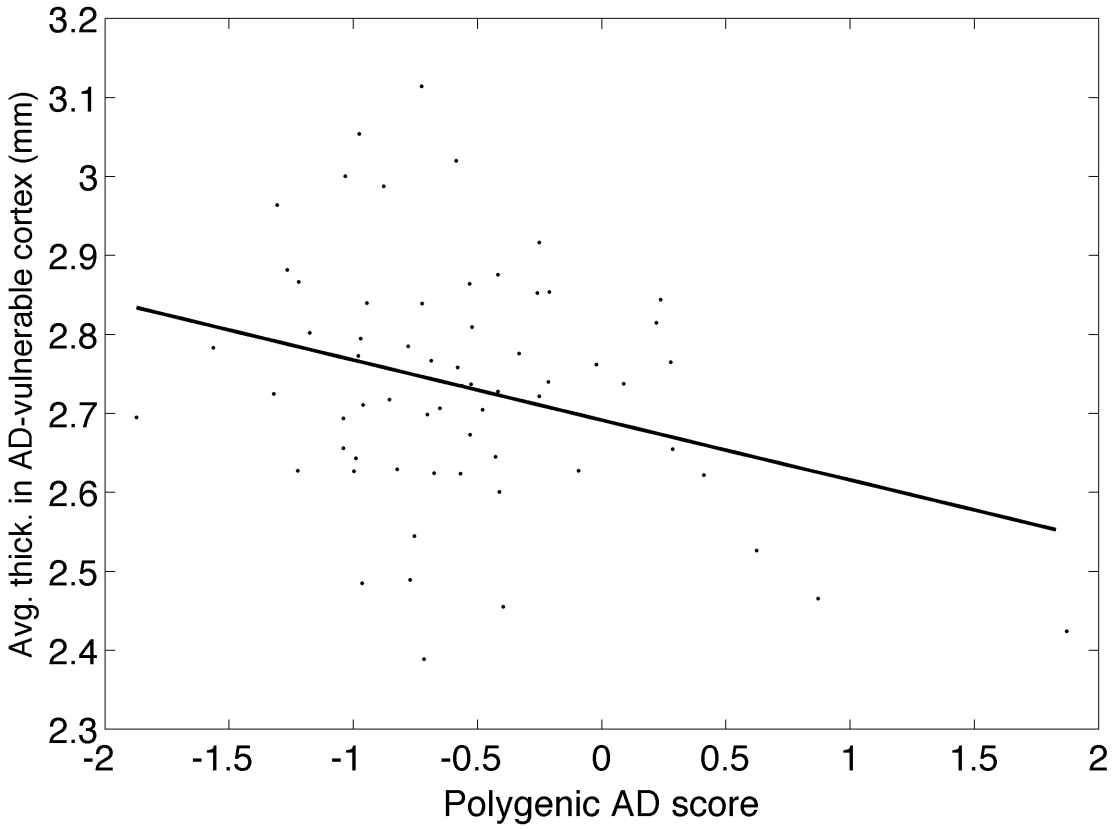
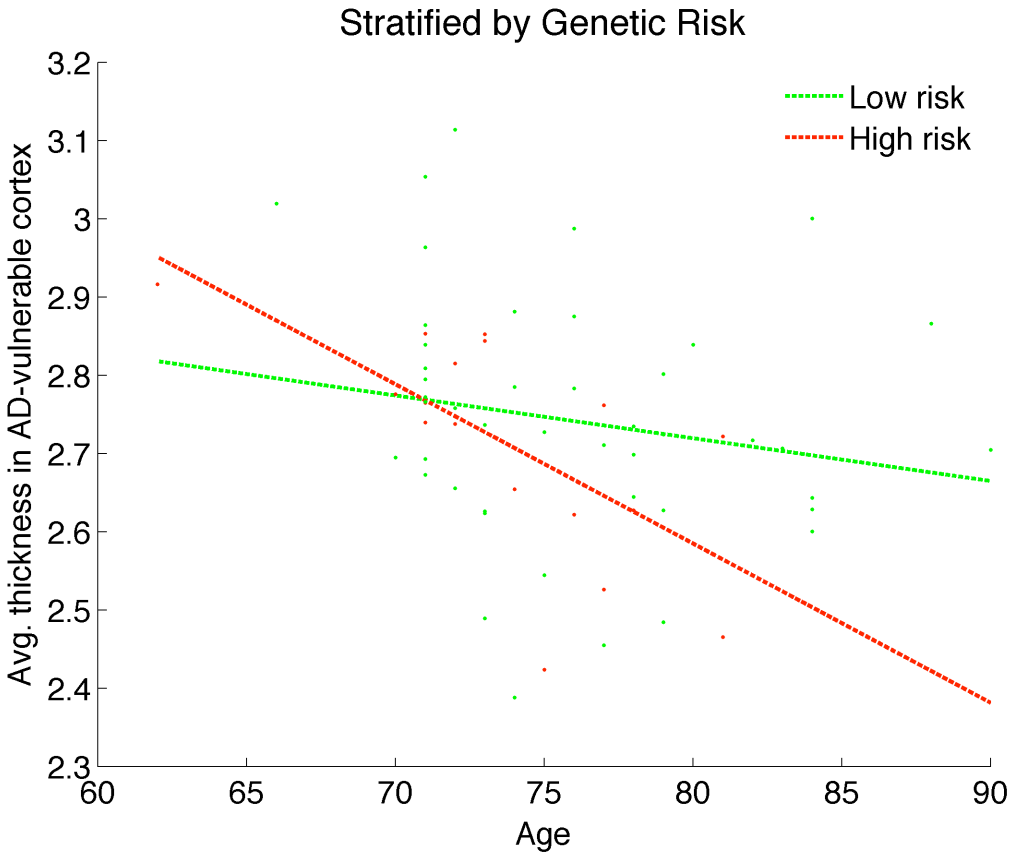


Figure 3. Cortical thickness versus age in CN subjects with sub-threshold levels of amyloid burden, stratified by polygenic score. The high risk group (N=17) has a significantly steeper slope than the low risk group (N=46) ($P < 0.05$).



Tables

Table 1. Summary statistics for the ADNI sample used in the analyses. Mean values are listed with standard deviations in parentheses. * indicates statistically significant group differences, all $P < 0.001$ (two-sampled t-test). Cortical thickness: average thickness in AD-vulnerable cortical regions, CN: Clinically Normal, AD: Alzheimer's patients.

Variable	**CN (N=104)**		**AD (N=100)**
Age	75.9 (5.1)		75.1 (7.8)
Female %	48		42
Education (yrs)	15.9 (2.7)		15.2 (3.3)
CDR-SB*	0.02 (0.1)		4.2 (1.5)
MMSE*	29.1 (1.0)		23.6 (1.9)
CSF Aβ1-42 (pg/ml)*	205.5 (55.5)		143.6 (40.7)
CSF t-tau (pg/ml)*	71.3 (31.0)		119.0 (59.9)
CSF p-tau (pg/ml)*	25.5 (15.1)		41.4 (19.7)
Cortical thickness (mm)*	2.71 (0.17)		2.41 (0.20)

Table 2. Partial correlation between polygenic score and average thickness of individual cortical ROIs in the CN group (N=104). Age, sex, education (years) and the first three principal components from the population substructure analysis were included as control variables. * indicates $P < 0.01$.

	Partial Correlation
Inf. Frontal Cortex	-0.17
Inf. Parietal Cortex	-0.10
Inf. Parietal Sulcus	-0.06
Lat. Temporal	-0.08
Medial Temporal Lobe	-0.02
Retrosplenial/Posterior Cingulate	-0.27*
Temporal Pole	-0.15

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