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## Association of Alzheimer disease GWAS loci with MRI-markers of brain aging

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## Abstract

Whether novel risk variants of Alzheimer's disease (AD) identified through genome-wide association studies (GWAS) also influence MRI-based intermediate phenotypes of AD in the general population is unclear. We studied association of 24 AD risk loci with intracranial volume (ICV), total brain volume (TBV), hippocampal volume (HV), white matter hyperintensity (WMH) burden, and brain infarcts in a meta-analysis of genetic association studies from large population-based samples (N=8,175–11,550). In single-SNP based tests, AD risk allele of *APOE* (rs2075650) was associated with smaller HV (p=0.0054) and *CD33* (rs3865444) with smaller ICV (p=0.0058). In gene-based tests, there was associations of *HLA-DRB1* with TBV (p=0.0006) and *BIN1* with HV (p=0.00089). A weighted AD genetic risk score was associated with smaller HV (beta±SE=−0.047±0.013, p=0.00041), even after excluding the *APOE* locus (p=0.029). However, only association of AD genetic risk score with HV, including *APOE*, was significant after multiple testing correction (including number of independent phenotypes tested). These results suggest that novel AD genetic risk variants may contribute to structural brain aging in non-demented older community persons.

## Keywords

Alzheimer; MRI-markers; genetic risk score; GWAS; hippocampal volume

## 1. INTRODUCTION

Alzheimer's disease (AD) is the leading cause of dementia and represents a major public health burden (Ballard, et al., 2011). Converging evidence suggests that pathological

processes leading to this progressive neurodegenerative disorder start many years before clinical diagnosis of dementia (Sperling, et al., 2011). MRI-markers of brain aging, including total brain volume (TBV) and hippocampal volume (HV), and markers of vascular brain injury, including white matter hyperintensities (WMH) and brain infarcts, are powerful predictors of dementia and may, at least in part, represent intermediate markers reflecting pathological processes leading to AD (DeBette and Markus, 2010, Jack, et al., 2013, Jack, et al., 2010, Kaye, et al., 1997, Sperling, et al., 2011, Vermeer, et al., 2007). Intracranial volume (ICV), an imaging marker reflecting brain growth during development and maturation, was suggested to be correlated with resilience to brain damage (Negash, et al., 2013).

Recently, large scale genome-wide association studies (GWAS) and candidate gene based studies have identified novel susceptibility loci for late-onset AD (Boada, et al., 2013, Carrasquillo, et al., 2009, Harold, et al., 2009, Hollingworth, et al., 2011, Jonsson, et al., 2012, Jonsson, et al., 2013, Lambert, et al., 2009, Lambert, et al., 2013, Naj, et al., 2011, Seshadri, et al., 2010). These AD risk variants have recently been used to examine the genotypic overlap between AD and other types of dementia (Carrasquillo, et al., 2014). Some of these variants have been studied with respect to various MRI measures in a mixed study sample of AD patients, mildly cognitive impaired and healthy controls (Biffi, et al., 2010, Furney, et al., 2011). They could also be implemented to explore the impact of genetic determinants of AD on MRI-markers of structural brain changes in non-demented community persons. Indeed, this could provide important information on the disease mechanisms through which these genes affect the risk of AD, and could be of interest for the design of preventative interventions. Whether all previously and newly discovered AD risk loci influence brain structure in advance of clinically detectable dementia has never been systematically investigated in large community samples to our knowledge. Our aim was to study association of known AD GWAS loci with ICV, TBV, HV, WMH burden and brain infarcts in non-demented participants from 10 population-based studies.

## 2. MATERIALS and METHODS

### 2.1. Population

Analyses were performed on 8,175 to 11,550 dementia free participants of European ancestry with quantitative brain MRI and genome-wide genotypes (N=8,175 for ICV, N=8,673 for TBV, N=11,550 for HV, N=9,361 for WMH burden and N=9,401 for brain infarcts), from up to 10 population-based cohort studies participating in the Cohorts of Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium: Aging Gene-Environment Susceptibility (AGES)–Reykjavik Study, Atherosclerosis Risk in Communities Study (ARIC), Austrian Stroke Prevention Study (ASPS), Cardiovascular Health Study (CHS), Framingham Heart Study (FHS), Rotterdam Study (RS), Erasmus Rucphen Family (ERF) study, Religious Order Study (ROS) & Rush Memory and Aging Project (MAP), Tasmanian Study of Cognition and Gait (TASCOG) and the 3C-Dijon study. Each study secured approval from institutional review boards, and all participants provided written informed consent for study participation, brain MRI, and use of DNA for genetic research. Individual studies are described in the Supplementary Appendix.

## 2.2. MRI scans

In each study, MRI scans were performed and interpreted in a standardized fashion, without reference to clinical or genetic information. Details on MRI parameters and phenotype definition are provided in the Supplementary Appendix. Briefly, automated or semi-quantitative post-processing software was used to measure ICV and TBV. TBV was expressed as percentage of ICV to correct for differences in head size (Ikram, et al., 2012). HV was evaluated using operator-defined boundaries drawn on serial coronal sections or automated methods (Bis, et al., 2012). WMH burden was estimated on a quantitative scale using custom-written computer programs in AGES-Reykjavik, ASPS, FHS, and RS; in ARIC and CHS, WMH burden was estimated on a semi-quantitative scale (Fornage, et al., 2011). Brain infarcts were defined as areas of abnormal signal intensity in a vascular distribution that lacked mass effect, 3–4 mm, distinct from dilated perivascular spaces (Debette, et al., 2010).

## 2.3. AD GWAS loci

We manually scanned the GWAS catalog (<http://www.genome.gov/gwastudies/>) and Alzgene ([www.alzgene.org/](http://www.alzgene.org/)) for GWAS on AD. We only chose studies performed on European subjects, including a replication stage, examining single marker based associations and having loci reaching genome wide significance ( $P < 5.0 \times 10^{-8}$ ). This led to the identification of 24 independent loci. Effect estimates for SNPs with the lowest p-value in each locus (defined as the index SNP of the locus) are presented in Supplementary Table 1. We included the *CD33* locus (rs3865444) despite absence of replication in the latest AD GWAS meta-analysis; (Lambert, et al., 2013) this locus was previously replicated in several AD GWAS, (Hollingworth, et al., 2011, Naj, et al., 2011) and recent functional studies provide strong evidence for involvement of rs3865444 and *CD33* in AD pathology (Bradshaw, et al., 2013). For the *APOE-ε* polymorphism we used rs2075650 as a proxy ( $r^2=0.48$  with rs429358, the *APOE-ε* SNP), because *APOE-ε* genotypes cannot be reliably imputed on commercial genome-wide chips. The AD risk variants near *HLA-DRB1* (Lambert, et al., 2013), *ATP5H/KCTD2* (Boada, et al., 2013), in *TREM2*, (Jonsson, et al., 2013), and *APP* (Jonsson, et al., 2012) were not included for single-SNP based association and genetic risk score based association as no index SNP or proxy ( $r^2 > 0.3$ ) was available among the genome-wide genotypes for MRI-markers of brain aging.

## 2.4. Power calculation

Quanto software (Gauderman, 2002a, Gauderman, 2002b) was used to compute power of of the five MRI marker studies assuming additive model of inheritance at  $\alpha=0.0025$  (Supplementary Figure 1). Power for the quantitative traits (ICV, TBV, HV, WMH burden) was computed for different percentage variance explained while for brain infarcts, a dichotomous trait, it was computed for different odds ratios at different allele frequencies.

## 2.5. Correlation between phenotypes and equivalent number of independent phenotypes

Correlation between the five MRI phenotypes in 3C-Dijon and FHS was calculated based on Pearson's correlation using the "rcorr" function in R. These correlations were used to compute the equivalent number of independent phenotypes using the online tool matSpDlite

(<http://neurogenetics.qimrberghofer.edu.au/matSpDlite/>). MatSpDlite which is based on the same principles used to identify number of independent SNPs in a locus, gives the equivalent number of independent variables in a correlation ( $r$ ) matrix, depending upon the ratio of observed eigenvalue variance (after spectral decomposition) to its theoretical maximum (Nyholt, 2004).

## 2.6. Association Analyses

Three analytical approaches were taken to examine the associations of interest.

**2.6.1. Single-SNP based association analysis**—We tested for association of AD GWAS loci with MRI-markers of brain aging using association estimates obtained from meta-analyses of GWAS for ICV(Ikram, et al., 2012), TBV(Ikram, et al., 2012), HV(Bis, et al., 2012), WMH burden (Fornage, et al., 2011) and brain infarcts (Debetto, et al., 2010) using genotypes imputed on the HapMap2 CEU reference panel. AD risk alleles, as described in the latest AD GWAS meta-analysis(Lambert, et al., 2013) were modeled as the effect alleles for associations with MRI-markers of brain aging. Logistic (brain infarcts) or linear (ICV, TBV, HV and WMH burden) regression was performed within each study, adjusting for age, gender, and principal components of population stratification, and for familial relationships or study center if relevant. For WMH burden, data was log transformed to achieve normal distribution and associations were additionally adjusted for ICV (except for studies measuring WMH burden on a semi-quantitative visual scale, visual grades being inherently normalized for brain size)(Fornage, et al., 2011). For most phenotypes (ICV, TBV, HV, and brain infarcts) meta-analyses were performed using fixed effects inverse variance weighted meta-analysis. For WMH burden, meta-analysis was performed using effective sample size weighted meta-analysis, because WMH burden was measured on different scales across studies. If the lead SNP at a specific AD GWAS locus was not available, a proxy SNP ( $r^2 > 0.70$  in 1000G CEU) of the lead SNP was used to check single-SNP based association results (Supplementary Table 1). After Bonferroni correction for testing 20 independent loci,  $p < 0.0025$  was considered significant for single-SNP based associations. However, application of a more stringent threshold additionally accounting for the number of independent phenotypes tested led to a Bonferroni correction of  $p < 0.000625$ .

**2.6.2. Gene-based association analysis**—Gene-based association tests can be more powerful in comparison to single-SNP based association tests when there are many causal variants in a gene with small effects (Liu, et al., 2010). Single-SNP based association results from the respective MRI-marker GWAS meta-analysis were used to compute gene-based association results using the Versatile Gene-Based Association Study2 (VEGAS2) software (<https://vegas2.qimrberghofer.edu.au/>) (Liu, et al., 2010). The gene annotations and LD calculation in VEGAS2 are based on 1000 genomes (phase 1 version 3). This tool annotated all but one gene (*MS4A4E*) within 50KB of the index SNPs. The test incorporates information from all markers within a gene and accounts for linkage disequilibrium (LD) between markers by using simulations from the multivariate normal distribution. Gene-based association analyses were performed for all protein coding genes (N=65 genes) which lie within a 50kb distance of index SNP of the AD risk loci. Gene boundaries were defined as 50kb upstream and downstream of the start and end of gene (Liu, et al., 2010). The choice

of 50 KB boundary to cover a gene was chosen as a trade-off between a longer boundary which would have caused excess overlap between nearby genes and a shorter boundary which would have ignored potential regulatory regions (Liu, et al., 2010). Maximum permutation limits were set to 1000,000. After correcting for the number of genes (N=65) tested the multiple testing threshold was  $p < 0.00077$ . A more stringent correction additionally accounting for number of independent phenotypes (N=4) tested, lead to a multiple testing threshold of  $p < 0.00019$  for gene based association.

**2.6.3. Construction of genetic risk score**—We constructed a genetic risk score comprising all selected AD risk variants from 20 independent AD risk loci to estimate joint effect of these SNPs on MRI-markers of brain aging. Methods have been recently developed to apply a genetic risk score to meta-analysis summary estimates without requiring access to raw data from individual studies (Dastani, et al., 2012). For each MRI-marker of interest, the beta-coefficient for a given SNP, as obtained from the GWAS meta-analysis for this MRI-marker, was weighted with the published AD beta-coefficient for the given SNP. The weighted sum of beta-coefficients for all 20 SNPs (Formula-i(a)) was used as the beta-coefficient of the genetic risk score. Similarly, for each MRI-marker of interest, the inverse of the variance for a given SNP (from the GWAS meta-analysis for this MRI-marker) was weighted by the square of the published AD beta-coefficient for the given SNP. These weighted inverse of variances were then summed and the inverse of this sum was used as the variance of the genetic risk score (Formula-i(b)). The Wald statistic was used to test for significance of associations between the genetic risk score and each MRI-marker (Dastani, et al., 2012). For WMH burden, betas and standard errors were estimated from Z-statistics provided by the effective sample size weighted meta-analysis using Formula-ii. AD beta-coefficients used as weights for the score were all drawn from the discovery stage of the recent largest AD GWAS meta-analysis (17,008 AD cases and 37,154 controls, Supplementary Table 1) (Lambert, et al., 2013). Associations with  $p < 0.05$  were considered significant for genetic risk score based associations.

$$\beta_{grs} = \frac{\sum_1^m w \beta SE^{-2}}{\sum_1^m w^2 SE^{-2}} \quad \text{Formula - i(a)}$$

$$SE^2_{grs} = \frac{1}{\sum_1^m w^2 SE^{-2}} \quad \text{Formula - i(b)}$$

$\beta_{grs}$ =beta of genetic risk score;  $SE_{grs}$ =SE of genetic risk score;  $w$ =weight applied (=SNP-specific beta of AD GWAS);  $\beta$ =SNP specific beta of association with MRI-phenotype;  $SE$ =SNP-specific SE of association with MRI-phenotype

$$SE \sim \sqrt{VP / (ES \times 2pq)} \quad \text{Formula - ii(a)}$$

$$Beta = SE \times Z \quad \text{Formula - ii(b)}$$

VP=phenotypic variance (approximated to 1); ES=Effective sample size; p=Minor allele frequency; q=Major allele frequency.

After correcting for four independent phenotypes tested, the multiple testing threshold for genetic risk score association was  $P < 0.0125$ .

### 3. RESULTS

#### 3.1. Correlation and heritability of the five MRI traits

Based on data from two studies which were part of the original meta-analysis the two MRI markers of structural brain aging, ICV and TBV showed high correlation with each other but were only moderately correlated with HV (Supplementary Table 2). The two MRI markers of vascular brain aging WMH burden and brain infarcts showed low correlation with each other and very little or no correlation with the three markers of structural brain aging. Depending upon this correlation the equivalent number of independent phenotypes calculated using matSpDlite was four for both studies. Published literature showed that the five MRI markers had moderate to high heritability (Supplementary Table 3).

#### 3.1. Single-SNP based associations

In total 9 out of 20 AD risk variants that could be analyzed showed association with at least one MRI-marker at  $p < 0.05$  (Table 1). With only 2 exceptions (*CD33* locus with brain infarcts ( $p = 0.048$ ) and *PTK2B* locus with ICV ( $p = 0.028$ )), betas were in the expected direction i.e. the AD risk allele was associated with increased risk for brain infarcts and with lower ICV, TBV and HV. The most significant associations were for *APOE*-rs2075650 with HV ( $\beta \pm SE = -0.042 \pm 0.015$ ,  $p = 0.0054$ ) and *CD33*-rs3865444 with ICV ( $\beta \pm SE = -5.209 \pm 1.886$ ,  $p = 0.0058$ ) (Table 1). However, none of the single-SNP based associations were significant after correcting for multiple testing. None of the AD risk variants showed associations with WMH burden.

#### 3.2. Gene-based associations

Out of the 24 loci investigated, 23 had at least one protein coding gene within 50kb distance. Only rs3851179 (11q14) had no protein coding gene within 50kb and was not represented in the gene-based association analysis (nearest genes: *PICALM* 87.72kb downstream and *EED* 86.95kb upstream). In total, 65 protein coding genes from 23 independent loci were assessed for gene-based association analyses (Supplementary Table 4).

A total of 27 protein coding genes within 50kb of 15 index SNPs were associated with ICV, TBV, HV or brain infarcts at  $p < 0.05$  (Table 2). For ICV we observed association with 13 genes within 50kb of five index SNPs (*MEF2C*, *NME8*, *PILRB*, *PILRA*, *ZCWPW1*, *MEPCE*, *PPP1R35*, *C7orf61*, *MS4A6A*, *PVRL2*, *TOMM40*, *APOE*, *APOC1*; p-range: 0.04–0.0078). Eight genes within 50kb of six index SNPs were associated with TBV (*CRI*, *HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1*, *TAS2R60*, *SCARA3*, *ICT1*, *CD33*; p-range: 0.047–0.0006). *BINI*, *TREML1* and *MS4A6A* were associated with HV ( $p = 0.00089$ , 0.03 and 0.048, respectively) while *MEF2C*, *AURKA*, *CSTF1* and *TAS2R60* showed association with brain infarcts (p-range: 0.049–0.033). For WMH burden we observed association with three genes from two



loci (*HLA-DQB1*, *HMHA1* and *ABCA7*;  $p=0.01$ ,  $0.046$  and  $0.049$  respectively). If we correct for the number of genes tested the association of *HLA-DRB1* with TBV remains significant but if we additionally correct for the number of phenotypes tested this association is not significant.

### 3.3. Genetic risk score based associations

The AD genetic risk score was associated with smaller HV ( $\beta \pm SE = -0.047 \pm 0.013$ ,  $p=0.00041$ ) (Table 3). This association was also observed after removing the *APOE* locus from the AD genetic score ( $\beta \pm SE = -0.050 \pm 0.023$ ,  $p=0.029$ ). There was also nominal association of the AD genetic risk score with smaller TBV ( $\beta \pm SE = -0.127 \pm 0.064$ ,  $P=0.046$ ) but this association was not significant after excluding the *APOE* locus from the genetic risk score ( $P=0.13$ ). Only association of the AD genetic risk score with HV including *APOE* locus was significant after correcting for the number of independent phenotypes tested.

## 4. DISCUSSION

We investigated associations of 24 genome-wide significant AD risk loci with five MRI-markers of brain structure and aging (ICV, TBV, HV, WMH burden and brain infarcts), in over 8,000 dementia free older community participants from the CHARGE consortium. Although no single SNP-based association met the significance threshold after correction for multiple testing, index AD risk variants mapping to eight of the 21 AD risk loci showed nominal association with at least one MRI-marker, the most interesting being association for *APOE* (rs2075650) with smaller HV and for *CD33* (rs3865444) with smaller ICV. In gene-based association analyses *HLA-DRB1* was significantly associated with TBV after correction for number of genes tested. A weighted AD genetic risk score was significantly associated with smaller HV.

In Single-SNP based associations none of the associations were significant after correcting for multiple testing. Nominally significant associations of an *APOE* risk variant with HV ( $P=0.0054$ ) and a *CD33* variant with ICV ( $P=0.0058$ ) were observed. Since the mid 1990's (Supplementary Table 5) some studies have described significant associations between the *APOE-ε4* allele and smaller HV (den Heijer, et al., 2002, Lehtovirta, et al., 1995, Lehtovirta, et al., 1996, Lind, et al., 2006, Liu, et al., 2014, Lu, et al., 2011, Morra, et al., 2009, O'Dwyer, et al., 2012, Plassman, et al., 1997, Schuff, et al., 2009, Soininen, et al., 1995), however other studies did not find such an association (Ferencz, et al., 2013, Khan, et al., 2014, Reiman, et al., 1998, Schmidt, et al., 1996). Using the largest sample size to date ( $N=11,550$ ), as previously reported by our group, our findings are supportive of an association of the *APOE-ε4* locus with smaller HV (Bis, et al., 2012). The rs3865444 (*CD33*) AD risk allele association with smaller ICV could perhaps be suggestive of an involvement of this locus in brain maturation and brain reserve. Recent reports suggest that rs3865444 influences *CD33* expression, including in young adults in their twenties (Bradshaw, et al., 2013), and is associated with diminished internalization of amyloid  $\beta_{42}$  peptide, and accumulation of neuritic amyloid pathology and fibrillar amyloid in vivo (Bradshaw, et al., 2013).

Gene-based analyses revealed significant associations of *HLA-DRBI* (index SNP rs9271192) with TBV. The *HLA-DRBI* locus was recently identified to be associated with AD in the largest meta-analysis of AD (Lambert, et al., 2013). This locus is part of the major histocompatibility complex, class II, and our findings add support to the role of autoimmunity in AD. The findings also suggest that the locus may be playing a role in pre-symptomatic stages of the disease, as we observe association with smaller brain volumes in non-demented older community persons.

When combined in a weighted genetic risk score, AD risk variants were associated cumulatively with decreased HV. Interestingly the association was maintained with a similar effect size, although less significant, after removing the *APOE* locus from the analysis, suggesting that, in aggregate, novel AD risk loci are associated with smaller HV in non-demented older community persons. The AD genetic risk score also showed nominal association with smaller TBV. Although this association was no longer significant after removing the *APOE* locus, other loci were contributing to this association, as the *APOE* risk variant alone was not significantly associated with TBV.

There were fewer associations with WMH burden and brain infarcts. Most associations with AD risk variants were observed for ICV, TBV, and HV. This may indicate that, even though they are strong predictors of dementia risk, (DeBette and Markus, 2010, Vermeer, et al., 2007) MRI-markers of vascular brain injury could have less shared genetic determinants with AD than MRI-markers of brain growth and brain atrophy, as suggested by others (Biffi, et al., 2010). Noteworthy, our study only tested for overlap of genome-wide significant AD risk variants, did not explore shared heritability and may have been underpowered for less common variants with smaller effect size (Supplementary Figure 1).

Our study has limitations. The 24 AD risk loci do not reflect the full spectrum of genetic susceptibility to AD and the index SNPs used may not be causal variants. The five GWAS of MRI-markers, although the largest of their kind, have fewer samples compared to the AD GWAS from which the loci have been obtained (Bis, et al., 2012, DeBette, et al., 2010, Fornage, et al., 2011, Ikram, et al., 2012, Lambert, et al., 2013). These five GWAS of MRI-markers were performed using imputed genotypes based on the HapMap2 panel, which has fewer markers with limited LD information, does not cover rare variants and has lower imputation accuracy, especially for lower allele frequencies, compared to the more recent 1000 genomes reference panels. We therefore couldn't analyze rare AD risk variants in the present study and we cannot exclude that the more limited LD information might have introduced some bias in the results of the gene-based analyses. In addition, despite major efforts to harmonize phenotype definitions across studies, there may be some residual heterogeneity in methods for quantifying MRI-markers of brain aging. These elements could have reduced our power to detect associations of AD GWAS loci with MRI-markers of brain aging. The choice of 50 KB window for a gene based test does not account for potential regulatory effects on more distant genes. Our findings cannot be generalized to populations of non-European ancestry. Ongoing, larger multi-ethnic GWAS of MRI-markers of brain aging, as well as sequencing projects searching for rare variants associated with AD risk and MRI phenotypes may enable us to expand our findings in the future.

## 5. Conclusion

In conclusion, we have shown that novel AD genetic risk variants are associated with MRI-markers of structural brain aging in older, non-demented community persons. In aggregate, novel AD genetic risk variants were associated with smaller brain volumes, especially HV. Significant gene-based associations and suggestive single SNP-based associations with ICV, TBV and HV also provide interesting hypotheses for mechanisms underlying genetic associations with AD

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

<b>AD</b>	Alzheimer’s disease
<b>AGES</b>	Aging Gene-Environment Susceptibility
<b>ARIC</b>	Atherosclerosis Risk in Communities Study
<b>ASPS</b>	Austrian Stroke Prevention Study
<b>CHARGE</b>	Cohorts of Heart and Aging Research in Genomic Epidemiology
<b>CHS</b>	Cardiovascular Health Study
<b>ERF</b>	Erasmus Rucphen Family
<b>FHS</b>	Framingham Heart Study
<b>GWAS</b>	Genome-wide association studies
<b>HV</b>	Hippocampal volume
<b>ICV</b>	Intra-cranial volume
<b>LD</b>	linkage disequilibrium

<b>MAP</b>	Rush Memory and Aging Project
<b>ROS</b>	Religious Order Study
<b>RS</b>	Rotterdam Study
<b>TASCOG</b>	Tasmanian Study of Cognition and Gait
<b>TBV</b>	Total brain volume
<b>VEGAS</b>	Versatile Gene-Based Association Study
<b>WMH</b>	White matter hyperintensity

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### Highlights

1. It is unknown if novel AD risk loci impact brain structure in non-demented elderly
2. We performed a meta-analysis of genetic association studies in non-demented elderly
3. AD risk variants were associated in aggregate with smaller HV
4. Gene-based tests were significant for *HLA-DRB1* with TBV and *BINI* with HV
5. Previously debated association of *APOE* risk variant with smaller HV was observed
6. Novel AD risk loci contribute to structural brain aging in older community persons

**Table 1**

Single-SNP based association of the AD loci with MRI markers of brain aging

Index SNP <sup>a</sup>	Proxy	Closest gene	Chr:position <sup>b</sup>	Distance from Gene <sup>c</sup>	Intra-Cranial Volume (in cm <sup>3</sup> )			Total Brain Volume (in % ICV)			Hippocampal Volume (in cm <sup>3</sup> )			WMH burden <sup>d</sup>			Brain Infarcts (yes/no)		
					$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	Z-statistics	p	$\beta$
rs2075650		<i>APOE</i>	19:45395619	13.39kb	4.405	2.605	0.091	-0.1	0.072	0.168	-0.042	0.015	0.0054	1.089	0.276	-0.081	0.062	0.195	
rs9331896	rs2279590	<i>CLU</i>	8:27467686	wg	-3.112	1.795	0.083	-0.104	0.051	0.04	-0.009	0.011	0.416	-1.546	0.122	-0.012	0.043	0.771	
rs10792832		<i>PICALM</i>	11:85867875	86.95kb	0.763	1.681	0.65	0.064	0.047	0.18	-0.001	0.01	0.863	1.243	0.214	0.003	0.04	0.932	
rs6656401		<i>CR1</i>	1:207692049	wg	-2.834	2.19	0.196	0.023	0.061	0.713	0.016	0.013	0.211	0.375	0.708	-0.069	0.054	0.197	
rs6733839	rs744373	<i>BIN1</i>	2:127892810	27.91kb	-1.943	1.862	0.297	-0.07	0.052	0.183	-0.024	0.011	0.027	-0.168	0.867	0.079	0.043	0.064	
rs4147929	rs3752246	<i>ABCA7</i>	19:1063443	wg	0.103	2.342	0.965	-0.018	0.065	0.786	-0.017	0.014	0.226	NA	NA	0.017	0.058	0.773	
rs983392	rs11230161	<i>MS4A6A</i>	11:59923508	15.57kb	-3.093	1.675	0.065	-0.059	0.047	0.214	-0.023	0.01	0.021	-1.42	0.156	-0.012	0.043	0.782	
rs10948363		<i>CD2AP</i>	6:47487762	wg	1.537	1.845	0.405	-0.017	0.052	0.742	0.003	0.011	0.87	1.089	0.276	-0.035	0.044	0.433	
rs11771145		<i>EPHA1</i>	7:143110762	4.78kb	3.353	1.901	0.078	-0.026	0.053	0.625	0.003	0.011	0.912	NA	NA	-0.023	0.042	0.592	
rs3865444		<i>CD33</i>	19:51727962	0.36kb	-5.209	1.886	0.0058	0.025	0.053	0.638	-0.019	0.011	0.087	-0.362	0.717	-0.088	0.045	0.048	
rs9271192		<i>HLA-DRB1<sup>e</sup></i>	6:32578530	20.92kb	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
rs28834970	rs2322599	<i>PTK2B</i>	8:27195121	wg	3.675	1.67	0.028	-0.006	0.047	0.898	-0.003	0.01	0.762	-0.824	0.41	-0.006	0.04	0.89	
rs11218343	rs7939826	<i>SORL1</i>	11:121435587	wg	4.525	6.239	0.468	-0.165	0.174	0.341	0.011	0.037	0.768	NA	NA	0.316	0.155	0.041	
rs10498633		<i>SLC24A4</i>	14:92926952	wg	-2.052	2.042	0.315	0.01	0.057	0.858	-0.012	0.012	0.329	0.363	0.717	0.049	0.048	0.304	
rs35349669	rs7607736	<i>INPP5D</i>	2:234068476	wg	-3.625	1.723	0.035	-0.063	0.048	0.196	-0.01	0.01	0.313	0.856	0.392	-0.003	0.041	0.935	
rs190982		<i>MEF2C</i>	5:88223420	23.50kb	-1.611	1.918	0.401	0.034	0.054	0.525	0.005	0.011	0.687	0.545	0.586	0.046	0.044	0.3	
rs2718058	rs12155159	<i>NME8</i>	7:37841534	46.67kb	2.168	1.762	0.218	0	0.05	0.994	0.012	0.01	0.271	-0.438	0.662	0.027	0.042	0.523	
rs1476679		<i>ZCWPW1</i>	7:100004446	wg	-0.22	1.8	0.903	-0.017	0.051	0.738	-0.01	0.011	0.36	-0.053	0.958	-0.014	0.044	0.754	
rs10838725	rs10838726	<i>CELF1</i>	11:47557871	wg	-0.433	1.795	0.809	0.085	0.05	0.092	0	0.011	0.992	-1.012	0.312	-0.068	0.043	0.115	
rs17125944		<i>FERMT2</i>	14:53400629	wg	0.465	2.767	0.867	-0.025	0.078	0.744	0.015	0.016	0.347	-0.574	0.566	0.069	0.071	0.332	
rs7274581	rs927174	<i>CASS4</i>	20:55018260	wg	-0.435	2.956	0.883	-0.119	0.083	0.152	-0.014	0.017	0.421	0.055	0.956	0.084	0.07	0.228	

Key:  $\beta$ , beta (meta-analysis effect estimate) per allele increase of the risk allele; Z-statistic, meta-analysis of Z-statistics (beta/SE) from each study, weighted by effective sample size and the ratio of the empirically observed dosage variance to the expected binomial dosage variance for imputed SNPs); WMH, white matter hyperintensities; SE, standard error

<sup>a</sup> Index SNP was defined as the SNP with the lowest p at the locus.

<sup>b</sup> Chr:position has been provided for the index SNP as per NCBI build 37 (GRCh37.p10).

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$d_c$  Distance from gene start or end (whichever is shortest) is provided in kilo bases (kb) and if within gene, wg notation used.

$d_p$  expressed in  $cm^3$  or on a semi-quantitative 10-point scale in the original study.

$\rho_2$  Neither the index SNP nor any SNP in LD with index SNP is available in the HapMap based imputed data meta-analysis results  $p < 0.0025$  ( $\alpha = 0.05/20$ ) was considered significant after correcting for number of independent loci tested

Table 2

Gene-based associations (P&lt;0.05) with MRI markers of brain aging for genes lying within 50kb of AD risk loci

Index-SNP (closest gene)	Gene	Chr	Start	Stop	p (Intra-cranial volume)	p (Total brain volume)	p (Hippocampal volume)	p (WMH burden)	p (brain infarcts)
rs6656401 (CRI)	<i>CRI</i>	1	207619472	207865110	0.271	0.0033	0.237	0.069	0.562
rs744373 (BINI)	<i>BINI</i>	2	127755598	127914903	0.612	0.782	0.00089	0.700	0.072
rs190982 (MEF2C)	<i>MEF2C</i>	5	87964057	88249922	0.020	0.815	0.134	0.180	0.033
rs9271192 (HLA-DRB1)	<i>HLA-DRB1</i>	6	32496546	32607613	0.467	0.00060	0.170	0.226	0.277
rs9271192 (HLA-DRB1)	<i>HLA-DQA1</i>	6	32555182	32661429	0.263	0.0014	0.108	0.059	0.310
rs9271192 (HLA-DRB1)	<i>HLA-DQB1</i>	6	32577240	32684466	0.179	0.0057	0.114	0.010	0.208
rs75932628 (TREM2)	<i>TREM1</i>	6	41066998	41172087	0.337	0.367	0.048	0.315	0.582
rs12155159 (NME8)	<i>NME8</i>	7	37838198	37990002	0.010	0.400	0.138	0.755	0.985
rs1476679 (ZCWPW1)	<i>PILRB</i>	7	99905625	100015454	0.0082	0.694	0.162	0.271	0.701
rs1476679 (ZCWPW1)	<i>PILRA</i>	7	99921067	100047722	0.0078	0.702	0.183	0.309	0.712
rs1476679 (ZCWPW1)	<i>ZCWPW1</i>	7	99948494	100076431	0.0078	0.672	0.196	0.363	0.751
rs1476679 (ZCWPW1)	<i>MEPCE</i>	7	99976412	100081749	0.0099	0.626	0.246	0.348	0.739
rs1476679 (ZCWPW1)	<i>PPP1R35</i>	7	99982911	100084094	0.0093	0.637	0.291	0.342	0.767
rs1476679 (ZCWPW1)	<i>C7orf61</i>	7	100004237	100111894	0.011	0.668	0.320	0.321	0.811
rs11771145 (EPHA1)	<i>TAS2R60</i>	7	143090545	143191502	0.931	0.012	0.393	0.086	0.049
rs2279590 (CLU)	<i>SCARA3</i>	8	27441576	27584286	0.060	0.031	0.367	0.440	0.651
rs11230161 (MS4A6A)	<i>MS4A6A</i>	11	59889079	60002139	0.035	0.375	0.030	0.358	0.804
rs11870474 (ATP5H/KCTD2)	<i>ICT1</i>	17	72958779	73067356	0.138	0.047	0.434	0.697	0.195
rs3752246 (ABCA7)	<i>ABCA7</i>	19	990101	1115570	0.497	0.589	0.799	0.049	0.301
rs3752246 (ABCA7)	<i>HMHA1</i>	19	1015921	1137830	0.337	0.577	0.724	0.046	0.128
rs2075650 (APOE)	<i>PVR2</i>	19	45299392	45442485	0.033	0.470	0.069	0.163	0.056
rs2075650 (APOE)	<i>TOMM40</i>	19	45344476	45456946	0.027	0.370	0.084	0.202	0.155
rs2075650 (APOE)	<i>APOE</i>	19	45359038	45462650	0.040	0.378	0.118	0.252	0.133
rs2075650 (APOE)	<i>APOC1</i>	19	45367920	45472606	0.030	0.488	0.106	0.274	0.163
rs3865444 (CD33)	<i>CD33</i>	19	51678334	51793274	0.179	0.046	0.463	0.968	0.100
rs927174 (CASS4)	<i>AURKA</i>	20	54894444	55017351	0.262	0.690	0.415	0.771	0.041

Index-SNP (closest gene)	Gene	Chr	Start	Stop	p (Intra-cranial volume)	p (Total brain volume)	p (Hippocampal volume)	p (WMH burden)	p (brain infarcts)
rs927174 (CASS4)	<i>CSTF1</i>	20	54917426	55029582	0.400	0.526	0.550	0.823	0.047

Key: WMH, white matter hyperintensities

p<0.0025 ( $\alpha=0.05/20$ ) was considered significant after correcting for number of independent loci tested; significant p-values after correcting for multiple testing are in bold; Gene-based association analysis was performed for genes within 50KB of index SNP. Only gene-based associations for those genes with p<0.05 with at least one MRI marker is presented. A complete list is presented in Supplementary Table 4.



**Table 3**  
Genetic risk score based association of the AD loci with MRI-markers of brain aging

	With <i>APOE</i>			Without <i>APOE</i>		
	Beta	SE	p	Beta	SE	p
Intra-cranial volume (in cm <sup>3</sup> )	1.179	2.174	0.59	-6.224	3.945	0.11
Total brain volume (in % ICV)	-0.120	0.061	0.048	-0.166	0.111	0.13
Hippocampal volume (in cm <sup>3</sup> )	-0.044	0.013	0.00042	-0.050	0.023	0.029
WMH burden <sup>a</sup>	0.013	0.020	0.52	-0.019	0.038	0.61
Brain infarcts (yes/no)	-0.039	0.052	0.45	0.055	0.094	0.56

Key: Beta, effect estimate, per allele increase of the risk allele; SE, standard error; WMH, white matter hyperintensities

<sup>a</sup> for WMH burden betas and SEs were estimated from the Z-statistics obtained in the WMH burden meta-analysis and do not reflect an interpretable effect size (as the WMH burden was estimated using different scales in participating studies) (Fornage, et al., 2011).