

The effect of increased genetic risk for Alzheimer's disease on hippocampal and amygdala volume

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Abstract

INTRODUCTION: Reduction in hippocampal and amygdala volume measured via structural MRI is an early marker of Alzheimer's disease (AD). Whether genetic risk factors for AD exert an effect on these subcortical structures independent of clinical status has not been fully investigated.

METHODS: We examine whether increased genetic risk for AD influences hippocampal and amygdala volumes in case-control and population cohorts at different ages, in 1674 Older (aged >53yrs; 17% AD, 39% MCI) and 467 young (16-30 yrs) adults.

RESULTS: An AD polygenic risk score (PRS) combining common risk variants excluding *APOE*, and a SNP in *TREM2*, were both associated with reduced hippocampal volume in healthy older adults and those with mild cognitive impairment (MCI). *APOE* ϵ 4 was associated with hippocampal and amygdala volume in those with AD and MCI, but was not associated in healthy older adults. No associations were found in young adults.

DISCUSSION: Genetic risk for AD affects the hippocampus before the clinical symptoms of AD, reflecting a neurodegenerative effect prior to clinical manifestations in older adults.

Key words

Alzheimer's disease; Polygenic risk score; *APOE*; *TREM2*; Hippocampus; Amygdala.

1. Introduction

The strongest identified genetic risk factor for Alzheimer's disease (AD) is the *APOE* $\epsilon 4$ allele (Genin, et al., 2011). Large-scale GWASMA (Genome Wide Association Study Meta Analyses) have identified an additional 19 common risk loci with small effects on AD risk (Lambert, et al., 2013). A low frequency missense variant in *TREM2* (p.R47H or rs75932628) substantially increases AD risk (Guerreiro, et al., 2013). Whether these variants exert an effect on disease related phenotypes (such as brain atrophy) in the early stages of AD, or before clinical onset is largely unknown.

The earliest histopathological changes in AD are typically seen within the medial temporal lobe, where neurofibrillary tangles and amyloid depositions first form. Beginning in the pre-clinical phase, these lesions lead to changes in regional brain volumes, in particular the hippocampus and amygdala (Yang, et al., 2012). Brain volume reduction is evident in other disorders (e.g. depression and anxiety) as well as in healthy aging, with the hippocampus being especially vulnerable (Small, et al., 2011). Determining how AD risk variants affect hippocampal and amygdala volume directly, and whether this is detectable before clinical manifestations of AD, will provide clues as to how they contribute to disease risk.

An effect of *APOE* status has been observed on structural brain changes in the elderly. Carriers of $\epsilon 4$ are generally found to have smaller hippocampal and amygdala volumes than homozygous $\epsilon 3$ subjects, but this is not consistently observed before the onset of MCI or AD (Hostage, et al., 2013, Khan, et al., 2014, Liu, et al., 2010). Effects have also been identified in young people (O'Dwyer, et al., 2012), though findings have also been inconsistent (Khan, et al., 2014). In addition, sex differences have been reported, with greater deleterious effect of *APOE* $\epsilon 4$ on hippocampal pathology in females (Fleisher, et al., 2005).

Other common AD genetic risk factors identified through GWAS studies have been investigated in relation to hippocampal and amygdala volume, through the use of polygenic

risk scores (PRS). A PRS allows the identification of phenotypic associations that would not be detectable using single variants with low effect size, as well as allowing a reduction in the number of statistical tests (Wray, et al., 2014). A PRS containing the first AD GWAS findings (three genome-wide associated variants) was found to be associated with clinical diagnosis and reduced hippocampal and amygdala volume in the AD case/control cohort ADNI (Alzheimer's Disease Neuroimaging Initiative) (Biffi, et al., 2010). A proxy for the rare *TREM2* risk variant (rs9394721) was also associated with smaller hippocampal volume and increased rate of temporal lobe atrophy in the ADNI cohort (Rajagopalan, et al., 2013).

A recent study assessed the effect of 20 AD risk variants combined in a PRS with various MRI markers of brain aging (intra-cranial volume, total brain volume, hippocampal volume, white matter hyperintensities and brain infarcts) in nondemented older community persons (Chauhan, et al., 2015). The PRS was applied to meta-analysis summary estimates from ten population based studies (total N=11,500). An association was observed with smaller hippocampal volume only, which remained significant after excluding *APOE*. Here we extend on this previous work by investigating the effect of AD genetic risk variants on hippocampal volume, and also investigate amygdala volume in our large sample (N>2000). By accessing the raw genotyping data, as opposed to meta-analysis summary estimates we are able to test for an effect in distinct diagnostic groups, including AD, MCI and healthy elderly to examine at which clinical stage effects can be seen. We also tested for any early effects of AD risk factors on hippocampal and amygdala volume in healthy young adults prior to substantial age related atrophy. Age and sex interaction effects were also investigated. We used the most recent GWAS findings identified by the International Genomics of Alzheimer's Project (IGAP) GWASMA which included 74,046 individuals (Lambert, et al., 2013) to select the nineteen genome wide significant AD risk variants to

include in the PRS. We also examine the effect of several additional PRS adding increasing numbers of SNPs at different P value thresholds of association. Inclusion of SNPs that do not pass the threshold for genome wide significance, but include a proportion of truly associated variants will give increased power to detect an association up to an optimal P value cut-off (Wray, et al., 2014).

2. Methods

2.1 Participants

Five cohorts, including two case-control and three population based, were used (Table 1. ADNI (Mueller, et al., 2005, Alzheimer's Disease Neuroimaging Initiative, www.adni-info.org) and AddNeuroMed (Westman, et al., 2011, Innovative Medicines (InnoMed) in Europe) are comprised of AD cases, MCI, and aged matched controls (Table 1). All AD cases met criteria for either probable or definite AD with inclusion criteria as previously described (Simmons, et al., 2011 and www.adni-info.org). MCI was assessed as having an abnormal memory complaint but with general cognition and functional performance sufficiently preserved such that a diagnosis of AD cannot be made. Elderly controls were screened for dementia.

The population cohorts were the Older Australian Twins Study (OATS) (Sachdev, et al., 2011), the Sydney Memory and Ageing Study (MAS) (Brodaty, et al., 2012), and the Queensland Twin Imaging (QTIM) cohort, of which the latter consists of young adults (Hibar, et al., 2013). For Sydney MAS and OATS diagnosis of MCI and AD were made with the most recent consensus criteria (Winblad, et al., 2004). For all those participants whose neuropsychological or functional profiles indicated the possibility of dementia, a diagnosis was made at a consensus meeting (for a detailed description of Sydney MAS and OATS methodologies see Sachdev, et al., 2009, Sachdev, et al., 2012). Both Sydney MAS and OATS

are longitudinal studies; here we utilized the MRI data and diagnosis of MCI or AD at baseline (i.e. on admission).

All cohorts are independent of the IGAP GWASMA except for ADNI, which contributed 1.6% of the AD cases and 0.5% of the controls (Lambert, et al., 2013). In this study we formed an All-Older group, aged 53-91 years, from four cohorts (ADNI, AddNeuroMed, OATS and Sydney MAS), and stratified into AD, MCI and Healthy Older groups. The QTIM cohort (aged 16-30 years) formed a separate Young Adult group (Table 1). As OATS and QTIM cohorts contain twins we omitted related individuals at random.

2.2 Hippocampal and Amygdala volumes

Subcortical volumes for the hippocampus and amygdala, and intracranial volume (ICV) were extracted from anatomical T1-weighted magnetic resonance images (Image acquisition is described in Supplementary Methods 1 and Supplementary table 1), using validated automated segmentation programs following the ENIGMA (Enhancing Neuro Imaging Genetics through Meta-Analysis) Consortium protocols (Stein, et al., 2012).

For the ADNI, AddNeuroMed and QTIM samples, bilateral amygdala and hippocampus volume segmentation was performed using the Freesurfer image analysis suite, previously reported in depth (Fischl, et al., 2002). Briefly, the T1 structural magnetic resonance imaging (MRI) scan is corrected for intensity bias, skull stripped, and transformed to Talairach space. Each voxel within the MRI volume is then assigned a neuroanatomic label (including left and right hippocampus and amygdala) based on probabilistic information estimated from a manually labelled training set. ICV was estimated based on the determinant of the transformation matrix used when transforming the MR volume to Talairach space.

FSL FIRST was utilised to segment subcortical structures for the Sydney MAS and OATS datasets as previously reported (Patenaude, et al., 2011). Input images were registered to MNI

space through two-stage linear transformation. Deformable mesh models, based on shape and intensity information from a manually segmented training set, were then used to segment bilateral hippocampus and amygdala volumes. ICV was calculated as the inverse of the determinant of the affine transformation matrix, multiplied by the size of the MNI template.

We calculated the mean of left and right hippocampal volume and amygdala volume. All subcortical volumes and ICV (intracranial volumes) outliers were winsorised to 4 standard deviations from the mean.

2.3 Genetic data

A polygenic risk score (PRS) was constructed from genome-wide SNP array data using 19 genome wide significant AD risk variants (from IGAP, PRS $P < 5 \times 10^{-8}$) (Lambert, et al., 2013). Scores were calculated by summing the number of risk alleles weighted by the effect size (log odds ratio) (Supplementary Table 2).

Threshold PRS were calculated with stage 1 summary data from IGAP using the method previously described (Purcell, et al., 2009) (see Supplementary Methods 3 for details of the IGAP discovery sample). SNPs within 500kb either side of the *APOE* locus were excluded to ensure all *APOE* associated signal was removed. LD-based clumping was carried out on all SNPs in the summary data, providing the most significantly associated SNP in each region of LD (using PLINK clumping command with a pairwise r^2 threshold of 0.2 and a physical distance threshold of 300kb). SNPs were checked for flip strands between the summary data and each cohort. We calculated the total score for each individual as the number of score alleles weighted by the log of the odds ratio from the discovery SNPs from the IGAP sample (using PLINK score function). The risk score calculation was repeated for P value thresholds of $P < 1 \times 10^{-6}$, $P < 1 \times 10^{-4}$, $P < 1 \times 10^{-3}$, $P < 0.01$, $P < 0.05$, $P < 0.1$, $P < 0.5$ and $P < 1$ (all SNPs). The number of SNPs included in each risk score is shown in Supplementary Table 3.

The PRS method assumes a polygenic disease model and is suitable for common variants with the assumptions of an additive effect and independent contribution to risk (Wray, et al., 2014). Both *APOE* and *TREM2* don't meet these assumptions and were assessed separately from the PRS. *APOE* $\epsilon 4$ allele is a diplotype acting under a co-dominant genetic model, and with a much larger effect size than the other common AD risk variants (Genin, et al., 2011). For the rare *TREM2* variant (p.R47H/rs75932628) we used rs9394721, the closest available imputed proxy ($r^2=0.492$) (Rajagopalan, et al., 2013). *APOE* genotyping was carried out as previously described (Jorm, et al., 2007). Haplotype $\epsilon 2/\epsilon 4$ carriers were excluded from the analysis due to the potential for counteracting effects of these alleles (1.7% of individuals).

2.4 Statistical Analyses

To assess how well the PRS predicted AD risk, we first tested for an association of each PRS with clinical status (excluding MCI) in the AddNeuroMed cohort which is independent from the IGAP discovery sample, using logistic regression (STATA version 11), controlling for age, sex and 4 ancestry principal components. Discriminative improvement of each PRS was assessed using receiver operating characteristic (ROC) curves. The equality of each area under the curve (AUC) for the ROC curves were testing using STAT “roccomp” taking into account the implicit correlation between curves (as the test is applied to the same sample). A covariance matrix is estimated using the method of structural components and the resulting test statistic has an asymptotically χ^2 distribution (DeLong, et al., 1988). We also tested the association between both the *APOE* $\epsilon 4$ genotype and the *TREM2* SNP and AD risk.

Next in each of the five cohorts we tested for an effect of PRS, the number of *TREM2* rs9394721 and *APOE* $\epsilon 4$ alleles on mean hippocampal and amygdala volumes, using linear

regression. Covariates included ICV, age, sex, and four ancestry principal components. Age², age*sex, age²*sex were also included as covariates if they showed evidence of an association (P<0.05).

We then combined all cohorts in a mega-analysis to test for associations in the All-Older group (a combined dataset incorporating the AD, MCI and Healthy Older groups shown in table 1), controlling for study and clinical status as well as stratifying by clinical status (AD, MCI and Healthy Older). Where there was a significant association we repeated the analyses testing for interaction effects of both sex and age, by including an interaction term for both sex and age with the independent variable (either number of *APOE* ε4 alleles, *TREM2* rs9394721 alleles and PRS) in the regression equation. Non-significant product terms were removed and the regression repeated. Interactions were identified by a significant product term and the nature of the interaction was investigated by testing the association in separate age and gender groups.

For all regression analysis the variance explained (R²) was calculated by taking the R² value of the full model (covariates and genotype/PRS) and subtracting the R² of the reduced model (covariates only). We also assessed the total variance explained by all the investigated AD risk variants by including PRS P<1x10⁻⁴, *APOE* ε4 and *TREM2* together within a single multiple regression and again subtracting the R² of the reduced model (covariates only) from the total R².

Due to ascertainment and measurement differences between cohorts we also carried out a meta-analysis (STATA METAN specifying a random effects model) and tested for study heterogeneity. Meta-analyses tested for an association between the number of *APOE* ε4 alleles, the PRS P<0.001 and *TREM2* rs9394721, with hippocampal volume and amygdala in the combined All-Older group (controlling for disease status), AD, MCI and Healthy Older groups.

3. Results

3.1 AD risk

The AD PRS was associated with AD risk in the AddNeuroMed cohort. The most significantly associated threshold was $P < 1 \times 10^{-3}$ (OR=1.51; $P=0.011$), though this had no more discriminative accuracy than a model with only age and sex covariates. However *APOE* $\epsilon 4$ genotype was highly associated with AD risk (OR=2.41 $p=1.64 \times 10^{-5}$) with significant improvement in discriminative accuracy over the covariates. In contrast *TREM2* rs9394721 was not associated with AD risk in this small sample (Table 2).

3.2 Hippocampal and amygdala volume

The effects of PRS, *TREM2* and *APOE* on hippocampal and amygdala volume in the mega-analysis are shown in Tables 3 and 4 and Figure 1, with Table 3 showing results for the combined All-Older and the young adults, and Table 4 stratifying the older group by clinical group (AD, MCI, Healthy Older). Results from each of the four older cohorts when analysed separately are presented in Supplementary Table 4.

APOE $\epsilon 4$ status was strongly associated with lower hippocampal and amygdala volumes in the All-Older group. However, when stratified by clinical status, *APOE* $\epsilon 4$ associated with lower volumes in the AD and MCI groups, but not in the Healthy Older group. In the AD group, this constitutes each *APOE* $\epsilon 4$ allele resulting in an average of 143 mm³ (4.8%) reduction in mean hippocampal volume and 47 mm³ in mean amygdala volume (3.8%). For MCI this was a 132 mm³ reduction in hippocampal volume (4.0%), and 37mm³ in amygdala volume (2.8%). No associations were found in the Young Adults.

We also found an association of PRS containing common AD risk variants of small effect with reduced hippocampal volume in the All-Older group. The strongest effect was for the

PRS containing SNPs below the $P < 10^{-4}$ threshold ($p = 0.004$). This association was suggestive in the stratified MCI ($N = 645$ PRS $P < 1 \times 10^{-4}$ $p = 0.057$) and Healthy Older ($N = 723$ PRS $P < 1 \times 10^{-3}$ $p = 0.075$) sub-groups, but not apparent in the smaller AD group. In the Young Adults there was no effect of the PRS on hippocampal volume, and no effect of PRS was identified for amygdala volume in any group.

TREM2 rs9394721 was associated with lower hippocampal volume in the All-Older group. It showed suggestive association in the MCI and Healthy Older groups (significant when not corrected for multiple testing), but not in the smaller AD group or Young Adults. No association was found between *TREM2* and amygdala volume.

The variance explained (R^2) by *APOE* $\epsilon 4$, PRS and *TREM2* rs9394721 on hippocampal and amygdala volume are shown for each regression in tables 4-5, and for hippocampal volume in figure 1. When we assessed the variance explained by all the genetic risk factors combined (by including *APOE* $\epsilon 4$, PRS $P < 1 \times 10^{-4}$ and *TREM2* rs9394721 within a single multiple regression) on hippocampal volume we found that they accounted for an R^2 of 1.6% in the All-Older group, 3% in the AD group, 3.2% in the MCI group and 0.3% in the Healthy Older group.

Age and sex interactions were identified and are shown as footnotes in Tables 3 and 4. The *APOE* effect was stronger in females (in the All-Older group $N = 817$, $\beta = -0.15$, $p = 3.4 \times 10^{-8}$), with an interaction found between *APOE* $\epsilon 4$ and sex in both AD and MCI groups. There was also an interaction between PRS (testing threshold $P < 1 \times 10^{-3}$) and sex, where the effect was again driven by the females (in the All-Older group: $N = 848$, $\beta = -0.08$, $p = 0.001$). For *TREM2* we identified an age*rs9394721 interaction in the All-Older group, with an association found for those ≤ 75 years ($N = 791$, $\beta = -0.10$, $P = 3.4 \times 10^{-4}$) but not > 75 years, and no frequency difference between the two age groups. When stratified by clinical status the age effect was only evident in the MCI group (≤ 75 years: $N = 292$, $\beta = -0.19$, $P = 0.003$).

The results for the meta-analysis are shown in Supplementary table 5 and Supplementary Figures 1-3). The association of *APOE* $\epsilon 4$ genotype and hippocampal volume in MCI and AD was confirmed but the association between *APOE* $\epsilon 4$ and amygdala volume was not significant in the meta-analysis, likely due to heterogeneity across studies in the measurement of this structure. The association of *TREM2* rs9394721 was confirmed in the All-Older group, but was not significant in any of the clinical groups. Similarly, the association between PRS and hippocampal volume was not significant, reflecting the small effect and loss of power due to the meta analysis.

4. Discussion

Chauhan et al. (2015) recently showed that an AD PRS constructed from 20 AD risk loci associated with reduced hippocampal volume in a large population based meta-analysis. Here we confirm these findings using raw genotype level data using a smaller sample size, but including a larger number of variants in the PRS identified in the largest GWASMA available (IGAP discovery sample: 17,008 cases, 37,154 controls (Lambert, et al., 2013)). We also investigate a variant in *TREM2*. Through stratification into AD, MCI and healthy older groups, we show at which disease stage associations can be identified.

In our cross-study mega-analyses we confirm the association between *APOE* and hippocampal volume in adults with Alzheimer's disease and mild cognitive impairment (MCI) ($\epsilon 4$ carriers having smaller volumes compared to non-carriers), but show no association in healthy older adults or in young adults. The same pattern of association, although to a lesser degree, was found for amygdala volume. In addition, AD polygenic risk scores (PRS, excluding *APOE*) and the rare variant *TREM2* were also found to be associated with hippocampal volume in older adults (i.e. All-Older group). The AD PRS association was driven by females, while the *TREM2* association was limited to those aged 75 years and

under, and both appear associated with volume loss during healthy aging and in cases of MCI. Notably, the PRS explain substantially less of the variance in hippocampal volume than *APOE* genotype. Nevertheless, In the AD and MCI cohorts, the combined effects of *APOE* $\epsilon 4$, the AD risk score, and *TREM2* accounted for more variance in hippocampal volume than *APOE* $\epsilon 4$ alone (fig 1). Combining the effects of the AD genetic risk variants (i.e. *APOE*, AD PRS, and *TREM2*), increases the total variance in hippocampal volume that can be explained in AD (to 3%) and in those with MCI (to 3.2%). However, in the healthy elderly *TREM2* is a better predictor alone (accounting for 0.3% of variance in hippocampal volume). Longitudinal studies exploring healthy aging and transition to MCI and AD will provide further clarity regarding these genetic profiles.

In contrast to the mega-analysis findings, meta-analyses only confirmed the strong association between *APOE* and hippocampal volume, and the *TREM2* rs9394721 in the larger combined All-older group. The reduction in significance in the groups with small sample sizes likely reflects heterogeneity across studies, and highlights the additional insights to be gained from mega-analyses.

The differing effects of AD risk variants at different disease stages may give insight into the mechanisms of how they contribute to AD risk. Shared mechanisms may drive brain aging and AD, with clinical onset resulting when brain aging surpasses a threshold (Swerdlow, 2011). Support for this hypothesis comes from gene expression studies that identify substantial overlap between expression changes with age, and in AD (Avramopoulos, et al., 2011). An opposing view is that volume differences associated with AD risk may be identifiable before clinical diagnosis because they are prodromal changes. Amyloid deposition and the specific pattern and acceleration of atrophy in AD compared to normal aging suggests that early AD is different from normal aging (Fjell, et al., 2014). Recent findings suggest that AD related hippocampal atrophy can be detected 4.2 years prior to onset

of clinical manifestations of dementia (Villemagne, et al., 2013). Testing the effect of these variants in middle age is the important next step in ascertaining how early the AD risk variant effect on degeneration can be identified. We found no association in young adulthood, although in a sub-sample of the same cohort previous work has found an association between the *CLU* genotype and white matter microstructure (Braskie, et al., 2011), suggesting other MRI measures may be more sensitive to early differences. This could include examination of the hippocampal microstructure, such as CA1 and subiculum regions, as patterns in volume reductions in subfields of the hippocampus together with entorhinal cortex may differentiate between AD and healthy aging (Wisse, et al., 2014).

We show that AD variants below genome wide significance contribute to the variance in hippocampal volume. The PRS containing SNPs below the $P < 10^{-4}$ threshold was the most highly associated with AD disease status and with hippocampal volume in older adults. The threshold that maximises the variance explained in the target sample depends on the size of the GWASMA discovery sample and the underlying genetic architecture (Wray, et al., 2014). Notably, in preliminary analyses we did not identify any association with PRS and hippocampal volume when using data from an earlier AD GWASMA with a smaller sample size (GERAD discovery sample: 3,941 cases, 7,848 controls (Harold, et al., 2009); data not shown). Large scale studies also using the GERAD discovery sample have found no association of AD PRS without *APOE* with memory or cognitive ability in people without dementia (Harris, et al., 2014, Verhaaren, et al., 2013). In light of our results these should now be tested using the latest IGAP GWASMA.

Our finding that PRS has a stronger effect on hippocampal volume in females adds to the literature showing the importance of taking sex into account in genetic association analysis in AD. The stratification of AD GWASMA by sex would be useful, allowing the generation of sex specific PRS (Altmann, et al., 2014, Azad, et al., 2007). All cohorts in the discovery

sample had excess females (58-68%) (Lambert, et al., 2013), so the PRS may be biased towards female genetic risk factors.

TREM2 is a receptor expressed on microglia that stimulates phagocytosis of cell debris and suppresses inflammatory reactivity. Over expression in the brains of AD transgenic mice ameliorates A β deposition, neuroinflammation, and neuronal loss (T. Jiang, et al., 2014). Thus, in humans, mutation carriers may have an inflammatory phenotype with impaired tissue debris clearance resulting in increased grey matter atrophy during aging. We build on the previous finding of an association of rs9394721, a R47H proxy with hippocampal volume in ADNI (Rajagopalan, et al., 2013), and show that the association is independent of clinical status and evidence of an association is detectable before onset of MCI. Indeed, we found associations to be limited to the Healthy Older adult and MCI groups, and the effect to be stronger in those aged ≤ 75 years. Previously the R47H genotype has been associated with lower cognitive function in those without AD and has been shown to confer increased risk for Parkinson's disease, frontotemporal dementia and amyotrophic lateral sclerosis (Jonsson, et al., 2013, Yaghmoor, et al., 2014). As rs9394721 is a relatively poor proxy for the disease causing variant ($r^2=0.492$) (Rajagopalan, et al., 2013), it is likely that a stronger association would be identified if direct genotyping results were available.

We did not find any association of PRS, or *TREM2* on amygdala volume, contrary to the association of PRS and amygdala volume reported in the ADNI case-control cohort (Biffi, et al., 2010). Amygdala volume is significantly reduced in AD in a similar magnitude to the hippocampus (Klein-Koerkamp, et al., 2014). However, we have previously shown that amygdala atrophy is not identified over a two year follow-up period in elderly population samples, which contrasts with significant atrophy in the hippocampus (J. Jiang, et al., 2014). Less age related atrophy in the amygdala and potential unreliability of segmentation of this small structure may reduce power to detect an effect.

APOE $\epsilon 4$ was associated with lower hippocampal and amygdala volumes in the combined All-Older group, with a large effect in those with AD and MCI ($\geq 4\%$ average difference in hippocampus and $\geq 2.8\%$ in amygdala per *APOE* $\epsilon 4$ allele). The association is stronger in females, as previously shown in those with MCI (Fleisher, et al., 2005). An increasing body of work shows an interaction between *APOE* and sex in AD risk which may be explained by the influence of estrogen levels acting in concert with *APOE* (Altmann, et al., 2014, Stone, et al., 1997). *APOE* $\epsilon 4$ was not associated with hippocampal volume in Healthy Older conflicting with some previous reports (Biffi, et al., 2010, Lind, et al., 2006, Reiman, et al., 1998, Wishart, et al., 2006) but in agreement with recent findings from other large scale studies, including both measures of volume and atrophy (Ferencz, et al., 2013, Manning, et al., 2014). Population studies of older people are likely to have a proportion of individuals with MCI, which may be the source of identified *APOE* association in conflicting reports. Even so, an effect in the healthy elderly may be detectable in a larger sample, especially as there is a reduced frequency of *APOE* $\epsilon 4$ alleles compared to AD and MCI groups. In cognitively healthy adults a large scale meta analysis showed that *APOE* $\epsilon 4$ carriers performed worse on measures of episodic memory, and global cognitive ability, with effect sizes increasing as age increases (Wisdom, et al., 2011). There are also conflicting reports on the effect of *APOE* on hippocampal volume in young people (Khan, et al., 2014, O'Dwyer, et al., 2012). In agreement with our result, a large study (N=1400) of 14 year olds also found no association (Khan, et al., 2014). Recent evidence of neurodevelopmental effects of *APOE* have been identified as affecting grey matter volumes in infants and neonates, suggesting that associations may be transient and could be clearer at a very young age (Dean, et al., 2014, Knickmeyer, et al., 2014).

In summary, in addition to the *APOE4* genotype, a PRS comprised of common AD risk variants of small effect, and *TREM2* associate with hippocampal volume independently of

clinical status in the elderly. *TREM2* is associated in Healthy Older and MCI individuals, with the AD PRS showing a trend nearing significance. This correlation with early MRI markers of AD shows evidence for a genetic modulation of neurodegeneration, and the potential for a combination of PRS and brain biomarkers to aid in the prediction of future cognitive decline and the development of AD.

A limitation of this study is in the combining of results across several cohorts with participant ascertainment and diagnostic adjudication as well as the use of multiple scanner platforms adding variability in the volumetric measures used. This is reflected as significant between study heterogeneity when the analysis is performed as a meta-analysis (Supplementary table 5). Replication in independent samples is required to confirm these findings, and testing the effect of these variants in middle age is the important next step to ascertain how early the prodromal AD risk variant effects on degeneration can be identified.

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Table 1. Demographics and descriptive statistics for (A) The five cohorts and (B) the derived AD, MCI, and Healthy Older and Young Adult groups.

A. Cohort	N (% Male)	Mean Age (SD, range)	% AD, MCI	APOE ϵ 4 Frequency [*] (N)	Mean Hippocampal Volume mm ³ (SD) [†]	Mean Amygdala Volume mm ³ (SD) [†]
ADNI	741 (59.9)	75.5 (6.8,55-91)	23.3, 48.7	0.300 (741)	3397 (574)	1488 (273)
AddNeuroMed	356 (43.5)	74.4 (6.1,53-89)	33.7, 32.0	0.254 (293)	3098 (613)	1176 (259)
Sydney MAS	542 (45.4)	78.4 (4.7,70-90)	0, 32.3	0.123 (534)	3371 (422)	1109 (248)
OATS	199 (32.7)	70.3 (5.2,65-89)	0.5, 12.7	0.169 (186)	3600 (436)	1195 (205)
QTIM	467 (37.9)	22.9 (3.3,16-30)	0,0	0.137 (417)	4095 (393)	1673 (239)
B, Group	N (% Male)	Mean Age (SD, range)		APOE ϵ 4 Frequency [*] (N)	Mean Hippocampal Volume mm ³ (SD) [†]	Mean Amygdala Volume mm ³ (SD) [†]
AD	280 (49.6)	75.5 (7.1, 55-91)		0.403 (267)	2933 (586)	1233 (328)
MCI	648 (56.8)	75.8 (6.7, 55-88)		0.264 (624)	3312 (522)	1336 (314)
Healthy Older	746 (42.2)	75.3 (5.9, 53-90)		0.138 (735)	3553 (453)	1291 (299)
Young Adults	467 (37.9)	22.9 (3.3,16-30)		0.137 (417)	4095 (393)	1673 (239)

AD, MCI and Healthy Older groups are comprised from ADNI and AddNeuroMed case/control cohorts and Sydney MAS and OATS population based cohorts.

All-Older group incorporates the AD, MCI and Healthy Older groups. Young Adults are from QTIM.

^{*}APOE genotyping and GWAS were not available for all subjects.

[†]Not corrected for ICV, sex, or age.

Abbreviations: SD, standard deviation; ICV, intracranial volume; AD, Alzheimer's disease; MCI, mild cognitive impairment; APOE, Apolipoprotein E; ADNI, Alzheimer's Disease Neuroimaging Initiative; Sydney MAS, Sydney Memory and Ageing Study; OATS, Older Australian Twins Study; QTIM, Queensland Twin Imaging.

Table 2. Association between AD Polygenic Risk Scores (PRS) and disease status (AD vs Control) in AddNeuroMed.

	N	OR	SE	P	Pseudo R ² *	AUC [†]
APOE ε4	188	2.41	0.49	1.64x10⁻⁵	0.086	0.800
PRS P<1	202	1.19	0.19	0.280	0.004	0.725
PRS P<0.5	202	1.18	0.19	0.318	0.004	0.727
PRS P<0.1	202	1.06	0.17	0.696	0.001	0.724
PRS P<0.05	202	1.07	0.17	0.657	0.001	0.724
PRS P<0.01	202	1.14	0.18	0.410	0.003	0.727
PRS P<1x10 ⁻³	202	1.41	0.23	0.032	0.017	0.734
PRS P<1x10 ⁻⁴	202	1.51	0.24	0.011	0.024	0.745
PRS P<1x10 ⁻⁶	202	1.37	0.22	0.040	0.016	0.737
PRS P<5x10 ⁻⁸	202	1.45	0.23	0.020	0.021	0.739
TREM2 rs9394721	202	0.96	0.14	0.784	0.000	0.722

APOE and GWAS data were not available for all subjects, and individuals with ε2/ε4 genotype were excluded for analysis including APOE genotype. The ratio of AD to controls is 1.05:1.

Abbreviations: APOE, Apolipoprotein E; N, number; OR, odds ratio; SE, standard error. AUC; Area under the ROC curves,

*The Pseudo R² is the McFadden's R² and is an estimation of the proportion of variance explained by the predictor.

†The AUC of the regression equation with covariates only (age, sex and 4 ancestry principle components have) is 0.721, with the APOE ε4 being the only variable which increases the AUC above using covariates alone at level which is statically significant ($\chi^2=1.62$ p=0.014) .

Table 3. Mega-analysis of the effect of AD genetic risk factors on hippocampal and amygdala volume in Older and Young Adults.

		Mean Hippocampus				Mean Amygdala			
		N	R ²	β (SE)	P	N	R ²	β (SE)	P
All-Older	<i>APOE</i> ε4	1611	0.011	-0.11 (0.02)	8.7x10⁻⁹^a	1607	0.003	-0.05 (0.02)	0.004
	PRS P<5x10 ⁻⁸	1664	0.000	-0.02 (0.02)	0.229	1660	0.000	0.02 (0.02)	0.381
	PRS P<1x10 ⁻⁶	1664	0.002	-0.04 (0.02)	0.030	1660	0.000	-0.01 (0.02)	0.607
	PRS P<1x10 ⁻⁴	1664	0.003	-0.05 (0.02)	0.004	1660	0.000	-0.01 (0.02)	0.746
	PRS P<1x10 ⁻³	1664	0.002	-0.05 (0.02)	0.007^b	1660	0.000	0.01 (0.02)	0.633
	PRS P<0.01	1664	0.000	-0.02 (0.02)	0.246	1660	0.000	0.00 (0.02)	0.919
	PRS P<0.05	1664	0.000	-0.02 (0.02)	0.315	1660	0.000	0.00 (0.02)	0.819
	PRS P<0.1	1664	0.000	-0.02 (0.02)	0.369	1660	0.000	0.01 (0.02)	0.793
	PRS P<0.5	1664	0.000	-0.01 (0.02)	0.480	1660	0.000	0.01 (0.02)	0.580
	PRS P<1	1664	0.000	-0.01 (0.02)	0.663	1660	0.000	0.01 (0.02)	0.725
	<i>TREM2</i> rs9394721	1664	0.004	-0.06 (0.02)	0.001^c	1660	0.000	-0.02 (0.02)	0.389
Young Adults	<i>APOE</i> ε4	401	0.000	0.02 (0.04)	0.653	404	0.006	-0.08 (0.05)	0.120
	PRS P<5x10 ⁻⁸	447	0.004	-0.06 (0.04)	0.126	450	0.006	-0.08 (0.05)	0.091
	PRS P<1x10 ⁻⁶	447	0.000	0.01 (0.04)	0.801	450	0.000	0.02 (0.05)	0.656
	PRS P<1x10 ⁻⁴	447	0.002	-0.04 (0.04)	0.291	450	0.001	0.03 (0.05)	0.522
	PRS P<1x10 ⁻³	447	0.004	-0.06 (0.04)	0.111	450	0.000	0.02 (0.05)	0.734
	PRS P<0.01	447	0.000	-0.02 (0.04)	0.646	450	0.001	0.03 (0.05)	0.468
	PRS P<0.05	447	0.001	0.02 (0.04)	0.562	450	0.002	0.04 (0.05)	0.351
	PRS P<0.1	447	0.000	0.02 (0.04)	0.651	450	0.002	0.04 (0.05)	0.395
	PRS P<0.5	447	0.000	0.00 (0.04)	0.972	450	0.000	-0.02 (0.05)	0.687
	PRS P<1	447	0.000	0.00 (0.04)	0.996	450	0.000	-0.02 (0.05)	0.690
	<i>TREM2</i> rs9394721	447	0.000	0.00 (0.04)	0.991	450	0.000	0.00 (0.05)	0.921

The combined All-Older group comprises the four elderly cohorts (ADNI, AddNeuroMed, OATS and Sydney MAS, including those with AD, MCI and Healthy Older). Cohort and disease status included as covariates along with standard covariates. The Young Adult group comprises the QTIM cohort. Significant associations are shown in bold.

^aStronger association in females (N=817 β=-0.15 p=3.4x10⁻⁸) compared to males (N=794 β=-0.08 p=0.004).

^bAssociation driven by females (Females; N=848, β=-0.08, p=0.001 and Males; N=816, β=-0.01, p=0.626).

^cAssociation found in those 75 years of age or younger (≤75 years: N=791, β=-0.10, P=3.4x10⁻⁴; >75 years; N=873, β=0.028, P=0.240).

Abbreviations: R², the variance explained by the genotype/PRS; β, standardised Beta; SE, standard error;

Table 4. Mega analysis of the effect of AD genetic risk factors on hippocampal and amygdala volume in the AD, MCI and Healthy Older groups.

		Mean Hippocampus				Mean Amygdala			
		N	R ²	β (SE)	P	N	R ²	β (SE)	P
AD	<i>APOE</i> ε4	267	0.027	-0.18 (0.04)	1.1 x10⁻⁴ ^a	268	0.008	-0.11 (0.05)	0.023
	PRS P<5x10 ⁻⁸	279	0.000	0.00 (0.04)	0.951	280	0.000	-0.01 (0.05)	0.760
	PRS P<1x10 ⁻⁶	279	0.000	0.00 (0.04)	0.932	280	0.007	-0.09 (0.05)	0.053
	PRS P<1x10 ⁻⁴	279	0.000	-0.02 (0.05)	0.655	280	0.001	-0.03 (0.05)	0.512
	PRS P<1x10 ⁻³	279	0.001	-0.03 (0.04)	0.442	280	0.000	-0.02 (0.05)	0.718
	PRS P<0.01	279	0.001	-0.02 (0.04)	0.600	280	0.000	-0.02 (0.05)	0.705
	PRS P<0.05	279	0.000	-0.01 (0.04)	0.780	280	0.000	0.01 (0.05)	0.860
	PRS P<0.1	279	0.000	-0.01 (0.04)	0.781	280	0.000	0.01 (0.05)	0.825
	PRS P<0.5	279	0.000	0.00 (0.04)	0.974	280	0.002	0.05 (0.05)	0.278
	PRS P<1	279	0.000	0.01 (0.04)	0.888	280	0.002	0.05 (0.05)	0.281
	<i>TREM2</i> rs9394721	279	0.004	-0.06 (0.04)	0.166	280	0.000	0.01 (0.04)	0.854
MCI	<i>APOE</i> ε4	621	0.024	-0.16 (0.03)	1.5x10⁻⁶ ^b	619	0.005	-0.08 (0.03)	0.012
	PRS P<5x10 ⁻⁸	645	0.000	-0.02 (0.03)	0.649	643	0.000	0.02 (0.03)	0.511
	PRS P<1x10 ⁻⁶	645	0.003	-0.05 (0.03)	0.121	643	0.000	0.00 (0.03)	0.910
	PRS P<1x10 ⁻⁴	645	0.004	-0.07 (0.03)	0.057	643	0.001	0.03 (0.03)	0.361
	PRS P<1x10 ⁻³	645	0.002	-0.04 (0.03)	0.192	643	0.000	0.01 (0.03)	0.674
	PRS P<0.01	645	0.000	0.01 (0.03)	0.687	643	0.000	-0.01 (0.03)	0.655
	PRS P<0.05	645	0.000	0.02 (0.03)	0.572	643	0.000	-0.02 (0.03)	0.518
	PRS P<0.1	645	0.000	0.01 (0.04)	0.825	643	0.001	-0.04 (0.03)	0.248
	PRS P<0.5	645	0.000	0.01 (0.03)	0.761	643	0.000	-0.02 (0.03)	0.612
	PRS P<1	645	0.000	0.01 (0.03)	0.716	643	0.000	-0.02 (0.03)	0.607
	<i>TREM2</i> rs9394721	645	0.004	-0.06 (0.03)	0.047^c	643	0.000	0.00 (0.03)	0.916
Healthy Older	<i>APOE</i> ε4	723	0.000	0.00 (0.03)	0.961	720	0.000	-0.01 (0.03)	0.775
	PRS P<5x10 ⁻⁸	740	0.002	-0.05 (0.03)	0.157	737	0.001	0.02 (0.03)	0.388
	PRS P<1x10 ⁻⁶	740	0.001	-0.04 (0.03)	0.289	737	0.000	0.02 (0.03)	0.552
	PRS P<1x10 ⁻⁴	740	0.002	-0.05 (0.03)	0.126	737	0.001	-0.03 (0.03)	0.298
	PRS P<1x10 ⁻³	740	0.003	-0.05 (0.03)	0.075	737	0.000	0.02 (0.03)	0.503
	PRS P<0.01	740	0.001	-0.03 (0.03)	0.331	737	0.000	0.01 (0.03)	0.599
	PRS P<0.05	740	0.001	-0.04 (0.03)	0.228	737	0.001	0.03 (0.03)	0.276
	PRS P<0.1	740	0.001	-0.03 (0.03)	0.416	737	0.001	0.03 (0.03)	0.208
	PRS P<0.5	740	0.001	-0.03 (0.03)	0.363	737	0.000	0.01 (0.03)	0.663
	PRS P<1	740	0.000	-0.02 (0.03)	0.490	737	0.000	0.00 (0.03)	0.910
	<i>TREM2</i> rs9394721	740	0.004	-0.06 (0.03)	0.042	737	0.001	-0.04 (0.03)	0.165

The AD group includes AD cases from ADNI and AddNeuromed, the MCI groups includes those with MCI from ADNI, AddNeuroMed, Sydney MAS and OATS, and Healthy Older includes healthy controls from ADNI and AddNeuroMed, and those with no diagnosis of MCI or dementia from Sydney MAS and OATS. P values are not correct for multiple testing. Associations with P<0.05 are shown in bold

^aStronger association in females (N=130, β=-0.27, p=7.3x10⁻⁵) compared to males (N=137, β=0.11, p=0.068).

^bStronger association in females (N=267, β=-0.21, p=1.7x10⁻⁴) compared to males (N=816, β=-0.01, p=0.626).

^c Association found in those 75 years of age or younger (≤75 years; N=292, β=-0.19, P=0.003 and N=353, β=0.015, P=0.699).

Abbreviations: R², the variance explained by the genotype

Figure Legends

Fig 1. Variance explained (R^2) by the effect of *APOE* $\epsilon 4$, PRS and *TREM2* rs9394721 on hippocampal volume in the Combined All-Older, and in separate clinical groups.

The combined variance explained (R^2) by *APOE* $\epsilon 4$, PRS $P < 1e-04$ and *TREM2* rs9394721 within a single multiple regression (representing all the variance explained by the AD risk variants investigated) totalled 0.016 in the All-Older group. For the separate clinical groups $R^2 = 0.030$ in the AD group, 0.032 in the MCI group and 0.003 in the Healthy Older group.

Ns represent the total N of the slightly differing sample sizes for each variant/score (each individual sample size is shown in tables 3 and 4) * $P < 0.05$, ** $P < 0.001$ represent significant P values for the association of the variant/score with hippocampal volume (not corrected for multiple testing).