nature genetics

Common variants at 6q22 and 17q21 are associated with intracranial volume

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During aging, intracranial volume remains unchanged and represents maximally attained brain size, while various interacting biological phenomena lead to brain volume loss. Consequently, intracranial volume and brain volume in late life reflect different genetic influences. Our genome-wide association study (GWAS) in 8,175 community-dwelling elderly persons did not reveal any associations at genome-wide significance ($P < 5 \times 10^{-8}$) for brain volume. In contrast, intracranial volume was significantly associated with two loci: rs4273712 ($P = 3.4 \times 10^{-11}$), a known height-associated locus on chromosome 6q22, and rs9915547 ($P = 1.5 \times 10^{-12}$), localized to the inversion on chromosome 17q21. We replicated the associations of these loci with intracranial volume in a separate sample of 1,752 elderly persons ($P = 1.1 \times 10^{-3}$ for 6q22 and 1.2×10^{-3} for 17q21). Furthermore, we also found suggestive associations of the 17q21 locus with head circumference in 10,768 children (mean age of 14.5 months). Our data identify two loci associated with head size, with the inversion at 17q21 also likely to be involved in attaining maximal brain size.

During early development and maturation, brain growth is the major force for increasing intracranial volume[1,2]. This process, which begins in utero, continues throughout childhood and ends in early adulthood, resulting in an intracranial volume that remains essentially unchanged throughout the remainder of life. During early life, intracranial volume is highly associated with brain volume. However, unlike intracranial volume, brain volume starts to decrease after early adulthood[3]. The greatest loss occurs in advanced age and is associated with disease states under polygenic and environmental influences, such as cerebrovascular and neurodegenerative diseases that result in brain atrophy[3,4]. As a consequence, the degree of association between intracranial volume and brain volume declines with advancing age. Although intracranial volume and brain volume are both highly heritable[5,6], the genetic influences on these measures may differ.

To assess genetic features underlying these traits, we performed a GWAS on cross-sectional measures of intracranial volume and brain volume in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium[7]. This consortium brings together six population-based cohort studies, of which five participated in the current analysis[8,9]: the Aging Gene-Environment Susceptibility–Reykjavik Study (AGES-RS), the Atherosclerosis Risk in Communities Study (ARIC), the Austrian Stroke Prevention Study (ASPS), the Framingham Heart Study (FHS) and the Rotterdam Study (RS). The current report includes 8,175 persons of European descent, each with genome-wide genotype data and a quantitative measure of intracranial volume based on magnetic resonance imaging (MRI). For brain volume analysis, we excluded persons diagnosed with dementia or those who showed a cortical infarct on MRI. Moreover, brain volume was expressed as a percentage of intracranial volume to exclude genetic influences of intracranial volume on brain volume. The ASPS only had data available for brain volume and not for intracranial volume, resulting in a total of 8,673 persons in the current GWAS on brain volume (**Table 1**).

Data for replication came from a separate sample of 1,752 elderly subjects aged 66–96 years from AGES-RS, who had undergone MRI but

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Received 22 June 2011; accepted 10 March 2012; published online 15 April 2012; corrected after print 27 April 2012; corrected after print 8 May 2013; doi:10.1038/ng.2245

Table 1 Characteristics of the study populations

	Total	AGES	ARIC	ASPS	FHS	RS I	RS II	RS III
Persons available for intracranial volume GWAS	8,175	2,671	359	NA	2,319	421	679	1,726
Mean age (s.d.)	67.5 (7.7)	76.2 (5.4)	72.7 (4.3)	65.1 (8.0)†	64.0 (11.4)	73.0 (7.7)	67.5 (5.6)	56.1 (5.5)
Female	56%	59%	60%	58%[a]	54%	52%	49%	55%
Mean intracranial volume in milliliters (s.d.)	1,301 (132)	1,499 (146)	1,463 (142)	NA	1,254 (131)	1,127 (116)	1,126 (117)	1,135 (117)
Excluded (due to pathology or technical artifacts)	227	0[b]	47	NA	77	20	26	57
Persons available for brain volume GWAS	8,673	2,671	312	725	2,242	401	653	1669
Mean brain volume, percentage of intracranial volume (s.d.)	78 (4)	72 (4)	74 (4)	79 (4)	79 (4)	78 (4)	82 (3)	85 (3)

[a]Values are reported for persons available for the brain volume GWAS. [b]Both the intracranial volume and brain volume analyses in AGES were performed after exclusion of persons with pathology or technical artifacts.

not genome-wide genotyping. Additionally, we investigated whether any of the loci significantly associated with intracranial volume or brain volume were also associated with head circumference in 10,768 children with an average age of 14.5 months from the Early Growth Genetics (EGG) Consortium[10]. Rather than being a formal replication, this analysis added support for association of the discovered loci with human brain development. Finally, because head size is a morphological phenotype related to adult height, we determined whether any significantly associated loci for intracranial volume were already implicated in height in a study by the Genetic Investigation of ANthropometric Traits (GIANT) Consortium[11]. We also performed additional height-adjusted analyses for the SNPs achieving genome-wide significance. In addition, we examined the association with intracranial volume of all known loci for adult height.

Within the CHARGE Consortium, after quality control, each individual study imputed genome-wide genotype data to 2.2 million HapMap Utah residents of Northern and Western European ancestry (CEU) SNPs using automated software. Subsequently, each study performed genome-wide association tests for intracranial volume and brain volume using linear regression. Each study used the same basic additive genetic model of dosages of the risk allele with a 1-degree-of-freedom trend test, adjusting for age, sex and familial relationships (in FHS). For each study, we examined quantile-quantile plots for brain volume and intracranial volume to ensure that the *P* value distributions conformed to a null distribution, the expected distribution of *P* values if no genetic association was present, at all but the extreme tail (**Supplementary Fig. 1**). Finally, after performing genomic control in each study, we conducted meta-analysis on results across studies using inverse-variance weighting. Examination of the quantile-quantile plots from meta-analysis revealed an excess of extreme *P* values but no evidence of systematic inflation of the genomic control inflation factor ($\lambda = 1.04$ for intracranial volume and 1.02 for brain volume; **Supplementary Fig. 2**). The genome-wide plots of *P* values for the individual SNPs against their genomic position are shown (**Fig. 1a,b**). We did not observe any association reaching genome-wide significance for brain volume, although 46 SNPs reached $P < 1 \times 10^{-5}$ (**Supplementary Table 1**). For intracranial volume, 720 SNPs located in two loci surpassed our preset threshold of genome-wide significance ($P < 5 \times 10^{-8}$). An additional 200 SNPs showed associations at $P < 1 \times 10^{-5}$ (**Supplementary Table 2**).

The most significant association with intracranial volume was identified for rs9915547 on chromosome 17. The minor allele of this SNP (minor allele frequency (MAF) = 0.22) was significantly associated with smaller intracranial volume ($P = 1.5 \times 10^{-12}$). This SNP belongs to an ~1-Mb large cluster of SNPs in very strong linkage disequilibrium (LD) with each other located in a region that has previously been identified as the chromosome 17q21 inversion (**Fig. 2a**). The chromosome 17q21 inversion consists of two haplotypes (H1 and H2), with the minor allele of rs9915547 corresponding to the H2 haplotype. Adjusting for height did not change this result ($P_{adjusted} = 1.8 \times 10^{-12}$).

The second association at genome-wide significance was found for rs4273712 on chromosome 6q22, with *RSPO3* and *CENPW* (also known as *C6orf173*) being the closest genes (**Fig. 2b**). The minor allele of this SNP (MAF = 0.27) was associated with larger intracranial volume ($P = 3.4 \times 10^{-11}$). This locus has previously been associated with adult height[11]. Of note, copy-number variations (CNVs) have been reported at this locus, with the closest CNVs to rs4273712 being described at positions 127,124,970–127,135,701 and 127,136,191–127,141,042. Both rs9915547 and rs4273712 were not associated with brain volume in our data ($P = 0.23$ and 0.67, respectively).

We sought to replicate the associations that were found at genome-wide significance in a sample of 1,752 elderly persons (66–96 years old) from AGES-RS who were not included in the discovery sample. We genotyped rs4273712 and rs9303525 ($P_{discovery} = 1.6 \times 10^{-12}$), which maps to the inversion and is in perfect LD with rs9915547, and replicated association with intracranial volume at $P = 1.1 \times 10^{-3}$ for rs4273712 and 1.2×10^{-3} for rs9303525); the meta–meta-analysis of the replication sample with the discovery samples yielded *P* values of 1.8×10^{-13} for the association with rs4273712 and 7.6×10^{-15} for the association with rs9303525 (**Table 2**).

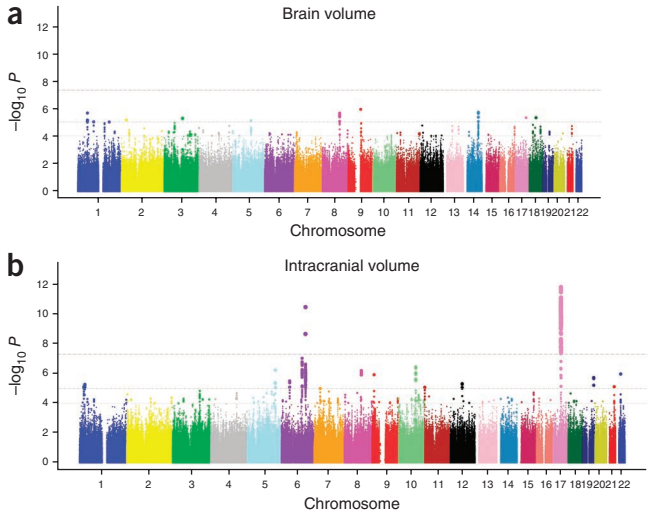

Figure 1 Genome-wide signal intensity (Manhattan) plots showing the individual *P* values (based on fixed-effects meta-analysis) against genomic position. (**a**,**b**) Association results are shown for brain volume (**a**) and intracranial volume (**b**). Within each chromosome, shown on the *x* axis, the results are plotted left to right from the p-terminal end. Horizontal dashed lines indicate *P* value thresholds of 1×10^{-4}, 1×10^{-5} and 5×10^{-8} (genome-wide significance).

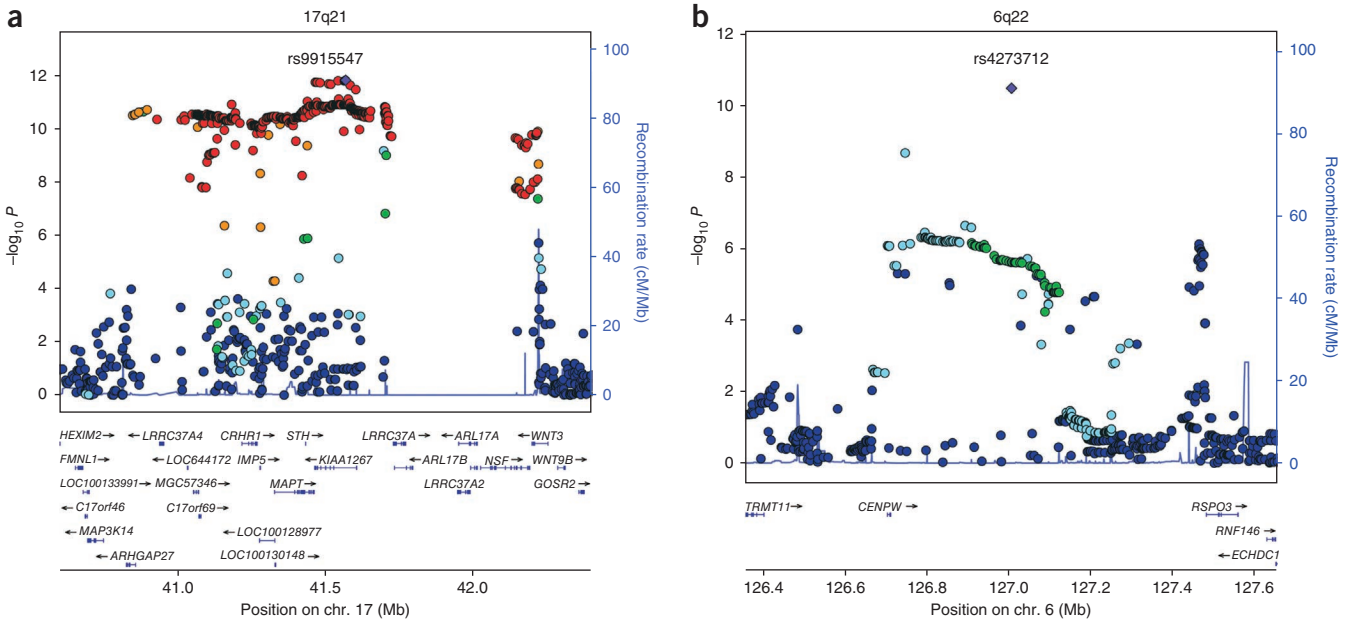

Figure 2 Regional plots for associations with intracranial volume at genome-wide significance. (**a**,**b**) All SNPs (circles) are plotted with meta-analysis *P* values against genomic position for chromosome 17q21 (**a**) and chromosome 6q22 (**b**). Symbols are colored according to the LD of the SNP with the top SNP (purple diamond). The blue line shows estimated recombination rates. Genes are shown as black arrows. We note that several CNVs have been reported at 6q22, the closest of which are downstream of rs4273712 at positions 127,124,970–127,135,701 and 127,136,191–127,141,042.

Finally, given the hypothesis that intracranial volume is reflective of maximum brain size during life, we reasoned that these loci may have a role in brain growth. Therefore, we investigated associations of these loci with head circumference in 10,768 children from the EGG Consortium[10], in whom head circumference is much more likely to be a direct marker for brain growth than in adults[1]. We found that rs9915547 was significantly associated with head circumference ($P = 5.3 \times 10^{-3}$). In the birth cohort studied, the most significant SNP within the 17q21 inversion was rs11655470, which was associated with head circumference at $P = 1.4 \times 10^{-6}$, although this SNP does not accurately map to the inversion itself (r^2 with the inversion = 0.22, based on HapMap CEU)[10]. Indeed, within the CHARGE Consortium, rs11655470 was only weakly associated with intracranial volume at $P = 0.04$. To investigate whether these signals were independent, we performed conditional analyses for rs9915547 and rs11655470 and found $P = 6.3 \times 10^{-15}$ and 0.38, respectively. The attenuation in the significance of the association for rs11655470 suggests that the association is primarily driven by rs9915547, which maps to the inversion.

No association was found for rs4273712 with head circumference in the EGG Consortium ($P = 0.16$). Because rs4273712 is located in a known height-associated locus, we also determined the association with intracranial volume of all 180 known loci for adult height[11] (**Supplementary Table 3**). Outside of the 6q22 locus, only rs1351394 on chromosome 12 was associated with intracranial volume with nominal significance ($P = 4.7 \times 10^{-6}$; threshold for Bonferroni-adjusted significance of $0.05/180 = 2.8 \times 10^{-4}$). Of note, another SNP at this locus (rs1042725) was associated with head circumference at genome-wide significance in the EGG Consortium ($P = 2.8 \times 10^{-10}$)[10]. The r^2 value between the rs1042725 and rs1351394 SNPs was 0.91, based on the CEU sample in HapMap Phase 2.

Several genes are located in the 17q21 inversion, which is tagged by the H1 and H2 haplotypes. The two haplotypes show different expression patterns of these genes, some of which have been implicated in neurodegenerative disorders in late life, particularly in individuals with the H1 haplotype[12]. This region includes *MAPT*, which encodes the tau protein, a major hallmark of various dementias, including Alzheimer's disease and frontotemporal dementia[12–14]. Mutations in *MAPT* have been consistently associated with frontotemporal dementia, progressive supranuclear palsy and Parkinson's disease[15]. This region also includes *GRN*, which encodes progranulin, with mutations in this gene also capable of causing frontotemporal degeneration[16,17]. Additional genes in the 17q21 inversion region include *CRHR1* (encoding corticotrophin releasing hormone receptor 1), a gene that has been associated with early brain development and that might mediate the response to environmental stress[18], and *STH* (encoding saitohin), which has been associated with increased risk of late-onset Alzheimer's disease and with progressive supranuclear palsy[14,15]. In addition, smaller intracranial volume has been consistently associated with increased risk for late-life dementia[19], suggesting that at least some of the findings related to intracranial volume and dementia risk may reflect the impact of this inversion on early development. Finally, several microdeletions

Table 2 Genome-wide significant loci for intracranial volume and results from various stages of the analysis

SNP					Discovery			Replication			Meta–meta-analysis	
Number	Locus	Strand	Minor allele	Major allele	MAF	β^a	*P*	MAF	β^a	*P*	β^a	*P*
rs4273712	6q22	+	G	A	0.27	12.2	3.4×10^{-11}	0.27	14.7	1.1×10^{-3}	12.5	1.8×10^{-13}
rs9303525	17q21	+	G	A	0.22	−14.7	1.6×10^{-12}	0.18	−16.5	1.2×10^{-3}	−14.9	7.6×10^{-15}

[a]β represents the difference in intracranial volume (in milliliters) per copy increase of the minor allele.

within the inversion have been shown to cause microencephaly and various childhood mental retardation syndromes[20,21].

The population genetics and evolutionary history of the 17q21 inversion are the object of ongoing research, with some studies suggesting that this inversion is approximately 3 million years old and almost exclusively present in the European population[22], although more recent work contests this notion[23]. Moreover, findings that the H2 haplotype is undergoing positive selection in some European populations[22] have also recently been challenged, with an alternative explanation being proposed in which higher H2 frequency is considered a founder effect[23]. The influence of the inversion on brain development, as shown in our study, might be hypothesized to be one mechanism explaining the putative positive selection for the H2 haplotype. However, this explanation is unlikely, as the H2 haplotype is associated with smaller intracranial volume, whereas intracranial volume has increased throughout evolution from primates to *Homo sapiens*[24]. Still, our findings provide further insights into the role of the 17q21 inversion in human biology. More research is needed to determine its exact role.

Thus far, in order to explain our findings regarding 17q21, we have focused primarily on neurological mechanisms, assuming that brain growth drives intracranial volume. An alternative explanation is that variations in bone growth determine intracranial volume and, thus, maximum brain volume. However, given that the 17q21 inversion does not seem to influence height[11] and that height is particularly influenced by bone growth, this alternative explanation seems unlikely.

Two loci implicated in height were also associated with intracranial volume. This suggests the possibility of multiple functions for the loci on chromosomes 6 and 12 associated with brain development. rs4273712 on chromosome 6 is close to the *RSPO3* gene, which encodes a protein of the thrombospondin type 1 repeat (TSR) superfamily, members of which seem to function in the development of the nervous system[25]. Another potentially relevant gene in this region is *RNF146*, which lies further downstream of *RSPO3* and has been shown to be upregulated in brains from individuals with Alzheimer's disease[26]. We also note that reported CNVs could explain the association pattern seen at 6q22, as the region showed little recombination, but without any SNP being in clear LD with rs4273712.

The chromosome 12 locus maps to a gene that encodes a protein belonging to the non-histone chromosomal high-mobility group (HMG) protein family. This gene has been implicated in height, adipogenesis and mesenchymal differentiation. Although the association at this locus did not reach genome-wide significance with intracranial volume, it was associated at genome-wide significance with head circumference in children[10]. This further shows overlap in genetic variants that influence head size in childhood, maximum head size in adulthood and, possibly, brain growth.

In conclusion, our GWAS determined that the chromosome 17 inversion was strongly associated with intracranial volume. We further found that this inversion may have a role in brain growth in early life. A second association at genome-wide significance was found for rs4273712 on chromosome 6 near *RSPO3* and *RNF146*, both of which potentially influence neuronal development and degeneration.

URLs. PLINK, http://pngu.mgh.harvard.edu/purcell/plink/; MaCH, http://www.sph.umich.edu/csg/abecasis/MACH/; R, http://r-project.org/.

METHODS

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

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

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

Atherosclerosis Risk in Communities Study (ARIC): The authors thank the staff and participants of the ARIC study for their important contributions. Research is carried out as a collaborative study supported by the US NHLBI (N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, N01-HC-55022, R01-HL087641 and R01-HL093029), the National Human Genome Research Institute (U01-HG004402) and the NIH (HHSN268200625226C). Infrastructure was partly supported by a component of the NIH and NIH Roadmap for Medical Research (UL1RR025005).

Austrian Stroke Prevention Study (ASPS): The authors thank the staff and participants of the ASPS for their valuable contributions. We thank B. Reinhart for her long-term administrative commitment and I.J. Semmler for technical assistance in creating the DNA bank. The research reported here was funded by the Austrian Science Fond (FWF) (P20545-P05 and P13180). The Medical University of Graz supports the databank of the ASPS.

Framingham Heart Study (FHS): This study is carried out by the US NHLBI of the NIH and Boston University School of Medicine. This work was supported by the Framingham Heart Study of the NHLBI (N01-HC-25195) and its contract with Affymetrix, Inc, for genotyping services (N02-HL-6-4278). A portion of this research used the Linux Cluster for Genetic Analysis (LinGA-II) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center. This study was also supported by grants from the National Institute of Neurological Disorders and Stroke (NS17950), the NHLBI (HL093029) and the NIA (AG08122, AG16495, AG033193, AG033040, AG031287 and P30AG013846).

Rotterdam Study (RS): The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists. The authors thank P. Arp, M. Jhamai, 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 creation and analysis of imputed data. The generation and management of GWAS genotype data for the Rotterdam Study were supported by the Netherlands Organisation of Scientific Research (NWO) Investments (nr. 175.010.2005.011 and 911-03-012). This study is funded by the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)- NWO project (nr. 050-060-810). The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University Organization for Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the 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 (nrs. 918-46-615, 904-61-096, 904-61-133 and 948-00-010), the Nederlandse Hartstichting (2009B102) and the Internationaal Parkinson Fonds.

EGG Consortium: We gratefully acknowledge the contribution of general practitioners, hospitals, midwives and pharmacies in Rotterdam. Financial support was received from the Academy of Finland (104781, 120315, 1114194 and Center of Excellence in Complex Disease Genetics), University Hospital Oulu, Biocenter, University of Oulu, the US NHLBI (5R01HL087679-02, through the STAMPEED program 1RL1MH083268-01), the ENGAGE project and grant agreement HEALTH-F4-2007-201413, the UK MRC (G0500539 and PrevMetSyn/Salve/MRC) and the Wellcome Trust (GR069224). DNA extraction, sample quality control, biobank upkeep and aliquotting were performed at the National Public Health Institute, Biomedicum Helsinki (Helsinki, Finland) and supported financially by the Academy of Finland and Biocentrum Helsinki. The Generation R Study is conducted by the Erasmus Medical Center in close collaboration with the School of Law and Faculty of Social Sciences of the Erasmus University Rotterdam, the Municipal Health Service Rotterdam area, the Rotterdam Homecare Foundation and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR). The Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam and the Netherlands Organization for Health Research and Development (ZonMw 21000074). V.J. received additional grants from the Netherlands Organization for Health Research and Development (ZonMw 90700303 and 916.10159). Additional support was provided by a grant from the Dutch Kidney Foundation (C08.2251).

The authors would like to thank all participating subjects and families from the Children's Hospital in Philadelphia. The research was financially supported by an Institute Development Award from the Children's Hospital of Philadelphia, a Research Development Award from the Cotswold Foundation and by the US NIH (1R01HD056465-01A1). The authors are grateful to the Raine Foundation, the RAINE Study families and the RAINE Study research staff. We gratefully acknowledge the assistance of the Western Australian Genetic Epidemiology Resource and the Western Australian DNA Bank (both National Health and Medical Research Council of Australia National Enabling Facilities). The authors also acknowledge the support of Healthway Western Australia, the National Health and Medical Research Council of Australia (572613) and the Canadian Institutes of Health Research (MOP 82893). We gratefully acknowledge the assistance of the Wind Over Water Foundation, the Telethon Institute for Child Health Research and the RAINE Medical Research Foundation of the University of Western Australia. The authors wish to acknowledge the following: Helmholtz Zentrum Muenchen–German Research Center for Environment and Health, Institute of Epidemiology; the Department of Pediatrics, University of Leipzig; Department of Pediatrics, Marien-Hospital; Bad Honnef; the Department of Human Exposure Research and Epidemiology, UFZ-Centre for Environmental Research Leipzig–Halle; the Department of Environmental Immunology, Zentrum für Umweltforschung (UFZ)-Centre for Environmental Research Leipzig–Halle; the Institut für Umweltmedizinische Forschung (IUF) and the Department of Pediatrics, Technical University. The UK MRC (74882), the Wellcome Trust (076467) and the University of Bristol provide core support for the Avon Longitudinal Study of Parents and Children (ALSPAC). We are extremely grateful to all the families who took part in the ALSPAC study, the midwives for their help in recruiting them and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

AUTHOR CONTRIBUTIONS

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

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Gale, C.R., O'Callaghan, F.J., Bredow, M. & Martyn, C.N. The influence of head growth in fetal life, infancy, and childhood on intelligence at the ages of 4 and 8 years. *Pediatrics* **118**, 1486–1492 (2006).
2. Gale, C.R., O'Callaghan, F.J., Godfrey, K.M., Law, C.M. & Martyn, C.N. Critical periods of brain growth and cognitive function in children. *Brain* **127**, 321–329 (2004).
3. DeCarli, C. *et al.* Measures of brain morphology and infarction in the framingham heart study: establishing what is normal. *Neurobiol. Aging* **26**, 491–510 (2005).
4. Ikram, M.A. *et al.* Brain tissue volumes in the general elderly population. The Rotterdam Scan Study. *Neurobiol. Aging* **29**, 882–890 (2008).
5. Carmelli, D. *et al.* Evidence for genetic variance in white matter hyperintensity volume in normal elderly male twins. *Stroke* **29**, 1177–1181 (1998).
6. Atwood, L.D. *et al.* Genetic variation in white matter hyperintensity volume in the Framingham Study. *Stroke* **35**, 1609–1613 (2004).
7. Psaty, B.M. *et al.* Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium. Design of prospective meta-analyses of genome-wide association studies from 5 cohorts. *Circ. Cardiovasc. Genet.* **2**, 73–80 (2009).
8. Seshadri, S. *et al.* Genome-wide analysis of genetic loci associated with Alzheimer disease. *J. Am. Med. Assoc.* **303**, 1832–1840 (2010).
9. Ikram, M.A. *et al.* Genomewide association studies of stroke. *N. Engl. J. Med.* **360**, 1718–1728 (2009).
10. Taal, H.R. *et al.* Common variants at 12q15 and 12q24 are associated with infant head circumference. *Nat. Genet.* published online (15 April 2012); doi:10.1038/ng.2238.
11. Lango Allen, H. *et al.* Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature* **467**, 832–838 (2010).
12. Di Maria, E. *et al.* The H1 haplotype of the *tau* gene (*MAPT*) is associated with mild cognitive impairment. *J. Alzheimers Dis.* **19**, 909–914 (2010).
13. Zody, M.C. *et al.* Evolutionary toggling of the *MAPT* 17q21.31 inversion region. *Nat. Genet.* **40**, 1076–1083 (2008).
14. Conrad, C. *et al.* Molecular evolution and genetics of the Saitohin gene and *tau* haplotype in Alzheimer's disease and argyrophilic grain disease. *J. Neurochem.* **89**, 179–188 (2004).
15. Levecque, C. *et al.* Association of polymorphisms in the *Tau* and *Saitohin* genes with Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* **75**, 478–480 (2004).
16. Gijselinck, I., Van Broeckhoven, C. & Cruts, M. Granulin mutations associated with frontotemporal lobar degeneration and related disorders: an update. *Hum. Mutat.* **29**, 1373–1386 (2008).
17. Gijselinck, I. *et al.* Progranulin locus deletion in frontotemporal dementia. *Hum. Mutat.* **29**, 53–58 (2008).
18. Hsuchou, H., Kastin, A.J., Wu, X., Tu, H. & Pan, W. Corticotropin-releasing hormone receptor–1 in cerebral microvessels changes during development and influences urocortin transport across the blood-brain barrier. *Endocrinology* **151**, 1221–1227 (2010).
19. Mortimer, J.A., Snowdon, D.A. & Markesbery, W.R. Head circumference, education and risk of dementia: findings from the Nun Study. *J. Clin. Exp. Neuropsychol.* **25**, 671–679 (2003).
20. Koolen, D.A. *et al.* A new chromosome 17q21.31 microdeletion syndrome associated with a common inversion polymorphism. *Nat. Genet.* **38**, 999–1001 (2006).
21. Koolen, D.A. *et al.* Clinical and molecular delineation of the 17q21.31 microdeletion syndrome. *J. Med. Genet.* **45**, 710–720 (2008).
22. Stefansson, H. *et al.* A common inversion under selection in Europeans. *Nat. Genet.* **37**, 129–137 (2005).
23. Donnelly, M.P. *et al.* The distribution and most recent common ancestor of the 17q21 inversion in humans. *Am. J. Hum. Genet.* **86**, 161–171 (2010).
24. Roth, G. & Dicke, U. Evolution of the brain and intelligence. *Trends Cogn. Sci.* **9**, 250–257 (2005).
25. Adams, J.C. & Tucker, R.P. The thrombospondin type 1 repeat (TSR) superfamily: diverse proteins with related roles in neuronal development. *Dev. Dyn.* **218**, 280–299 (2000).
26. von Rotz, R.C., Kins, S., Hipfel, R., von der Kammer, H. & Nitsch, R.M. The novel cytosolic RING finger protein dactylidin is up-regulated in brains of patients with Alzheimer's disease. *Eur. J. Neurosci.* **21**, 1289–1298 (2005).

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ONLINE METHODS

Participating cohorts. Analyses were performed among participants of studies in the CHARGE Consortium[7]. Details on the six participating discovery samples and the replication and extension samples 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.

Measurement of intracranial volume and brain volume by MRI. Each study performed brain MRI on scanners ranging from 0.5 to 1.5 T in field strength. Each study used its own custom-made or freely downloadable fully automatic or semiquantitative MRI post-processing software to measure intracranial volume and brain volume. Brain volume was expressed as percentage of intracranial volume in order to correct for differences in individual head size in all studies. As a result of the software used, the ASPS only had measurements available for brain volume. All software has been extensively validated against manual tracings, which are the gold standard for automated MRI post-processing algorithms. We note that any remaining heterogeneity across studies due to differences in post-processing algorithms would lead to lower power; this in turn could lead to false negatives but would not invalidate the associations that were found.

Specific details for each study's MRI protocol are provided in the **Supplementary Note**. The correlation between intracranial volume (in milliliters) and brain volume (in milliliters) was 0.83 for AGES, 0.88 for ARIC, 0.89 for FHS, 0.91 for RS I, 0.92 for RS II and 0.95 for RS III. In contrast, when using brain volume expressed as percentage of intracranial volume, as was done in our GWAS, the correlations were strongly attenuated and became −0.35 for AGES, −0.29 for ARIC, −0.01 for FHS, 0.04 for RS I, −0.19 for RS II and −0.17 for RS III.

Genotyping, quality controls and imputation. The CHARGE consortium was formed after the individual studies had finalized their GWAS platforms, and the six studies included used different platforms, including the Illumina Human370-Duo BeadChip for AGES-RS, the Affymetrix GeneChip SNP Array 6.0 for ARIC; the Illumina Human610-Quad BeadChip for ASPS, the Affymetrix GeneChip Human Mapping 500K Array Set and 50K Human Gene Focused Panel for FHS and the Illumina HumanHap550 Duo BeadChip for RS.

As detailed previously[8,9], participant-specific quality controls included filters for call rate, heterozygosity and number of Mendelian errors per individual. SNP-specific quality controls included filters for call rate, MAF, Hardy-Weinberg equilibrium and differential missingness by outcome or genotype (mishap test in PLINK).

The set of genotyped input SNPs used for imputation in each study was selected on the basis of their highest quality data in the GWAS. We used SNPs with call rates of >95% in ARIC, >97% in FHS and >98% in AGES-RS, ASPS and RS; MAFs of >0.01 in each study; Hardy-Weinberg equilibrium P values of $>1 \times 10^{-6}$ in AGES-RS, ARIC, ASPS, FHS and RS; and P values of $>1 \times 10^{-9}$ in the test of differential missingness from the mishap test in PLINK in each study. We used the Markov Chain Haplotyping (MaCH) package (version 1.0.15 or 1.0.16) for AGES-RS, ARIC, ASPS, FHS and RS imputed to the plus strand of NCBI Build 36 using HapMap release 22. Imputation of genotypes provided a dosage value for every SNP between zero and two, indicating the expected value of a SNP being homozygous for the reference allele. For each imputed SNP, the informativeness of the imputation was estimated (as the ratio of the empirically observed dosage variance to the expected binomial dosage variance, O/E ratio). In the primary meta-analysis using inverse-variance weighting, less weight was given to imputed SNPs with low O/E ratios, resulting in higher variance of the estimate.

Studies were screened for latent population substructure[8,9], including cryptic relatedness, using suitable programs that included EIGENSTRAT[27] in ARIC, FHS and AGES-RS and an identity-by-descent (IBD) matrix in ASPS and RS. When appropriate, components related to intracranial volume or brain volume were included as covariates in linear regression.

We studied quantile-quantile plots to ensure that the P-value distributions in each of the cohorts conformed to a null distribution at all but the extreme tail.

We also calculated the genomic inflation factor λ, which measures overdispersion of test statistics from association tests, indicating population stratification, and can be used to apply genomic control.

Analysis within studies. Each study ran 1-degree-of-freedom additive association models of the GWAS with intracranial volume and brain volume. Alleles were used as dosages of 0, 1 or 2 copies of the risk allele. Intracranial volume was expressed in milliliters, whereas brain volume was expressed as percentage of intracranial volume to correct for individual head size differences. We note that the SIENAX software used in ASPS only provided this adjusted estimate of atrophy and no absolute volume in milliliters. All models were adjusted for age and sex and, in FHS, for familial relationships. In secondary analyses, we also adjusted for height.

Discovery meta-analysis. After quality control and filtering within each study, AGES-RS had either genotyped or imputed data for 2,408,992 SNPs, ARIC 2,298,221 SNPs, ASPS 2,317,924 SNPs, FHS 2,543,888 SNPs, RS I 2,543,888 SNPs, the RS II 2,543,888 SNPs and RS III 2,543,888 SNPs. We restricted the present meta-analysis to the 2,229,753 autosomal SNPs common to all studies. For our discovery meta-analysis, we used the technique of inverse-variance weighting, assuming fixed-effects, after applying genomic control within each individual study. β estimates were weighted by their inverse variance, and a combined estimate was obtained by summing the weighted β estimates and dividing by the summed weights. Hence results for SNPs imputed with low certainty were down-weighted, because low reliability of imputation ensures a large variance. In contrast, studies with large sample sizes and directly genotyped or well-imputed SNPs had greater effects on the meta-analyses P values because of small variances. We undertook the meta-analysis at Rotterdam using the R package MetABEL.

We estimated the genomic inflation factor λ after meta-analysis. The estimate of λ was 1.039 for intracranial volume and 1.019 for brain volume, indicating no significant inflation of P values. We also examined quantile-quantile plots of the results from the meta-analysis. For intracranial volume, the original quantile-quantile plot was driven in large part by the chromosome 17 inversion and the numerous SNPs in close LD in that region; therefore, we recalculated the quantile-quantile plot after excluding the chromosome 17 inversion.

Replication analysis and extension analysis. The replication sample consisted of a random subset of 1,752 European participants from the AGES-RS, who had not undergone genome-wide genotyping at the time of the study but had MRI scans with measurement of intracranial volume available. Methods for measurement of intracranial volume were the same as for the rest of the participants from AGES-RS. Using TaqMan assays, we genotyped rs4273712 and rs9303535 ($P_{\text{discovery}} = 1.6 \times 10^{-12}$), which maps to the chromosome 17 inversion in perfect LD with rs9915547 ($r^2 = 1$ and $D' = 1$ between these two SNPs). We performed 1-degree-of-freedom additive association relating these SNPs to intracranial volume. The threshold for statistical significance was set at $P < 0.025$ (Bonferroni correction for two SNPs). Subsequently, we performed meta-analysis on the results from the replication sample with those from the discovery sample using fixed-effect inverse-variance meta-analysis.

For extension of our samples, we examined loci that were associated with genome-wide significance in the discovery analysis in an existing meta-analysis on head circumference during infancy in 10,768 children (mean age of 14.5 months)[10]. In this analysis, the threshold for statistical significance was also set at $P < 0.025$. Head circumference was measured manually.

Association of reported height-associated loci with intracranial volume. Because head size is a morphological phenotype related to adult height, we also examined all loci that were recently reported to be associated with adult height for their association with intracranial volume[11]. A total of 180 SNPs were tested, resulting in a Bonferroni-corrected P-value threshold of $0.05/180 = 2.8 \times 10^{-4}$ for significance.

27. Price, A.L. *et al. Nat. Genet.* **38**, 904–909 (2006).

Erratum: Common variants at 6q22 and 17q21 are associated with intracranial volume

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***Nat. Genet.* 44, 539–544 (2012); published online 15 April 2012; corrected online 27 April 2012**

In the version of this article initially published online, the percentage of females in the AGES study in Table 1 was left blank. This number should be 59%. The error has been corrected for the PDF and HTML versions of this article.

Corrigendum: Common variants at 6q22 and 17q21 are associated with intracranial volume

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***Nat. Genet.* 44, 539–544 (2012); published online 15 April 2012; corrected after print 27 April 2012; corrected after print 8 May 2013**

In the version of this article initially published, Thorkild I.A. Sørensen was listed incorrectly as a contributing member of the EGG Consortium. The error has been corrected for the HTML and PDF versions of this article.