



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



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### ABSTRACT

Reduction in hippocampal and amygdala volume measured via structural magnetic resonance imaging 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. 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 >53 years; 17% AD, 39% mild cognitive impairment [MCI]) and 467 young (16–30 years) adults. An AD polygenic risk score combining common risk variants excluding apolipoprotein E (APOE), and a single nucleotide polymorphism in TREM2, were both associated with reduced hippocampal volume in healthy older adults and those with MCI. APOE ε4 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. Genetic risk for AD affects the hippocampus before the clinical symptoms of AD, reflecting a neurodegenerative effect before clinical manifestations in older adults.

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## 1. Introduction

The strongest identified genetic risk factor for Alzheimer's disease (AD) is the apolipoprotein E (*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 preclinical 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 mild cognitive impairment (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 (PRSs). 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 (3 genome-wide associated variants) was found to be associated with clinical diagnosis and reduced hippocampal and amygdala volume in the AD case and/or control cohort Alzheimer's Disease

Neuroimaging Initiative (ADNI; 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 magnetic resonance imaging (MRI) markers of brain aging (intracranial volume, total brain volume, hippocampal volume, white matter hyperintensities, and brain infarcts) in non-demented older community persons (Chauhan et al., 2015). The PRS was applied to meta-analysis summary estimates from 10 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 before 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 19 genome-wide significant AD risk variants to include in the PRS. We also examine the effect of several additional PRS adding increasing numbers of single nucleotide polymorphisms (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 cutoff (Wray et al., 2014).

## 2. Methods

### 2.1. Participants

Five cohorts, including two case-control and 3 population based, were used (Table 1). ADNI (Mueller et al., 2005; Alzheimer's Disease Neuroimaging Initiative, [www.adni-info.org](http://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

**Table 1**  
Demographics and descriptive statistics for (A) The 5 cohorts and (B) the derived AD, MCI, and healthy older and young adult groups

A. cohort	N (% male)	Mean age (SD, range)	% AD, MCI	<i>APOE</i> $\epsilon 4$ frequency <sup>a</sup> (N)	Mean hippocampal volume mm <sup>3</sup> (SD) <sup>b</sup>	Mean amygdala volume mm <sup>3</sup> (SD) <sup>b</sup>
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)	<i>APOE</i> $\epsilon 4$ frequency <sup>a</sup> (N)	Mean hHippocampal volume mm <sup>3</sup> (SD) <sup>b</sup>	Mean amygdala volume mm <sup>3</sup> (SD) <sup>b</sup>	
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.

Key: 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; GWAS, Genome Wide Association Study.

<sup>a</sup> *APOE* genotyping and GWAS were not available for all subjects.

<sup>b</sup> Not corrected for ICV, sex, or age.

with inclusion criteria as previously described (Simmons et al., 2011 and [www.adni-info.org](http://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, 2012). Both Sydney MAS and OATS are longitudinal studies; here, we used 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 4 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 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 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 labeled 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 used 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 2-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 outliers were winsorized to 4 standard deviations from the mean.

## 2.3. Genetic data

A 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 [OR]; Supplementary Table 2).

Threshold PRS was 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 500 kb 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 300 kb). 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 OR 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 codominant 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 curves. The equality of each area under the curve for the receiver operating characteristic 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  $\chi^2$  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 5 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 4 ancestry principal components. Age<sup>2</sup>, age  $\times$  sex, age<sup>2</sup>  $\times$  sex interactions 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*  $\epsilon 4$  alleles, *TREM2* rs9394721 alleles, and PRS) in the

regression equation. Nonsignificant 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 ( $R^2$ ) was calculated by taking the  $R^2$  value of the full model (covariates and genotype/PRS) and subtracting the  $R^2$  of the reduced model (covariates only). We also assessed the total variance explained by all the investigated AD risk variants by including PRS  $p < 1 \times 10^{-4}$ , APOE  $\epsilon 4$ , and TREM2 together within a single multiple regression and again subtracting the  $R^2$  of the reduced model (covariates only) from the total  $R^2$ .

Owing 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  $\epsilon 4$  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 Fig. 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, and healthy older). Results from each of the 4 older cohorts when analyzed 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 mm<sup>3</sup> (4.8%) reduction in mean hippocampal volume and 47 mm<sup>3</sup> in mean amygdala volume (3.8%). For MCI, this was a 132 mm<sup>3</sup> reduction in hippocampal volume (4.0%), and 37 mm<sup>3</sup> 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 less than the  $p < 10^{-4}$  threshold ( $p = 0.004$ ). This association was suggestive in the stratified MCI ( $N = 645$  PRS  $p < 1 \times 10^{-4}$  and  $p = 0.057$ ) and healthy older ( $N = 723$ , PRS,  $p < 1 \times 10^{-3}$  and  $p = 0.075$ ) subgroups, 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 3 and 4, and for hippocampal volume in Fig. 1. When we assessed the variance explained by all the genetic risk factors combined (by including APOE  $\epsilon 4$ , PRS  $P < 1e04$  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  $\times$  rs9394721 interaction in the all-older group, with an association found for those  $\leq 75$  years ( $N = 791$ ,  $\beta = -0.10$ ,  $p = 3.4 \times 10^{-4}$ ) but not  $> 75$  years, and no frequency difference between the 2 age groups. When stratified by clinical status the age effect was only evident in the MCI group ( $\leq 75$  years:  $N = 292$ ,  $\beta = -0.19$ ,  $p = 0.003$ ).

The results for the meta-analysis are shown in Supplementary Table 5 and Supplementary Figs 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

**Table 2**

Association between AD polygenic risk scores (PRS) and disease status (AD vs. control) in AddNeuroMed

Genetic variant/PRS	N	OR	SE	<i>p</i>	Pseudo $R^2$ <sup>a</sup>	AUC <sup>b</sup>
APOE $\epsilon 4$	188	2.41	0.49	$1.64 \times 10^{-5}$	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 $p < 1 \times 10^{-3}$	202	1.41	0.23	0.032	0.017	0.734
PRS $p < 1 \times 10^{-4}$	202	1.51	0.24	0.011	0.024	0.745
PRS $p < 1 \times 10^{-6}$	202	1.37	0.22	0.040	0.016	0.737
PRS $p < 5 \times 10^{-8}$	202	1.45	0.23	0.020	0.021	0.739
TREM2 rs9394721	202	0.96	0.14	0.784	0.000	0.722

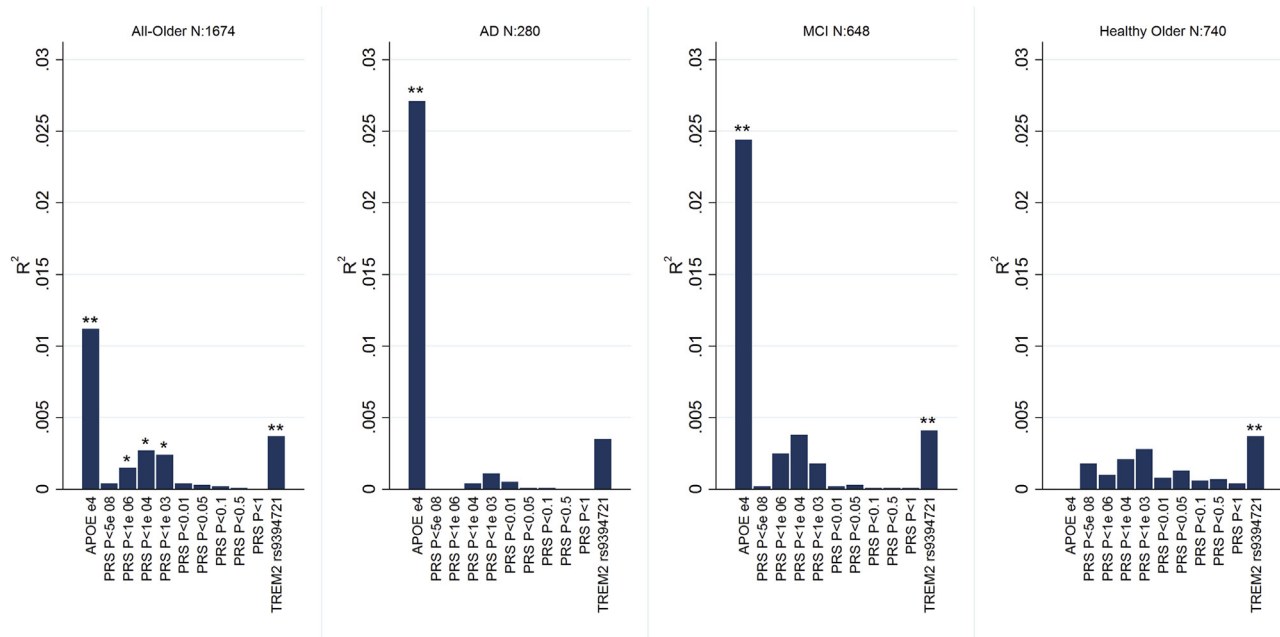
APOE and GWAS data were not available for all subjects, and individuals with  $\epsilon 2/\epsilon 4$  genotype were excluded for analysis including APOE genotype. The ratio of AD to controls is 1.05:1.

Key: APOE, apolipoprotein E; AUC, area under the ROC curves; GWAS, Genome Wide Association Study; N, number; OR, odds ratio; SE, standard error.

<sup>a</sup> The Pseudo  $R^2$  is the McFadden's  $R^2$  and is an estimation of the proportion of variance explained by the predictor.

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





**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  $p < 1e04$ , and *TREM2* rs9394721 within a single multiple regression (representing all the variance explained by the AD risk variants investigated) totaled 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). \* $p < 0.05$  and \*\* $p < 0.001$  represent significant  $p$  values for the association of the variant/score with hippocampal volume (not corrected for multiple testing). Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; MCI, mild cognitive impairment;  $R^2$ , the variance explained by the genotype.

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.

**Table 3**  
Mega-analysis of the effect of AD genetic risk factors on hippocampal and amygdala volume in older and young adults

Participant group	Genetic variant/PRS	Mean hippocampus				Mean amygdala			
		N	$R^2$	$\beta$ (SE)	$p$	N	$R^2$	$\beta$ (SE)	$p$
All older	<i>APOE</i> $\epsilon 4$	<b>1611</b>	<b>0.011</b>	<b>-0.11 (0.02)</b>	<b><math>8.7 \times 10^{-9a}</math></b>	<b>1607</b>	<b>0.003</b>	<b>-0.05 (0.02)</b>	<b>0.004</b>
	PRS $p < 5 \times 10^{-8}$	1664	0.000	-0.02 (0.02)	0.229	1660	0.000	0.02 (0.02)	0.381
	PRS $p < 1 \times 10^{-6}$	<b>1664</b>	<b>0.002</b>	<b>-0.04 (0.02)</b>	<b>0.030</b>	1660	0.000	-0.01 (0.02)	0.607
	PRS $p < 1 \times 10^{-4}$	<b>1664</b>	<b>0.003</b>	<b>-0.05 (0.02)</b>	<b>0.004</b>	1660	0.000	-0.01 (0.02)	0.746
	PRS $p < 1 \times 10^{-3}$	<b>1664</b>	<b>0.002</b>	<b>-0.05 (0.02)</b>	<b>0.007<sup>b</sup></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
Young Adults	<i>TREM2</i> rs9394721	<b>1664</b>	<b>0.004</b>	<b>-0.06 (0.02)</b>	<b>0.001<sup>c</sup></b>	1660	0.000	-0.02 (0.02)	0.389
	<i>APOE</i> $\epsilon 4$	401	0.000	0.02 (0.04)	0.653	404	0.006	-0.08 (0.05)	0.120
	PRS $p < 5 \times 10^{-8}$	447	0.004	-0.06 (0.04)	0.126	450	0.006	-0.08 (0.05)	0.091
	PRS $p < 1 \times 10^{-6}$	447	0.000	0.01 (0.04)	0.801	450	0.000	0.02 (0.05)	0.656
	PRS $p < 1 \times 10^{-4}$	447	0.002	-0.04 (0.04)	0.291	450	0.001	0.03 (0.05)	0.522
	PRS $p < 1 \times 10^{-3}$	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 4 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.

Key:  $\beta$ , standardized beta; AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; *APOE*, apolipoprotein E; MCI, mild cognitive impairment; OATS, Older Australian Twins Study; PRS, polygenic risk scores; QTIM, Queensland Twin Imaging;  $R^2$ , the variance explained by the genotype/PRS; SE, standard error; Sydney MAS, Sydney Memory and Ageing Study.

<sup>a</sup> Stronger association in females ( $N = 817$ ,  $\beta = -0.15$ ,  $p = 3.4 \times 10^{-8}$ ) compared to males ( $N = 794$ ,  $\beta = -0.08$ ,  $p = 0.004$ ).

<sup>b</sup> Association driven by females (Females;  $N = 848$ ,  $\beta = -0.08$ ,  $p = 0.001$  and Males;  $N = 816$ ,  $\beta = -0.01$ ,  $p = 0.626$ ).

<sup>c</sup> Association found in those 75 years of age or younger ( $\leq 75$  years:  $N = 791$ ,  $\beta = -0.10$ ,  $p = 3.4 \times 10^{-4}$ ;  $> 75$  years:  $N = 873$ ,  $\beta = 0.028$ ,  $p = 0.240$ ).

**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

Participant group	Genetic variant/PRS	Mean hippocampus				Mean amygdala				
		N	R <sup>2</sup>	β (SE)	p	N	R <sup>2</sup>	β (SE)	p	
AD	<i>APOE</i> ε4	<b>267</b>	<b>0.027</b>	<b>-0.18 (0.04)</b>	<b>1.1 × 10<sup>-4a</sup></b>	<b>268</b>	<b>0.008</b>	<b>-0.11 (0.05)</b>	<b>0.023</b>	
	PRS <i>p</i> < 5 × 10 <sup>-8</sup>	279	0.000	0.00 (0.04)	0.951	280	0.000	-0.01 (0.05)	0.760	
	PRS <i>p</i> < 1 × 10 <sup>-6</sup>	279	0.000	0.00 (0.04)	0.932	280	0.007	-0.09 (0.05)	0.053	
	PRS <i>p</i> < 1 × 10 <sup>-4</sup>	279	0.000	-0.02 (0.05)	0.655	280	0.001	-0.03 (0.05)	0.512	
	PRS <i>p</i> < 1 × 10 <sup>-3</sup>	279	0.001	-0.03 (0.04)	0.442	280	0.000	-0.02 (0.05)	0.718	
	PRS <i>p</i> < 0.01	279	0.001	-0.02 (0.04)	0.600	280	0.000	-0.02 (0.05)	0.705	
	PRS <i>p</i> < 0.05	279	0.000	-0.01 (0.04)	0.780	280	0.000	0.01 (0.05)	0.860	
	PRS <i>p</i> < 0.1	279	0.000	-0.01 (0.04)	0.781	280	0.000	0.01 (0.05)	0.825	
	PRS <i>p</i> < 0.5	279	0.000	0.00 (0.04)	0.974	280	0.002	0.05 (0.05)	0.278	
	PRS <i>p</i> < 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	<b>621</b>	<b>0.024</b>	<b>-0.16 (0.03)</b>	<b>1.5 × 10<sup>-6b</sup></b>	<b>619</b>	<b>0.005</b>	<b>-0.08 (0.03)</b>	<b>0.012</b>
		PRS <i>p</i> < 5 × 10 <sup>-8</sup>	645	0.000	-0.02 (0.03)	0.649	643	0.000	0.02 (0.03)	0.511
PRS <i>p</i> < 1 × 10 <sup>-6</sup>		645	0.003	-0.05 (0.03)	0.121	643	0.000	0.00 (0.03)	0.910	
PRS <i>p</i> < 1 × 10 <sup>-4</sup>		645	0.004	-0.07 (0.03)	0.057	643	0.001	0.03 (0.03)	0.361	
PRS <i>p</i> < 1 × 10 <sup>-3</sup>		645	0.002	-0.04 (0.03)	0.192	643	0.000	0.01 (0.03)	0.674	
PRS <i>p</i> < 0.01		645	0.000	0.01 (0.03)	0.687	643	0.000	-0.01 (0.03)	0.655	
PRS <i>p</i> < 0.05		645	0.000	0.02 (0.03)	0.572	643	0.000	-0.02 (0.03)	0.518	
PRS <i>p</i> < 0.1		645	0.000	0.01 (0.04)	0.825	643	0.001	-0.04 (0.03)	0.248	
PRS <i>p</i> < 0.5		645	0.000	0.01 (0.03)	0.761	643	0.000	-0.02 (0.03)	0.612	
PRS <i>p</i> < 1		645	0.000	0.01 (0.03)	0.716	643	0.000	-0.02 (0.03)	0.607	
<i>TREM2</i> rs9394721		<b>645</b>	<b>0.004</b>	<b>-0.06 (0.03)</b>	<b>0.047<sup>c</sup></b>	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 <i>p</i> < 5 × 10 <sup>-8</sup>	740	0.002	-0.05 (0.03)	0.157	737	0.001	0.02 (0.03)	0.388
	PRS <i>p</i> < 1 × 10 <sup>-6</sup>	740	0.001	-0.04 (0.03)	0.289	737	0.000	0.02 (0.03)	0.552	
	PRS <i>p</i> < 1 × 10 <sup>-4</sup>	740	0.002	-0.05 (0.03)	0.126	737	0.001	-0.03 (0.03)	0.298	
	PRS <i>p</i> < 1 × 10 <sup>-3</sup>	740	0.003	-0.05 (0.03)	0.075	737	0.000	0.02 (0.03)	0.503	
	PRS <i>p</i> < 0.01	740	0.001	-0.03 (0.03)	0.331	737	0.000	0.01 (0.03)	0.599	
	PRS <i>p</i> < 0.05	740	0.001	-0.04 (0.03)	0.228	737	0.001	0.03 (0.03)	0.276	
	PRS <i>p</i> < 0.1	740	0.001	-0.03 (0.03)	0.416	737	0.001	0.03 (0.03)	0.208	
	PRS <i>p</i> < 0.5	740	0.001	-0.03 (0.03)	0.363	737	0.000	0.01 (0.03)	0.663	
	PRS <i>p</i> < 1	740	0.000	-0.02 (0.03)	0.490	737	0.000	0.00 (0.03)	0.910	
	<i>TREM2</i> rs9394721	<b>740</b>	<b>0.004</b>	<b>-0.06 (0.03)</b>	<b>0.042</b>	737	0.001	-0.04 (0.03)	0.165	

The AD group includes AD cases from ADNI and AddNeuroMed; the MCI groups include 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.

Key: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; *APOE*, apolipoprotein E; MCI, mild cognitive impairment; OATS, Older Australian Twins Study; PRS, polygenic risk scores; QTIM, Queensland Twin Imaging; R<sup>2</sup>, the variance explained by the genotype; SE, standard error; Sydney MAS, Sydney Memory and Ageing Study.

<sup>a</sup> Stronger association in females (N = 130, β = -0.27, *p* = 7.3 × 10<sup>-5</sup>) compared to males (N = 137, β = 0.11, *p* = 0.068).

<sup>b</sup> Stronger association in females (N = 267, β = -0.21, *p* = 1.7 × 10<sup>-4</sup>) compared to males (N = 816, β = -0.01, *p* = 0.626).

<sup>c</sup> 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).

In our cross-study mega-analyses, we confirm the association between *APOE* and hippocampal volume in adults with AD and MCI (ε4 carriers having smaller volumes compared to noncarriers), 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 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, whereas 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* ε4, the AD risk score, and *TREM2* accounted for more variance in hippocampal volume than *APOE* ε4 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 suggest 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 before 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 subsample 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 lower than genome-wide significance contribute to the variance in hippocampal volume. The PRS containing SNPs lower than the  $p < 10^{-4}$  threshold was the most highly associated with AD disease status and with hippocampal volume in older adults. The threshold that maximizes 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: 3941 cases, 7848 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 toward 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 $\beta$  deposition, neuroinflammation, and neuronal loss (Jiang et al., 2014b). Thus, in humans, mutation carriers may have an inflammatory phenotype with impaired tissue debris clearance resulting in increased gray 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  $\leq 75$  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 2 year follow-up period in elderly population samples, which contrasts with significant atrophy in the hippocampus (Jiang et al., 2014a). 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 gray 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 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.

## Disclosure statement

The authors have no conflicts of interest to disclose.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2015.12.023>.

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