Common variants at 12q14 and 12q24 are associated with hippocampal volume

Aging is associated with reductions in hippocampal volume that are accelerated by Alzheimer’s disease and vascular risk factors. Our genome-wide association study (GWAS) of dementia-free persons (n = 9,232) identified 46 SNPs at four loci with \( P \) values of \( < 4.0 \times 10^{-7} \). In two additional samples (n = 2,318), associations were replicated at 12q14 within MSRB3-WIF1 (discovery and replication; \( r_s17178006; P = 5.3 \times 10^{-11} \)) and at 12q24 near HRK-FBXW8 (\( r_{s7294919}; P = 2.9 \times 10^{-11} \)). Remaining associations included one SNP at 2q24 within DPP4 (\( r_{s6741949}; P = 2.9 \times 10^{-7} \)) and nine SNPs at 9p33 within ASTN2 (\( r_{s7852872}; P = 1.0 \times 10^{-7} \)); along with the chromosome 12 associations, these loci were also associated with hippocampal volume (\( P < 0.05 \)) in a third younger, more heterogeneous sample (n = 7,794). The SNP in ASTN2 also showed suggestive association with decline in cognition in a largely independent sample (n = 1,563). These associations implicate genes related to apoptosis (HRK), development (WIF1), oxidative stress (MSRB3), ubiquitination (FBXW8) and neuronal migration (ASTN2), as well as enzymes targeted by new diabetes medications (DPP4), indicating new genetic influences on hippocampal size and possibly the risk of cognitive decline and dementia.

Differences in hippocampal volume that appear with advancing age represent cumulative effects of early-life factors, life-course events and disease. Hippocampal atrophy is a recognized biological marker of Alzheimer’s disease\(^1\)–\(^2\); however, it is influenced by various vascular and metabolic factors\(^3\)–\(^4\). Because hippocampal volume is a heritable\(^5\), widely measurable trait that shows meaningful detectable changes throughout life, it is a suitable endophenotype for aging-related physiological processes and presymptomatic diseases, improving the power to detect genetic associations.

We explored genetic influences on hippocampal volume by conducting a cross-sectional genome-wide association analysis in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium\(^6\) among 9,232 dementia-free persons from 8 community-based studies whose mean age ranged from 56 to 84 years (weighted average of 67.1 years). Each study imputed to a common set of SNPs from the Phase 2 HapMap Centre d’Etude du Polymorphisme Humain (CEPH) Utah residents of Northern and Western European ancestry (CEU) population using genotype data from Illumina or Affymetrix arrays; additive genetic models associating total hippocampal volume and genotype dosage were fitted with adjustment for age, sex and familial relationships (if applicable; Supplementary Note); and genomic control was applied. Study-specific results were combined in an inverse variance–weighted meta-analysis.

We then conducted in silico replication of associations that reached genome-wide significance and sought additional evidence for suggestive associations in a second-stage targeted meta-analysis of 2,318 subjects from two community-based studies: the Three City Study and an independent sample from the third expansion of the Rotterdam Study. Characteristics of the discovery and replication samples are given (Supplementary Table 1).

A Manhattan plot of \(-\log_{10} (P)\) values from the discovery analysis is shown (Fig. 1), where \( P \) values for 46 SNPs at 4 loci (Supplementary Table 2) surpassed our replication threshold of \( P < 4.0 \times 10^{-7} \) corresponding to 1 expected false positive. Of these, 18 SNPs at 2 loci surpassed a genome-wide significance threshold of \( P < 5.0 \times 10^{-8} \); the 12q14 locus, which included WIF1, LEMD3 and MSRB3, and the 12q24 locus, which included HRK and FBXW8. We found evidence of replication (\( P < 0.01 \)) for both associations. The remaining suggestive associations included SNPs at 2q24 within DPP4 and at 9p33 within ASTN2, which had consistent directions of association in the discovery and replication phases but did not attain genome-wide significance in a combined analysis. Estimates for each stage are shown (Table 1). Discovery GWAS results for the region around each signal were annotated with recombination rates and known genes (Fig. 2), and study-specific findings are shown (Fig. 3).

**Figure 1** Genome-wide Manhattan plot for hippocampal volume. The plot shows individual \( P \) values (based on the discovery meta-analysis) against genomic position for association with hippocampal volume. Within each chromosome (x-axis), the results are plotted left to right from the p-terminal end. The dashed line indicates the threshold for replication of \( P < 4 \times 10^{-7} \), and the solid line indicates the threshold for genome-wide significance of \( P < 5 \times 10^{-8} \). The nearest genes are indicated above SNPs that reached the significance threshold for replication.

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Received 6 September 2011; accepted 6 March 2012; published online 15 April 2012; doi:10.1038/ng.2237
Table 1 Discovery, replication and combined meta-analysis

| Locus  | SNP        | Gene     | Allele 1/2 | Allele 1 frequency | 
|--------|------------|----------|------------|--------------------|
| 2q24   | rs6741949  | DPP4     | G/C        | 0.53               |
|        | rs87852872 | ASTN2    | C/G        | 0.62               |
| 12q14  | rs17178006 | MSRB3    | G/T        | 0.10               |
|        | rs6581612  | WIF1     | C/A        | 0.27               |
| 12q24  | rs7294919  | HRK      | T/C        | 0.91               |

& The SNP is located within the indicated gene.

Allele 1, coded (risk)/non-coded allele; β, association estimate in mm^3; N, effective sample size; (imputation quality × N).

We present association estimates for a meta-analysis combining the discovery and replication results for these four loci. To contextualize the magnitude of a SNP’s association with hippocampal volume, we divided the regression coefficient for the allele by the mean decrease in hippocampal volume for each year of chronological age (−27.4 mm^3 per year, estimated within the Framingham Heart Study).

The strongest association was for rs7294919, located at 12q24 between HRK and FBXW8, where each copy of the T allele (allele frequency (AF) = 0.91) was associated with lower hippocampal volume (regression coefficient beta, β = −107.8 mm^3; P = 2.9 × 10^{-11}), equivalent to 3.9 years of aging.

**HRK** is expressed throughout the brain, with the highest levels in the amygdala, entorhinal cortex and hippocampus. The HRK protein acts as a key regulator of apoptosis, a complex pathway associated with aging, ischemia and Alzheimer’s disease, through its interaction with the death-repressor proteins Bcl-2 and Bcl-X, (ref. 10). In rat neuronal cell cultures, a homologous protein, DP5 (with 72% identity), is induced during β-amylloid-mediated cytotoxicity, withdrawal of nerve growth factor (NGF) and induced global ischemia. Although treatment strategies aimed at modifying the apoptotic pathway have yet to achieve success, our findings suggest that this area of therapeutics might remain promising.

**FBXW8** encodes the substrate-recognition component of a Skp1-Cullin-F-box protein (SCF) E3 ubiquitin ligase found in the Golgi apparatus of neurons. Different E3 ligase complexes target specific sub-structures for polyubiquitination and proteasome-mediated degradation, suggesting a role for FBXW8 in clearing abnormal and potentially toxic protein aggregates, particularly hyperphosphorylated tau.

The described role of FBXW8 in presynaptic development, synapse formation, neurotransmitter release and promotion of dendrite growth in hippocampal neurons makes a genetic association with hippocampal volume plausible. Whether one or both of the HRK and FBXW8 genes are involved in determining hippocampal volume is unclear, as rs7294919 is an expression SNP (eSNP) associated with changes in the expression of both.

At the 12q14 locus, the G allele of rs17178006 (AF = 0.10), intronic within MSRB3, was associated with decreased hippocampal volume (β = −123.8 mm^3; P = 5.3 × 10^{-11}) equivalent to 4.5 years of aging. MSRB3 catalyzes the reduction of methionine sulfoxide residues in proteins and requires zinc or selenium as a cofactor. Thus, the association of lower selenium levels with elevated plasma homocysteine—which in turn has been associated with increased risk of Alzheimer’s disease and hippocampal atrophy—may be mediated by suppression of Msrb proteins in various organs, including the brain. Several SNPs in low linkage disequilibrium (LD; r^2 = 0.7) with rs17178006 were also associated with decreased hippocampal volume, including rs6581612 (AF = 0.27; β = −63.3 mm^3; P = 7.2 × 10^{-11}) between WIF1 and LEMD3. The WIF1 protein inhibits extracellular Wnt signaling proteins, which have a role in embryonic development, along with β-catenin, and hippocampal aging. Changes in Wnt signaling mimic the effects of environmental enrichment increasing hippocampal synaptic densities. LEMD3 encodes a transforming growth factor-β antagonist expressed in the hippocampus and is upregulated protectively during ischemia and epileptogenesis. Further, the LEMD3 protein interacts with progerin, the abnormal form of lamin A responsible for premature aging in progeria (Hutchinson-Gilford syndrome).

In testing for independent effects of these two SNPs in conditional models, both associations were attenuated, but only that at rs17178006 remained significant (P < 0.05; Supplementary Fig. 1), suggesting that the SNPs mark a single locus. Whereas MSRB3 may be most influential, it remains possible that more than one gene in this region is involved.
associated with hippocampal volume. For example, eight eSNPs in the vicinity of this locus (Supplementary Table 3) were associated with hippocampal volume at \( P \leq 5.3 \times 10^{-4} \) and have been reported to modify LEMD3 expression.20

In addition to these findings, SNPs at two additional loci showed suggestive evidence for association but did not reach genome-wide significance in our combined meta-analysis. The first was rs6741949 in an intron of DPP4 on chromosome 2q24, where the G allele (AF = 0.53) was associated with smaller hippocampal volume \((\beta = -52.8 \text{ mm}^3; P = 2.9 \times 10^{-7})\). Many bioactive peptides whose levels are altered in Alzheimer’s disease and vascular brain injury are substrates for the DPP4 protein32; and DPP4 reduces extracellular \( \beta \)-amyloid deposition in mouse models of Alzheimer’s disease33. Further, DPP4 is an intrinsic membrane glycoprotein and a widely expressed serine exopeptidase33. It is also an adipokine that is overexpressed in the visceral adipose tissue of obese persons and in individuals with diabetes33,34, conditions associated with smaller hippocampal volume.3,36

A new class of antidiabetic medications (sitagliptin and related incretin compounds) inhibits DPP4 to improve insulin sensitivity and glucose tolerance through increased levels of glucagon-like protein–1 (GLP-1) and GLP-2. Of note, endogenous incretin, GLP-1, is also strongly expressed in some hippocampal neurons and has neuroprotective properties.37-39

The second suggestive association was for rs7852872, located in an intron of ASTN2 on chromosome 9, where the C allele (AF = 0.63) was associated with lower hippocampal volume \((\beta = -47.7 \text{ mm}^3; P = 1.0 \times 10^{-7})\). The ASTN2 protein is a cell adhesion molecule expressed in neurons, including those in the dentate gyrus, and is hypothesized to function in glial-guided neuronal migration40,41.

We sought additional replication of our significant and suggestive associations by testing the lead (or proxy) SNP from each locus in data from the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) Consortium. Briefly, samples from this group \((n = 7,794; \text{mean age of 39.9 years})\) came from multiple studies including normal older individuals, a developmental sample and individuals symptomatic for cognitive or affective diseases. Among the ENIGMA sample, we observed a consistent direction of association for all cross-study comparisons. For the loci where the lead SNP was available in ENIGMA, replication was strongest at HRK-FBXW8 (rs7294919; \( P = 1.6 \times 10^{-7} \)) and nominal at DPP4 (rs6741949; \( P = 0.04 \)). Although the lead SNPs were not available at the other loci, one proxy SNP in weak LD \((r^2 = 0.3)\) with rs17178006 (MSRB3) and another in strong LD \((r^2 = 0.96)\) with rs7852872 (ASTN2) both had \( P \) values of <0.05 in ENIGMA data (Table 2).

Because the ENIGMA and CHARGE samples differed in two key aspects—the ENIGMA sample included younger adults (8 of 13 studies had no participants older than 65 years of age) and some persons with cognitive impairment and dementia (13% of the sample)—we examined the top loci in subsamples of healthy persons \((n = 5,775; \text{mean age of 34.8 years})\) and cognitively intact older persons \((n = 816; \text{mean age of 67.2 years})\). Association estimates were generally similar to those of the full sample (Supplementary Table 4).

Given the established relationship between hippocampal atrophy and Alzheimer’s disease, we investigated whether SNPs from published Alzheimer’s disease GWAS42-46 were associated with hippocampal volume in our discovery meta-analysis (Supplementary Table 5). We found nominal association with smaller hippocampal volume of risk alleles in four genes associated with Alzheimer’s disease: APOE \((P = 0.005)\), BIN1 \((P = 0.02)\), MS4A4E \((P = 0.001)\) and TOMM40 \((P = 0.01)\). However, in aggregate, various SNPs known to be associated with Alzheimer’s disease explained less than 1% of the observed variance in hippocampal volume.

We also examined our five lead SNPs for association with cognitive decline in 1,593

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**Table 2 Replication results in the ENIGMA sample**

| Locus | SNP     | Gene   | Allele 1/2 | Allele 1 frequency | \( \beta \) s.e.m. | \( P \) | \( N \)  |
|-------|---------|--------|------------|-------------------|-----------------|-----|------|
| 2q24  | rs6741949| DPP4a  | G/C        | 0.58              | -28.2           | 14.0 | 0.04 | 7,794 |
| 9q33  | rs7852872| ASTN2b | T/C        | 0.64              | -29.0           | 12.8 | 0.02 | 7,794 |
| 12q14 | rs7040792a| (\( r^2 = 1.00 \)) | T/C    | 0.24              | -32.4           | 14.6 | 0.08 | 7,794 |
|       | rs17178006| MSRB3b | G/T        | 0.25              | -25.4           | 14.6 | 0.08 | 7,794 |
|       | rs1370938b| (\( r^2 = 0.30 \)) | A/C    | 0.25              | -25.4           | 14.6 | 0.08 | 7,794 |
|       | rs5681612 | WIFI   | C/A        | 0.25              | -25.4           | 14.6 | 0.08 | 7,794 |
|       | rs1498792a| (\( r^2 = 0.96 \)) | T/C    | 0.90              | -112.2          | 21.4 | 1.6 \times 10^{-7} | 7,794 |

 Allele 1/2 encoded (risk)/non-coding allele; \( \beta \), association estimate in mm\(^3\); \( N \), effective sample size.

a) Proxy SNPs; \( r^2 \) is the correlation between the proxy SNP and the lead SNP in the HapMap Phase 2 CEU sample. b) The SNP is located within the indicated gene.
participants (mean age of 78.6 years) in the Religious Orders Study and the Rush Memory and Aging Project\(^{37}\) (Supplementary Table 6) and found that rs7852872 (ASTN2) was associated with accelerated rates of global cognitive decline (P = 0.009) and memory loss (P = 0.01) (Supplementary Fig. 2 and Supplementary Table 7). The magnitude of effect was comparable to that noted previously for a SNP in CR1 (rs6656401) in the same sample\(^{28}\), providing evidence for the potential importance of this region.

The strengths of the current study include the large, population-based sample. In the discovery sample, our power to detect association at genome-wide significance on the order of 0.2 s.d. in hippocampal volume (~128 mm\(^3\) in the largest single sample, the Aging Gene-Environment Susceptibility–Reykjavik Study (AGES)) was modest for rare variants (68% for those with minor allele frequency (MAF) of 0.05) and strong for more common variants (>99% for those with MAF of 0.10). Additional power estimates are shown (Supplementary Fig. 3). The concordance of these associations in ENIGMA data provides additional biological validation in a population that included younger persons, suggesting that these genes are developmentally important and may be related to maximal adult hippocampal volume. The ENIGMA sample also has a substantial proportion of persons with dementia, which indicates that these genes may remain important in regulating the response to injury. Analysis exploring the association of our lead SNPs with cognitive decline provided further context for our findings. Finally, we showed modest association between previously described SNPs for Alzheimer’s disease risk and smaller hippocampal volume in our samples.

The current study also has limitations. A single cross-sectional assessment was used in all studies, and magnetic resonance imaging (MRI) and reading protocols varied across participating studies—some studies used manually traced boundaries (the gold standard), whereas others used computerized algorithms. Although correlation between these two methods was good (Pearson’s r = 0.7)\(^{48}\), the heterogeneity of measurement techniques may have compromised our ability to detect small associations. Although our sample size was reasonably large, we may have missed associations with small effect sizes, as well as rare variants not covered by commercial genotyping arrays.

Previous studies have suggested that cognitive, neuropathological and MRI endophenotypes of Alzheimer’s disease might be early and more sensitive markers of genetic risk than clinical dementia. Hence, it could be argued that genes associated with risk for Alzheimer’s disease should also be associated with hippocampal volume, even in our dementia-free sample. Although four Alzheimer’s disease risk genes were associated with hippocampal volume, several were not; therefore, in this study, hippocampal volume was not a more sensitive measure than clinical Alzheimer’s disease. It is clear that genetic analysis of MRI endophenotypes within a community-based cohort study of healthy older individuals is not an ideal study design to identify all the genes associated with clinical Alzheimer’s disease. Our aim, however, was to identify genetic influences on hippocampal development and response to aging, not Alzheimer’s disease per se.

In summary, we detected four genetic loci associated with hippocampal volume in a large, population-based, dementia-free sample. Two of these loci replicated in independent community-based samples, as well as in a mixed-age sample from the ENIGMA Consortium that included some participants with cognitive impairment, indicating that these loci may have broad implications for determining the integrity of the hippocampus across a range of ages and cognitive capacities. Findings from this study identified a series of relevant and potentially important genes associated with hippocampal volume during development and aging and in the presence of Alzheimer’s disease.

Exploration of these genomic regions in dense-genotyping, expression and translational studies will be required to understand the role of these genes in determining hippocampal volume.

**METHODS**

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

**ACKNOWLEDGMENTS**

The authors thank the ENIGMA Consortium, which is represented by J.L.S., S.E.M., A.D.V., D.P.H., M.J.W., B.F., N.G.M. and P.M.T.

**Aging Gene-Environment Susceptibility–Reykjavik Study (AGES):** Research was funded by the US National Institute on Aging (NIA; N01–AG–12100), with contributions from the National Eye Institute (NEI), the National Institute on Deafness and Other Communication Disorders (NIDCD), the US National Heart, Lung, and Blood Institute (NHLBI), the NIA Intramural Research Program, Hjartavélin (the Icelandic Heart Association) and the Alþingi (the Icelandic Parliament).

**The Atherosclerosis Risk in Communities Study (ARIC):** The authors thank the staff and participants of the ARIC study for their important contributions. Research was carried out as a collaborative study supported by the US NHLBI (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, HHSN268201100012C, HL087641, HL59367, HL086694 and HL7825); the National Human Genome Research Institute (U01HG04402) and the NIH (HHSN268200625226C). Infrastructure was partly supported by a component of the NIH and the NIH Roadmap for Medical Research (UL1RR025005). This project was also supported by NHLBI grant HL093029.

**The Cardiovascular Health Study (CHS):** Coauthors were supported in part by US NHLBI grants (HL087652 and HL105756), as well as by NIA grants (AG02098 and AG05133).

**The Austrian Stroke Prevention Study (ASPS):** The authors thank the staff and participants of ASPS for their valuable contributions. Research was carried out as a collaborative study supported by the US NHLBI (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, HHSN268201100012C, HL087641, HL59367, HL086694 and HL7825); the National Human Genome Research Institute (U01HG04402) and the NIH (HHSN268200625226C). Infrastructure was partly supported by a component of the NIH and the NIH Roadmap for Medical Research (UL1RR025005). This project was also supported by NHLBI grant HL093029.

**The European Collection of Animal Cell Cultures:** Coauthors were supported in part by US NHLBI grants (HL087652 and HL105756), as well as by NIA grants (AG02098 and AG05133).

**The Framingham Heart Study (FHS):** This work was supported by the National Heart, Lung and Blood Institute's Framingham Heart Study (contract N01-HC-25359) and its collaboration with Agency for Healthcare Research and Quality, AHRQ (U18 HS19912).

**The Religious Order Study and the Rush Memory and Aging Project:** Development and aging and in the presence of Alzheimer’s disease.

**URLs**

ENIGMA Consortium, http://enigma.loni.ucla.edu; eqtl.ucichicago.edu; http://eqtl.ucichicago.edu/cgi-bin/gbrowse/eqtl/; SNAP, http://www.broadinstitute.org/mpg/snap/; GeneCruiser, http://genecruiser.broadinstitute.org/genecruiser3/

**Note:** Supplementary information is available on the Nature Genetics website.
pharmacists. The authors also thank P. Arp, M. Jhami, M. Verkerk, L. Herrera and M. Peters for their help in creating the GWAS database and K. Estrada and M.V. Struchalin for their support in the creation and analysis of imputed data. The genotyping and management of GWAS genotype data for the Rotterdam Study are supported by NWo Investments (nr. 175.010.2005.011 and 911-03-012). This study is funded by the Research Institute for Diseases in the Elderly (RIDE2: 014-93-015) and the NCI-NWO project (nr. 050-060-810). The Rotterdam Study is funded by the Erasmus Medical Center and Erasmus University, Rotterdam, the Netherlands Organisation for Health Research and Development (ZonMw), RIDE2, the Dutch Ministry of Education, Culture and Science, the Dutch Ministry for Health, Welfare and Sports, the European Commission (DG XII) and the Municipality of Rotterdam. The Rotterdam Scan Study is supported by the NWO (project nrs. 918-46-615, 904-61-096, 904-61-133 and 948-00-010), Nederlandse Hartstichting (2009B102) and Internationaal Parkinson Fonds.

The Tasmanian Study of Gait and Cognition (TASCOG): This study is supported by project grants from the National Health and Medical Research Council of Australia (NHMRC; 403000, 491109 and 606543) and a grant from the Wicking Dementia Education and Research Centre, Hobart. V.S. is supported by an NHMRC–National Heart Foundation Career Development Fellowship (606544). M.A.B. is supported by an NHMRC Senior Principal Research Fellowship (APP1024879).

Three City Study (3C): We thank the staff and participants of the 3C Study for their important contributions. We also thank A. Boland for her technical help in preparing the DNA samples for analyses. The 3C Study is conducted under a partnership agreement between INSERM, Victor Segalen–Bordeaux II University and Sanofi-Aventis. The Fondation pour la Recherche Médicale funded the preparation and initiation of the study. The 3C Study is also supported by the Caisse Nationale Maladie des Travailleurs Salariés, Direction Générale de la Santé, Mutuelle Générale de l’Éducation Nationale (MGEN), Institut de la Longévité, Conseils Régiaux d’Aquitaine et Bourgogne, Fondation de France and the French Ministry of Research–INSERM Programme Cohortes and Collections de Données Biologiques. This work was supported by the National Foundation for Alzheimer’s Disease and Related Disorders, the Institut Pasteur de Lille and the Centre National de Génotypage.

AUTHOR CONTRIBUTIONS

Study concept and design were performed by J.C.B., C. DeCarli, M.A.v.B., C. Dufouil, P.A., A.G.U., M.M.B.B., F.E., M.A.B., C.M.v.D., T.H.M., C.T., L.J.L., M.A.I. and S. Seshadri. Acquisition of data was carried out by F.v.d.L., F.C., H.S., V.S., M. Schuur, S. Sigurdsson, B.F.J.V., J.-C.L., C.R.I., J.S.F., D.E., T.H.M., B.M., F.H.C., C.E., P.D., K.A., M.A.v.B., A.B., C. Dufouil, J.H., W.J.N., G.K.G., P.A., K.F.B., T.G.P., B.A.O., A.G.U., R.A., A.E., R.I.B., J.C.v.S., M.M.B.B., M.W.V., P.A.W., A.v.d.L., V.G., M.A.B., D.A.B., C.M.v.D., T.H.M., R.S., C.T., L.J.L., M.A.I. and S. Seshadri. Statistical analysis and interpretation of the data were performed by J.C.B., C. DeCarli, A.V.S., F.v.d.L., F.M., S.D., J.M.S., H.S., V.S., M. Schuur, L.Y., S.H.C., B.F.J.V, A.L.D., M. Struchalin, J.S., C.A.I.-V., N.A., R.F.A., D.G.K., M.C., R.T., L.B.C., M.A.B., D.A.B., R.S., L.J.L. and M.A.I. The manuscript was drafted by J.C.B., C. DeCarli, M.A.v.B., M.A.B., B.M., P.D.L., L.J.I.L. and S. Seshadri. This manuscript revision was performed by J.C.B., C. DeCarli, F.v.d.L., M.E., S.D., J.M.S., H.S., S.-H.C., S. Sigurdsson, A.L.D., J.-C.L., J.S., C.A.I.-V., A.Z., T.H., L.H.C., E.C., P.D., C. Dufouil, M.C., W.T., J.N., E.Y.C., A.H., P.F.B., K.B.F., T.G.P., J.L.S., S.E.M., A.A.V., D.P.H., M.J.W., B.F., N.G.M., P.M.T., M.A.N., G.U., A.E., R.I.B., O.L.L., T.B.H., V.C., M.M.B.B., J.T.B., M.W.V., D.K., F.F., P.A.W., A.v.d.L., V.G., W.T.L., M.A.B., C.M.v.D., T.H.M., R.S., C.T., L.J.L., M.A.I. and S. Seshadri. Funding was obtained by M.F., H.S., V.S., C. Dufouil, W.J.N., A.H., B.A.O., A.G.U., J.C.v.S., T.H., V.C., M.M.B.B., F.E., P.A.W., A.v.d.L., V.G., D.A.B., C.M.v.D., T.H.M., R.S., C.T., L.J.L and S. Seshadri.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Participating studies. Our analyses were performed among dementia-free participants within the setting of the CHARGE Consortium. The ten discovery samples included the Aging Gene-Environment Susceptibility—Reykjavik Study (AGES), the Atherosclerosis Risk in Communities Study (ARIC), the Austrian Stroke Prevention Study (ASP), the Erasmus Rucphen Family Study (ERF), the Framingham Heart Study (FHS), the Religious Order Study & Rush Memory and Aging Project (RUSH), three independent phases of the Rotterdam Study (RS I, RS II and RS III) and the Tasmanian Study of Cognition and Gait (TASCOG). The two second-stage replication samples included the Three City Study (3C) and another independent sample of the third expansion of the Rotterdam Study (RS R). Details of the discovery samples and second-stage studies can be found in the Supplementary Note. Each study has an Institutional Review Board that approved the consent procedures, examination components, data security processes, genotyping protocols and current study design. All participants gave written informed consent for study participation and for use of DNA for genetic research.

Hippocampal volume phenotypes. Each study evaluated the total hippocampal volume using 1T, 1.5T or 3T MRI and either operator-defined, manually traced boundaries drawn on serial coronal sections or automated methods, according to previously described image reading protocols. For these analyses, we used data from the baseline examination or the first examination in which an MRI measurement was obtained. Specific details for each study’s MRI protocol are provided in the Supplementary Note.

Genotyping and imputation. The studies in these analyses used commercial genotyping platforms available from Illumina or Affymetrix. Each study performed genotyping quality control checks and imputed the approximately 2.5 million polymorphic autosomal SNPs described in the HapMap CEU population for each participant using available imputation methods. Details of per-study genotyping, imputation and quality control procedures are available in the Supplementary Note.

Statistical analysis within studies. Each study independently implemented a predefined GWAS analysis plan. For the continuous measure of hippocampal volume, we evaluated cross-sectional associations of hippocampal volume and genetic variation using linear regression models (or linear mixed-effects models, in the Framingham Heart Study and the Erasmus Rucphen Family Study to account for family relatedness). For each of the 2.5 million SNPs, each study fit additive genetic models, regressing trait on genotype dosage (zero to two copies of the variant allele). In our primary analyses, all studies were adjusted for age and sex. Some studies made additional adjustments, including for study site or familial structure or for whether the DNA had been whole-genome amplified. Additional details of the statistical analyses are available in the Supplementary Note.

Discovery meta-analysis. We conducted a meta-analysis of regression estimates and standard errors using an inverse-variance weighting approach as implemented in METAL. After verification of strand-alignement across studies, quality control, filtering and imputation within each study, we restricted our meta-analysis to autosomal SNPs that were reported in at least two studies and that had an average MAF of at least 1%. Before meta-analysis, we calculated a genomic inflation factor ($\lambda_{GC}$) for each study to screen for cryptic population substructure or undiagnosed irregularities that might have inflated the test statistics. Inflation was low, with $\lambda_{GC}$ below 1.05 in all studies. We applied genomic control to each study whose genomic inflation factor was greater than 1.00 by multiplying all of the standard errors by the square root of the study-specific $\lambda_{GC}$. We expressed the association of each SNP with hippocampal volume as the regression slope ($\beta$), its standard error (SE($\beta$)) and a corresponding P value. Standardized gene and SNP annotations were created using a PERL program.

For follow up, we decided a priori on a significance threshold of $P < 4 \times 10^{-7}$, which corresponds to no more than one expected false positive finding over 2.5 million tests.

Replication meta-analysis. Replication samples were drawn from external studies with available genetic data and measures of hippocampal volume. We provided each collaborating second-stage study with a list of signal SNPs that attained a P value of $P < 4 \times 10^{-7}$ and combined the results from these studies using a fixed-effects meta-analysis.

Combined meta-analysis. We combined results from the discovery and second-stage analyses using inverse-variance weighting and considered SNPs with a P value of <5 $\times 10^{-8}$ to have reached genome-wide significance.

External validation. We sought external replication for our significant and suggestive loci in the ENIGMA Consortium, the details of which can be found in the accompanying paper by Stein et al. The international ENIGMA Consortium comprises a wide variety of studies that all have GWAS and hippocampal volume measures. The sample includes case-control studies of Alzheimer’s disease and depression and family-based and sibling pair samples, as well as population-based samples of varying ages and ancestries (European, African and Hispanic). ENIGMA assesses brain volume using Freesurfer/FSL-FIRST (the Oxford Centre for Functional MRI Brain (FMRIB) Software Limited–FMRIB’s Integrated Registration and Segmentation Tool) protocols in most samples but also uses other protocols in a few samples. Hence, we chose to compare the results from ENIGMA and CHARGE in a qualitative manner, as these two studies varied in the composition of the study participants as well as in the methods used to assess hippocampal volume. We considered replication to be represented by a P value of <0.05 with a consistent direction of association.

Exploration of loci for eQTLs and functional variants. We examined the four loci identified as associated with hippocampal volume for the presence of cis eQTL associations using uchicago.edu. We also searched for functional SNPs in LD with the five index SNPs. We identified over 70 SNPs with $r^2 > 0.4$ that were within 500 kb of each index SNP using the SNAP proxy tool and annotated these SNPs using GeneCruiser; none of these SNPs were exonic, nonsynonymous coding SNPs.

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