nature genetics

Common variants at 12q15 and 12q24 are associated with infant head circumference

To identify genetic variants associated with head circumference in infancy, we performed a meta-analysis of seven genome-wide association studies (GWAS) (N = 10,768 individuals of European ancestry enrolled in pregnancy and/or birth cohorts) and followed up three lead signals in six replication studies (combined N = 19,089). rs7980687 on chromosome 12q24 ($P = 8.1 \times 10^{-9}$) and rs1042725 on chromosome 12q15 ($P = 2.8 \times 10^{-10}$) were robustly associated with head circumference in infancy. Although these loci have previously been associated with adult height[1], their effects on infant head circumference were largely independent of height ($P = 3.8 \times 10^{-7}$ for rs7980687 and $P = 1.3 \times 10^{-7}$ for rs1042725 after adjustment for infant height). A third signal, rs11655470 on chromosome 17q21, showed suggestive evidence of association with head circumference ($P = 3.9 \times 10^{-6}$). SNPs correlated to the 17q21 signal have shown genome-wide association with adult intracranial volume[2], Parkinson's disease and other neurodegenerative diseases[3–5], indicating that a common genetic variant in this region might link early brain growth with neurological disease in later life.

Head circumference in infancy is used as a measure for brain size and development[6,7]. Normal variation in head circumference seems to be associated with cognitive and behavioral development[8–10]. Larger head circumference in infancy is associated with higher IQ scores in childhood[10–12]. The underlying mechanisms, however, are poorly understood. Head circumference is a complex trait, with a high heritability of approximately 0.7–0.9 (ref. 13). Several rare mutations with large effects on head circumference have been identified[14–17], including those resulting in microcephaly and intellectual disability[15–17]. Common genetic variants that influence normal variation in head circumference in early life have not yet been identified.

To search for common genetic variants associated with head circumference in infancy, we performed a meta-analysis of multiple GWAS. We reasoned that finding such common variants might lead to an enhanced understanding of molecular mechanisms important for variation in brain development.

We calculated meta-analysis association statistics from ~2.5 million directly genotyped and imputed SNPs in infants of European descent from seven discovery GWAS (N = 10,768; **Supplementary Table 1**). In all studies, head circumference in infancy (age 18 months, range 6 to 30 months) was measured from the occipital protuberance to the forehead, using a flexible, non-stretching measuring tape according to standardized procedures. If multiple measurements were available for one individual in this time frame, only the measurement performed closest to the age of 18 months was used (**Supplementary Tables 1** and **2**). Because the relationship between head circumference and age during infancy is nonlinear and the variance increases with age, we calculated sex- and age-adjusted standard deviation (s.d.) scores of head circumference in each study separately[18].

In the discovery phase, we identified three lead signals (shown in the Manhattan plot in **Supplementary Fig. 1**); two independent loci on chromosome 12 and one on chromosome 17 showed suggestive evidence for association with head circumference in infancy. These three loci represent the first three independent loci of the discovery analysis and were at 12q24.31 in *SBNO1* (rs7980687; $P_{discovery} = 3.3 \times 10^{-7}$; **Fig. 1a**), at 12q15 near *HMGA2* (rs1042725; $P_{discovery} = 6.6 \times 10^{-7}$; **Fig. 1b**) and at 17q21.1 near *CRHR1-MAPT* (rs11655470; $P_{discovery} = 1.4 \times 10^{-6}$; **Fig. 1c**). Other loci with suggestive evidence of association with infant head circumference ($P < 1 \times 10^{-5}$) are described in **Supplementary Table 3**.

The associations of these three lead SNPs in each cohort are shown (**Table 1**). We followed up these three associations in six independent replication samples of European descent (N = 8,321; **Supplementary Table 2**). We genotyped the most strongly associated SNP from each locus (rs7980687 from 12q24.31, rs1042725 from 12q15 and rs11655470 from 17q21.1) or a closely correlated proxy SNP (selected by HapMap r^2 value). Consistent associations were observed for both signals on chromosome 12 in the replication samples (P = 0.003 and 8.1×10^{-5} for rs7980687 and rs1042725, respectively). Marginal evidence of association for rs11655470 was seen in the replication samples (P = 0.093). Genomic control correction was applied during the discovery meta-analysis stage to adjust the statistics generated within each cohort (λ values ranged from 1.007–1.054; **Supplementary Table 1**). Results from the replication cohorts were combined with the genomic control–corrected discovery results to generate overall meta-analysis results. Combining discovery and replication samples (N = 19,089; **Table 1**), each A allele of rs7980687 in *SBNO1* was robustly associated with 0.074 s.d. larger head circumference (95% confidence interval (CI) = 0.049 to 0.099; $P = 8.1 \times 10^{-9}$; explained variance = 0.24%), and each T allele of rs1042725 near *HMGA2* was associated with 0.065 s.d. smaller head circumference (95% CI = −0.085 to −0.045; $P = 2.8 \times 10^{-10}$; explained variance = 0.33%). This reflects differences of ~1.2 and ~1.0 mm in head circumference, respectively. The effect of each T allele of rs11655470 near *CRHR1-MAPT* did not reach genome-wide significance in the

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Received 22 June 2011; accepted 7 March 2012; published online 15 April 2012; doi:10.1038/ng.2238

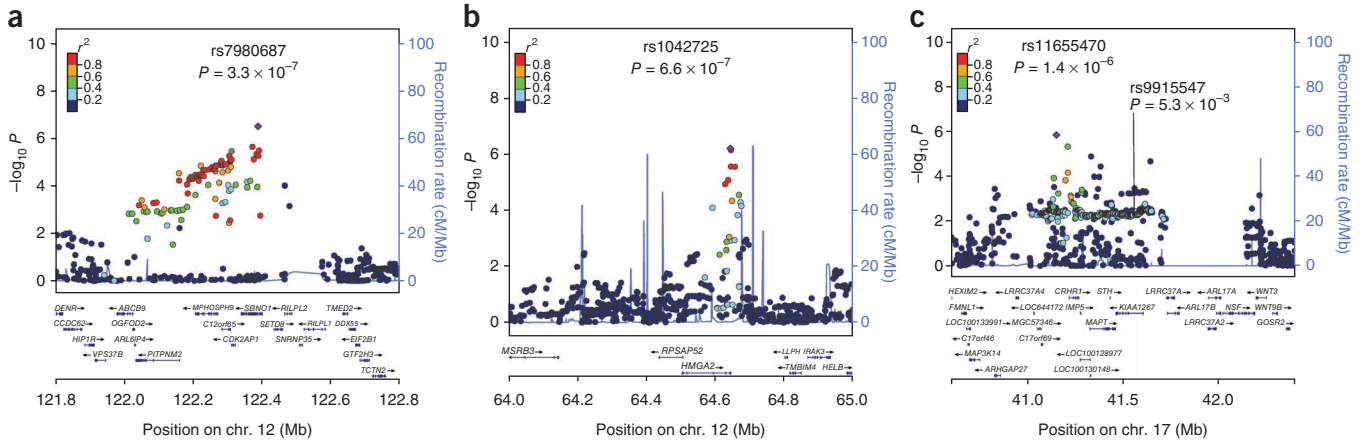

Figure 1 Regional association plots of the three lead signals. (**a**–**c**) Directly genotyped and imputed SNPs are plotted using filled circles with their meta-analysis *P* values ($-\log_{10}$ values) as a function of genomic position (NCBI Build 36). In each plot, the discovery stage SNP taken forward to replication is represented by a purple diamond (defining a global meta-analysis *P* value). Local LD structure is reflected by the plotted estimated recombination rates (from HapMap) in the region around the associated SNP and its correlated proxies. The correlations of the lead SNP to other SNPs at the locus are indicated by color. The recombination rates (light blue line, second *y* axis) are superimposed on the plot. Gene annotations are shown as dark blue arrows. Regional association plots are shown for the 12q24.31 (**a**), 12q15 (**b**) and 17q21.1 (**c**) loci. rs9915547 ($r^2 = 0.22$ with rs11655470 in HapMap CEU) is indicated in **c** downstream of the main signal and showed association with genome-wide significance with adult intracranial volume ($P = 1.5 \times 10^{-12}$)[2]. Regional plots were drawn using LocusZoom software[36].

combined analysis (effect of 0.048 s.d. larger head circumference; 95% CI = 0.028 to 0.068; $P = 3.8 \times 10^{-6}$; explained variance = 0.21%). These three associations showed low heterogeneity ($P > 0.1$, heterogeneity statistic (I^2) = 5–33%).

Additionally, the signals in *SBNO1* and near *HMGA2* but not the one near *CRHR1-MAPT* were associated with height measured at the same time as head circumference (**Supplementary Table 4**). When we adjusted the model for current height, the associations of rs7980687 and rs1042725 with head circumference were slightly attenuated (effect size of 0.057 s.d.; 95% CI = 0.035 to 0.080; $P = 3.8 \times 10^{-7}$ and effect size of −0.048 s.d.; 95% CI = −0.066 to −0.030; $P = 1.3 \times 10^{-7}$ for rs7980687 and rs1042725, respectively; **Supplementary Table 5**). The association of the third signal near *CRHR1-MAPT* was unaffected. In-depth mediation analysis showed that the effects of rs7980687 and rs1042725 on head circumference were only partly explained by height (12% and 24%, respectively) (**Supplementary Fig. 2** and **Supplementary Table 6**). The effect of rs11655470 was a completely direct effect of the SNP on head circumference (**Supplementary Table 6**). To further adjust for possible population stratification, we added principal components to the model in cohorts where these measures were available (total $N = 12{,}763$). This did not materially change the effect on head circumference, indicating that the association tests used were robust to population stratification (**Supplementary Table 7**). The three variants were not associated with other covariates, such as breastfeeding, socioeconomic status or educational level (data not shown). We did not find evidence for an interaction of these variants with infant sex or breastfeeding after Bonferroni correction ($P > 0.017$; **Supplementary Tables 8** and **9**).

Table 1 Individual association results by study and meta-analysis

Study type	Study	Year(s) of birth	Median age (months)	Total (*N*)	Male (%)	rs7980687[A] at 12q24 (*SBNO1*)				rs1042725[T] at 12q15 (nearest gene *HMGA2*)				rs11655470[T] at 17q21 (nearest genes *CRHR1-MAPT*)			
						MAF	*β*	S.E.	*P*	MAF	*β*	S.E.	*P*	MAF	*β*	S.E.	*P*
	ALSPAC (D)	1991–1992	18.9	1,748	53	0.19	0.105	0.038	6×10^{-3}	0.47	−0.071	0.031	0.02	0.41	0.114	0.031	3×10^{-4}
	CHOP	2006–2010	18.5	1,008	59	0.20	0.041	0.058	0.48	0.48	−0.017	0.046	0.72	0.39	0.036	0.048	0.45
	COPSAC	1998–2001	18.1	369	49	0.19	0.083	0.086	0.33	0.47	−0.026	0.065	0.69	0.45	0.159	0.063	0.01
Discovery	Generation R	2002–2006	13.1	2,240	52	0.21	0.064	0.031	0.04	0.49	−0.059	0.026	0.02	0.42	0.060	0.026	0.02
	LISA (D)	1998–1999	11.8	357	56	0.21	−0.045	0.077	0.56	0.48	−0.059	0.060	0.33	0.39	0.068	0.061	0.26
	NFBC1966	1966	12.3	4,287	49	0.20	0.181	0.041	1×10^{-5}	0.49	−0.074	0.033	0.02	0.49	0.068	0.033	0.04
	RAINE	1989–1991	13.1	759	53	0.19	0.108	0.058	0.06	0.50	−0.179	0.043	4×10^{-5}	0.41	−0.001	0.044	0.09
Discovery meta-analysis				10,768			0.091	0.018	3.3×10^{-7}		−0.072	0.014	6.6×10^{-7}		0.070	0.015	1.4×10^{-6}
	ALSPAC (R)	1991–1992	18.9	3,163	51	0.20	0.042	0.030	0.16	0.49	−0.088	0.024	3×10^{-4}	0.40	0.044	0.024	6×10^{-4}
	DNBC	1996–2002	12.1	531	54	0.20	0.120	0.070	0.09	0.45	−0.049	0.058	0.40	0.45	0.060	0.058	0.30
Replication	EFSOCH	2000–2004	12.1	703	52	0.20	0.054	0.061	0.37	0.50	−0.019	0.046	0.67	0.41	0.027	0.046	0.56
	INMA	2004–2007	13.9	693	53	0.16	0.020	0.062	0.75	0.44	−0.029	0.045	0.52	0.36	0.022	0.046	0.64
	GINI+LISA (R)	1995–1999	11.8	698	51	0.21	0.020	0.060	0.74	0.50	−0.092	0.049	0.06	0.40	−0.070	0.050	0.16
	NFBC1986	1985–1986	12.0	2,533	48	0.22	0.082	0.035	0.02	0.49	−0.034	0.029	0.25	0.50	0.019	0.287	0.51
Replication meta-analysis				8,321			0.055	0.018	2.5×10^{-3}		−0.058	0.015	8.3×10^{-5}		0.025	0.015	0.093
Overall meta-analysis				19,089			0.074	0.013	8.1×10^{-9}		−0.065	0.010	2.8×10^{-10}		0.048	0.010	3.6×10^{-6}

MAF, minor allele frequency; S.E., standard error; D, discovery cohort; R, replication cohort. *β* reflects differences in head circumference s.d. score per minor allele (additive model). *P* values are obtained from linear regression of each SNP against the head circumference s.d. score (additive model). All study samples were of European descent.

Table 2 Association of the three lead signals related to head circumference with other phenotypes

	Head circumference in third trimester of pregnancy (s.d. score)				Head circumference at birth (s.d. score)				Intracranial volume (ml)				
Marker	Total (*N*)	*β*	S.E.	*P*	Total (*N*)	*β*	S.E.	*P*	Total (*N*)	Mean age at measurement (years)	*β*	S.E.	*P*
rs7980687[A] at 12q24	3,781	0.089	0.029	1.9×10^{-3}	17,330	0.050	0.012	5.2×10^{-5}	8,175	67.5	0.72	2.03	0.72
rs1042725[T] at 12q15	3,781	−0.075	0.023	9.9×10^{-4}	17,074	−0.031	0.010	1.9×10^{-3}	8,175	67.5	−7.18	1.61	8.8×10^{-6}
rs11655470[T] at 17q21	3,781	0.049	0.024	0.037	17,695	0.030	0.010	2.0×10^{-3}	8,175	67.5	3.54	1.69	0.036[a]

S.E., standard error. *β* reflects differences in the head circumference s.d. score per minor allele or differences in intracranial volume per minor allele (additive model). *P* values are obtained from linear regression of each SNP and sex against the head circumference s.d. score in fetal life (additive model); SNP, sex and gestational age against birth head circumference s.d. score at birth (additive model); and SNP, age and sex against Intracranial volume (additive model)[2]. All study samples were of European descent.
[a]A variant further downstream (rs9915547; $r^2 = 0.22$ with HapMap CEU) showed association at genome-wide significance ($P = 1.5 \times 10^{-12}$) with adult intracranial volume[2].

In order to further investigate the effect of the three lead signals on fetal head growth, we assessed the associations of the variants with head circumference using third trimester fetal ultrasound data ($N = 3,781$) and head circumference measured at birth ($N = 17,695$) in discovery and replication cohorts that had these data available (**Supplementary Table 2**). All three signals showed evidence of association with head circumference in the third trimester of pregnancy and at birth (**Table 2**). The directions of the effects were consistent with those in infancy.

Next, we assessed the associations of the three lead signals with intracranial volume (ICV) in adulthood, measured by magnetic resonance imaging (MRI) in 8,175 individuals in the CHARGE Consortium[2]. There was evidence of association between the signals near *HMGA2* and *CRHR1-MAPT* and ICV (**Table 2**). For the signal near *CRHR1-MAPT*, a variant further downstream (rs9915547; $r^2 = 0.22$ in the HapMap Utah residents of Northern and Western European ancestry (CEU) population) showed an association at genome-wide significance ($P < 5 \times 10^{-8}$). All directions of the effects were consistent with the observed associations for head circumference in infancy (**Table 2**).

We also assessed whether there were functional common variants in linkage disequilibrium (LD; $r^2 > 0.50$) with our three lead SNPs that were either nonsynonymous SNPs or expression quantitative trait loci (eQTLs). One variant, rs1060105, in high LD with our lead signal (rs7980687; HapMap $r^2 = 0.89$), was a nonsynonymous SNP located in exon 5 of *SBNO1* (missense mutation c.2186G>A (encoding p.Ser729Asn)). The rs1060105[A] minor allele was associated with increased head circumference in infancy (effect size = 0.081 s.d.; 95% CI = 0.048 to 0.115; $P = 2.4 \times 10^{-6}$ ($N = 10,768$)). The underlying mechanism for this is unknown. Considering that transcription regulation is highly cell type specific, we next evaluated whether we could find known eQTLs in brain tissue but did not find any eQTLs in publicly available brain expression data[19]. Subsequently, we also explored eQTL databases from other tissues and identified three SNPs in LD with rs7980687 ($r^2 > 0.7$ with HapMap CEU) associated with gene transcript expression of *CDK2AP1* and *MPHOSPH9* in liver tissue, monocytes and lymphoblastoid cell lines[20–22]. Little is known about these genes, except that both are involved in cell cycle regulation (**Supplementary Table 10**)[23,24].

To our knowledge, this is the first GWAS on head circumference in infancy. The top two signals associated with infant head circumference (rs7980687 in *SBNO1* and rs1042725 near *HMGA2*) have previously been associated with adult height[1]. Therefore, we also assessed the association between the 180 known height variants and head circumference during infancy[1]. A strong deviation from the null hypothesis of no association was observed on the quantile-quantile plot (**Supplementary Fig. 3**). Besides *SBNO1* and *HMGA2*, 23 other height variants were nominally associated with head circumference in infancy (**Supplementary Table 11**). After applying Bonferroni correction for multiple testing in this candidate gene analysis ($P < 2.8 \times 10^{-4}$), markers in or near *ZNFX1* ($P = 6.1 \times 10^{-6}$), *OR2J3* ($P = 1.8 \times 10^{-5}$) and *ZBTB38* ($P = 1.8 \times 10^{-4}$) still showed statistically significant association with head circumference in infancy.

The relative effect size of rs1042725 near *HMGA2* was similar for infant head circumference (0.065 s.d.) and adult height (0.060 s.d.). However, the effect size of rs7980687 in *SBNO1* on infant head circumference (0.074 s.d.) was considerably larger than for adult height (0.035 s.d.). As head size is correlated with total body size[25], it might be the case that the top two loci have a more general regulatory role in skeletal growth and bone development. It also could be possible that variants in *SBNO1* affect brain growth and concurrent head circumference or that they affect skull growth rather than skeletal growth. The *SBNO1* gene is involved in the Notch signaling pathway[26]. In *Drosophila melanogaster*, a similar gene (*sno*) is required for early embryogenesis, and absence of this gene leads to maldevelopment of the central nervous system[26]. In humans, *SBNO1* has been implicated in oncogenic processes[27,28].

The variant near *HMGA2* was one of the first to be associated with adult height. Deletions and truncations in the *HMGA2* gene in mice and humans have been associated with small and large stature[29,30]. The effect of *HMGA2* is similar for head circumference and adult height; thus, it seems likely that it has a more general role in skeletal growth.

The variant (rs11655470) in the promoter region of *CRHR1-MAPT* was also related to head circumference, although this signal did not reach genome-wide significance. rs11655470 lies within the 17q21 inversion but is not strongly correlated with the inversion ($r^2 = 0.22$ with HapMap CEU). The 900-kb region corresponding to the conversion contains several genes. The SNP is closely related to the *CRHR1* gene ($r^2 = 0.59$ with rs171440 in HapMap CEU). Variants in or near *CRHR1* have been associated with brain development and bone mineral density[31,32], although the underlying mechanisms are largely unknown. Another gene included in the 17q21 inversion is *MAPT* ($r^2 = 0.22$ with HapMap CEU). Both common variants and mutations in *MAPT* are known to be associated with Parkinson's disease and other neurodegenerative diseases[3–5,33,34]. Other genes in this region are *STH* (encoding saitohin) and *GRN* (encoding granulin). *STH* has been associated with progressive supranuclear palsy and increased risk of late-onset Alzheimer's disease[35,36]. Mutations in *GRN* have been shown to cause frontotemporal degeneration[37]. It might be the case that common genetic variants in or near *CRHR1-MAPT* affect early brain development by altering the stability and assembly of microtubules. In an accompanying paper, Ikram *et al.*[2] show that a correlated SNP in the same region (rs9303525; HapMap $r^2 = 0.22$ with rs11655470) is associated with adult intracranial volume with genome-wide significance. Because the LD between the variants is low, it is possible that they represent separate, independent effects on different phenotypes. When we adjusted the effect of rs11655470

on infant head circumference for the CHARGE Consortium ICV signal (rs9915547), the effect was attenuated but remained significant (0.059 s.d.; $P = 1.0 \times 10^{-5}$ and 0.037 s.d.; $P = 7.3 \times 10^{-3}$ before and after adjustment for rs9915547, respectively), suggesting that these signals both represent a third marker influencing both phenotypes (**Supplementary Table 12**). However, although the association attenuates after conditioning on the CHARGE Consortium ICV signal, the two signals might still independently mark different causal variants in the region, and the attenuation might be due to the weak LD between the two signals caused by proximity. The marker associated with head circumference is in low LD with the chromosome 17q21 inversion, whereas the CHARGE Consortium ICV signal is in high LD with the inversion. Therefore, it does not seem likely that the 17q21 inversion is causally related to infant head circumference. The biological mechanisms underlying these associations are largely unknown.

Our study highlights the early effect of variants in or near *SBNO1* and *HMGA2* on head circumference in fetal life and infancy and shows that a variant near *CRHR1-MAPT* is marginally associated with head circumference in infancy. Our findings suggest that the genetic variants in the *CRHR1-MAPT* region might link early brain growth with neurological disease in later life. Further research is needed to elucidate whether these variants influence brain growth and neurodevelopment in early life.

URLs. SIMBioMS, http://www.simbioms.org/; The International HapMap Project, http://hapmap.ncbi.nlm.nih.gov/; Growth Analyser 3.0, http://www.growthanalyser.org/; METAL, http://www.sph.umich.edu/csg/abecasis/metal/index.html; SNAP, http://www.broadinstitute.org/mpg/snap/; GTEx eQTL browser, http://www.ncbi.nlm.nih.gov/gtex/test/GTEX2/gtex.cgi; eqtl.uchicago.edu, http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/.

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

We thank A. Sayers for helpful discussions with respect to the conducted mediation analysis. Major funding for the research in this paper is from the Academy of Finland (project grants 104781, 120315, 129269, 1114194, 134839, 129287 and Center of Excellence in Complex Disease Genetics); Biocentrum Helsinki; Biocenter, University of Oulu, Finland; the British Heart Foundation; the Canadian Institutes of Health Research (grant MOP 82893); The Children's Hospital of Philadelphia (Institute Development Award); the Cotswold Foundation (Research Development Award); the Darlington Trust; the Dutch Asthma Foundation; the Dutch Ministry of the Environment; Erasmus Medical Center Rotterdam; Erasmus University Rotterdam; The European Community's Seventh Framework Programme (FP7/2007-2013), ENGAGE project and grant agreement HEALTH-F4-2007- 201413; Exeter National Health Service (NHS) Research and Development; Fundació La Marató de TV3 (Televisió de Catalunya); Helmholtz Zentrum Muenchen, the German Research Center for Environment and Health, Institute of Epidemiology I, Neuherberg; Instituto de Salud Carlos III (FIS PI081151 and PS09/00432); Institut für Umweltmedizinische Forschung (IUF) Düsseldorf; Marien-Hospital Wesel; the UK MRC (G0500539, G0600331, PrevMetSyn/Salve/MRC and G0600705); the Municipal Health Service Rotterdam; the National Health and Medical Research Council of Australia (403981 and 003209); the National Public Health Institute, Helsinki, Finland; the Netherlands Organisation for Scientific Research (NWO) and the Netherlands Organisation for Health Research and Development (ZonMw) (grants SPI 56-464-14192, 904-61-090, 904-61-193, 912-03-031, 480-04-004 and 400-05-717); the US NHLBI (grant 5R01HL087679-02 through the STAMPEED program (1RL1MH083268-01)); the US NIH (grant 1R01HD056465-01A1); the Peninsula NIHR Clinical Research Facility; the RAINE Medical Research Foundation; the Rotterdam Homecare Foundation; South West NHS Research and Development; Stichting Astmabestrijding; Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR) Rotterdam; Technical University Munich; the Telethon Institute for Child Health Research; UFZ–Centre for Environmental Research Leipzig–Halle; University Hospital Oulu, Finland; University of Bristol; University of Leipzig; the Wellcome Trust (project grant GR069224); the Western Australian DNA Bank; the Western Australian Genetic Epidemiology Resource and ZonMW (grant 21000074). Data exchange and deposition has been facilitated by the SIMBioMS platform. Personal funding was provided by the Dutch Kidney Foundation (C08.2251 to H.R.T.), the MRC UK (G0500539, PrevMetSyn and PS0476 to S. Das), a Sir Henry Wellcome Postdoctoral Fellowship (Wellcome Trust grant 085541/Z/08/Z to R.M.F.), a MRC New Investigator Award (MRC G0800582 to D.M.E.) and Wellcome Trust 4-year PhD studentships (WT083431MA to J.P.K. and WT088431MA to J.L.B.). I.P. and J.F.-B. are in part supported by the European Community's ENGAGE grant HEALTH-F4-2007-201413, A.T.H. is employed as a core member of the Peninsula NIHR Clinical Research Facility and V.W.V.J. is funded by the Netherlands Organisation for Health Research (ZonMw 90700303 and 916.10159). Detailed acknowledgments by study are given in the **Supplementary Note**.

AUTHOR CONTRIBUTIONS

Project design was carried out by H.R.T., J.P.B., F.G., M. Kerkhof, A.H., E.A.P.S., J.S., M.M.B.B., L.J.L., A.v.d.L., T.H.M., S.S., A.V.S., B.F., S.F.A.G., A.J.v.d.H., J.P.N., L.J.P., H.-E.W., C.D., M.I.M., G.H.K., X.E., A.T.H., M. Melbye, H.B., C.E.P., H.H., G.D.S., J.H., M.-R.J. and V.W.V.J. Sample collection and phenotyping was performed by H.R.T., M. Kaakinen, M.G., M. Kerkhof, M.A.I., K.B., B.L.K.C., J.E., F.D.M., I.P., J.S., S. Debette, M.F., V.G., S.S., M.W.V., J.F.-B., R.M.C., A.-L.H., C.I., A.M., J.P.N., A. Pouta., U.S., M.S., N.H.V., G.H.K., A.T.H., H.H., M.-R.J. and V.W.V.J. Genotyping was performed by R.M.F., L.J.B., J.L.B., C.E.K., S.J.L., N.K., M.M.-N., F.R., C.T., A.I.F.B., A.-L.H., M.L., W.L.M., J.P.N., L.J.P., A. Palotie, S.M.R., A.G.U., C.M.v.D., X.E., C.E.P., E.W. and M.-R.J. Statistical analysis was performed by H.R.T., B.S.P., E.T., S. Das, D.O.M.-K., N.M.W., M. Kaakinen, E.K.-M., J.P.B., R.M.F., F.G., D.L.C., M. Kerkhof, N.J.T., M.A.I., P.C., D.M.E., J.P.K., J.L., G.M., P.F.O., H.Y., S.S., B.F., S.F.A.G., U.S. and C.D. The manuscript was written by H.R.T., B.S.P., E.T., S. Das, D.O.M.-K., G.D.S., J.H., M.-R.J. and V.W.V.J. The EGG, EAGLE and CHARGE consortia provided the infrastructure for conducting the genome-wide association meta-analysis, collaboration and discussion.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Lango Allen, H. *et al.* Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature* **467**, 832–838 (2010).
2. Ikram, M.A. *et al.* Common variants at 6q22 and 17q21 are associated with intracranial volume. *Nat. Genet.* **44**, 539–544 (2012).
3. Simón-Sánchez, J. *et al.* Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat. Genet.* **41**, 1308–1312 (2009).
4. International Parkinson Disease Genomics Consortium. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet* **377**, 641–649 (2011).
5. Webb, A. *et al.* Role of the tau gene region chromosome inversion in progressive supranuclear palsy, corticobasal degeneration, and related disorders. *Arch. Neurol.* **65**, 1473–1478 (2008).
6. Ivanovic, D.M. *et al.* Head size and intelligence, learning, nutritional status and brain development. Head, IQ, learning, nutrition and brain. *Neuropsychologia* **42**, 1118–1131 (2004).
7. Castellanos, F.X. *et al.* Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder. *Arch. Gen. Psychiatry* **53**, 607–616 (1996).
8. Ivanovic, D.M. *et al.* Long-term effects of severe undernutrition during the first year of life on brain development and learning in Chilean high-school graduates. *Nutrition* **16**, 1056–1063 (2000).
9. Wiles, N.J. *et al.* Fetal growth and childhood behavioral problems: results from the ALSPAC cohort. *Am. J. Epidemiol.* **163**, 829–837 (2006).
10. Gale, C.R. *et al.* 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).
11. 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).
12. Fisch, R.O., Bilek, M.K., Horrobin, J.M. & Chang, P.N. Children with superior intelligence at 7 years of age: a prospective study of the influence of perinatal, medical, and socioeconomic factors. *Am. J. Dis. Child.* **130**, 481–487 (1976).
13. Smit, D.J. *et al.* Heritability of head size in Dutch and Australian twin families at ages 0–50 years. *Twin Res. Hum. Genet.* **13**, 370–380 (2010).

14. Williams, C.A., Dagli, A. & Battaglia, A. Genetic disorders associated with macrocephaly. *Am. J. Med. Genet. A* **146A**, 2023–2037 (2008).
15. Kaindl, A.M. *et al.* Many roads lead to primary autosomal recessive microcephaly. *Prog. Neurobiol.* **90**, 363–383 (2010).
16. Cox, J., Jackson, A.P., Bond, J. & Woods, C.G. What primary microcephaly can tell us about brain growth. *Trends Mol. Med.* **12**, 358–366 (2006).
17. Kumar, A., Girimaji, S.C., Duvvari, M.R. & Blanton, S.H. Mutations in STIL, encoding a pericentriolar and centrosomal protein, cause primary microcephaly. *Am. J. Hum. Genet.* **84**, 286–290 (2009).
18. Fredriks, A.M. *et al.* Continuing positive secular growth change in The Netherlands 1955–1997. *Pediatr. Res.* **47**, 316–323 (2000).
19. Gibbs, J.R. *et al.* Abundant quantitative trait loci exist for DNA methylation and gene expression in human brain. *PLoS Genet.* **6**, e1000952 (2010).
20. Veyrieras, J.B. *et al.* High-resolution mapping of expression-QTLs yields insight into human gene regulation. *PLoS Genet.* **4**, e1000214 (2008).
21. Zeller, T. *et al.* Genetics and beyond–the transcriptome of human monocytes and disease susceptibility. *PLoS ONE* **5**, e10693 (2010).
22. Schadt, E.E. *et al.* Mapping the genetic architecture of gene expression in human liver. *PLoS Biol.* **6**, e107 (2008).
23. Shintani, S. *et al.* p12(DOC-1) is a novel cyclin-dependent kinase 2-associated protein. *Mol. Cell. Biol.* **20**, 6300–6307 (2000).
24. Matsumoto-Taniura, N., Pirollet, F., Monroe, R., Gerace, L. & Westendorf, J.M. Identification of novel M phase phosphoproteins by expression cloning. *Mol. Biol. Cell* **7**, 1455–1469 (1996).
25. Saunders, C.L., Lejarraga, H. & del Pino, M. Assessment of head size adjusted for height: an anthropometric tool for clinical use based on Argentinian data. *Ann. Hum. Biol.* **33**, 415–423 (2006).
26. Coyle-Thompson, C.A. & Banerjee, U. The strawberry notch gene functions with Notch in common developmental pathways. *Development* **119**, 377–395 (1993).
27. Suzuki, C. *et al.* Identification of Myc-associated protein with JmjC domain as a novel therapeutic target oncogene for lung cancer. *Mol. Cancer Ther.* **6**, 542–551 (2007).
28. Radtke, F. & Raj, K. The role of Notch in tumorigenesis: oncogene or tumour suppressor? *Nat. Rev. Cancer* **3**, 756–767 (2003).
29. Ligon, A.H. *et al.* Constitutional rearrangement of the architectural factor HMGA2: a novel human phenotype including overgrowth and lipomas. *Am. J. Hum. Genet.* **76**, 340–348 (2005).
30. Zhou, X., Benson, K.F., Ashar, H.R. & Chada, K. Mutation responsible for the mouse pygmy phenotype in the developmentally regulated factor HMGI-C. *Nature* **376**, 771–774 (1995).
31. Rivadeneira, F. *et al.* Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. *Nat. Genet.* **41**, 1199–1206 (2009).
32. 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).
33. Wolfe, M.S. Tau mutations in neurodegenerative diseases. *J. Biol. Chem.* **284**, 6021–6025 (2009).
34. Samaranch, L. *et al.* The effect of MAPT H1 and APOE epsilon4 on transition from mild cognitive impairment to dementia. *J. Alzheimers Dis.* **22**, 1065–1071 (2010).
35. 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).
36. 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).
37. 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).

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ONLINE METHODS

Stage 1: GWAS meta-analysis of head circumference. *Discovery samples, genotyping and imputation.* We selected seven population-based studies with head circumference measured in infancy (study cohort–specific median age range of 11–18 months) and GWAS data available by the beginning of March 2010 (combined $N = 10{,}768$), including the Avon Longitudinal Study of Parents And Children (ALSPAC; $N = 1{,}748$); The Children's Hospital of Philadelphia (CHOP; $N = 1{,}008$); the Copenhagen Study on Asthma in Childhood (COPSAC; $N = 369$); The Generation R Study (Generation R; $N = 2{,}240$); the Lifestyle–Immune System–Allergy Study (LISA; $N = 357$); the Northern Finland 1966 Birth Cohort (NFBC1966; $N = 4{,}287$) and the Western Australian Pregnancy Study (RAINE; $N = 759$). Genotypes were obtained using high-density SNP arrays and were then imputed for ~2.4 million HapMap SNPs (Phase 2, release 21/22). The basic characteristics, exclusions (for example, samples of non-European ancestry), genotyping, quality control and imputation methods for each discovery sample are presented in **Supplementary Table 1**.

Statistical analysis within discovery samples. Head circumference was measured in infancy (age window: 6–30 months). If multiple measurements were available for one individual within this age range, the measurement taken closest to 18 months was used. Sex- and age-adjusted s.d. scores were constructed using Growth Analyser 3.0 (from the Dutch Growth Research Foundation) in each study separately[18]. The association between each SNP and head circumference was assessed in each study sample using linear regression of head circumference s.d. score against genotype, assuming an additive model. Imputed genotypes were only used where directly assayed genotypes were unavailable.

Meta-analysis of discovery samples. Data exchange was facilitated by the SIMBioMS platform[38]. Before meta-analysis, SNPs with MAF of <1% and poorly imputed SNPs (proper_info of ≤0.4 (SNPTEST) or R2 of ≤0.3 (MACH2QTL)) were filtered out. Fixed-effects meta-analyses were independently conducted by two investigators. Meta-analysis was performed using the METAL software package, and genomic control[39] was applied during the meta-analysis stage to adjust the statistics generated within each cohort (see **Supplementary Table 1** for individual study λ values; the discovery meta-analysis λ value was 1.043). Meta-analysis was performed using the inverse-variance method; a fixed-effects model was assumed. SNPs available in less than four discovery cohorts were excluded. Final meta-analysis results were obtained for 2,449,806 SNPs. We considered the top three lead signals (representing three distinct genomic regions on chromosomes 12 and 17) in the discovery analysis for follow-up in additional samples. The two loci on chromosome 12 reached the threshold of $P < 1 \times 10^{-6}$ and were therefore selected for replication, and the third locus on chromosome 17 was just above that threshold ($P = 1.4 \times 10^{-6}$) and was selected because of previous knowledge of a nearby association at genome-wide significance with intracranial volume described by Ikram *et al.*[2]

Stage 2: follow-up of three lead signals in additional samples. *Follow-up samples, genotyping and analysis.* We used six independent study samples (combined $N = 8{,}321$) to follow up the three lead signals from the GWAS meta-analysis (represented by index SNPs rs7980687, rs1042725 and rs11655470). Details of these study samples are presented in **Supplementary Table 2**. If the index SNP was unavailable, a closely correlated proxy was substituted (rs12322888 or rs12316131 for rs7980687 (HapMap $r^2 = 0.95$ for both SNPs); rs7970350 or rs1351394 for rs1042725 (HapMap $r^2 = 1$ and 0.91, respectively); rs12938031 for rs11655470 (HapMap $r^2 = 0.58$)). In three of the replication studies, the index SNPs were imputed from genome-wide genotype data (see **Supplementary Table 2**). Head circumference analysis was performed within each study sample as in the discovery phase.

Statistical analysis. *Meta-analyses of discovery and replication samples.* We performed fixed-effects inverse-variance meta-analyses of the head circumference association results for the three lead signals in the seven discovery samples and six replication samples combined. Fixed-effects meta-analyses were conducted independently by two investigators using RMeta in R (v.2.7.0). We used the Cochran Q test and the I^2 statistic[40] to assess evidence of between-study heterogeneity of effect sizes.

Informed consent (or parental consent, as appropriate) was obtained from all discovery and follow-up study participants, and study protocols were approved by the local ethics committees.

Analyses of potential confounders. To verify that the investigated lead SNPs were not associated with other covariates that could theoretically confound the observed associations with head circumference (including height, weight and age at measurement; sex; breastfeeding and maternal educational level), we used linear or logistic regression models to assess the association between each covariate and genotype in all discovery and replication samples. For height and weight, we constructed sex- and age-adjusted s.d. scores using Growth Analyser 3.0 in each study separately, similar to the head circumference s.d. score. To investigate possible effects of the three lead signals on head circumference through height, we first conducted linear regression analysis, with and without adjustment for height s.d. score. Next, we conducted a mediation analysis and assessed direct and indirect SNP effects (mediated through height) on head circumference for each of the signals using a seemingly unrelated regression model (STATA, StataCorp LP) or a simple path analysis model (MPLUS, Muthen & Muthen), which gave identical effect estimates. To investigate whether the associations between genotypes and infant head circumference were similar in the sexes, we repeated the analyses in males and females separately. Furthermore, we evaluated possible effect modification by breastfeeding status for each of the SNPs. Where possible, we performed meta-analysis on the results to assess overall evidence of association.

Analysis of fetal head circumference and intracranial volume. We explored associations of rs7980687, rs1042725 and rs11655470 with third trimester fetal head circumference and head circumference at birth, assuming an additive model using linear regression. Fetal head circumference was measured by ultrasound in three studies (combined $N = 3{,}781$ singleton pregnancies) in the third trimester of pregnancy (gestational age window of 27–36 weeks). Only one measurement per subject was included in the time window. If multiple measurements were available within this time frame, the one taken closest to the median gestational age of 32 weeks was used. We calculated gestational age–specific s.d. scores using previously published growth charts[41]. This analysis was adjusted for sex. Head circumference was measured at birth or within 31 days of life in 12 studies ($N = 13{,}775$; **Supplementary Table 2**). We created s.d. scores for head circumference within each of the cohorts and assessed the association with each SNP, adjusted for sex and gestational age. If head circumference was measured in the first month, we used gestational age at birth + age (weeks) at measurement in the first month. Combined effect estimates were calculated using fixed-effects meta-analyses.

We used the meta-analysis on intracranial volume in adults, measured by MRI, in the CHARGE Consortium[42] as a third additional phenotype. Data collection methods, phenotype definition, baseline characteristics and results of the meta-analysis are described elsewhere[2,43].

Analysis of known adult height variants with infant head circumference. We used the discovery meta-analyses to assess the associations of the previously identified 180 known adult height–associated loci[1] with head circumference in infancy, using the same model. We also determined whether very closely related SNPs (HapMap $r^2 > 0.95$) showed higher significance levels than the originally reported SNPs. SNPs with a P value lower than 2.8×10^{-4} (0.05/180) were considered significant.

Variance explained. To estimate the percentage of variation in birth weight explained by each of the associated loci, we obtained adjusted R^2 from univariate linear regression models of head circumference against genotype. We then calculated a mean value from all discovery and replication studies weighted by sample size.

Nonsynonymous SNPs and eQTLs. We assessed SNPs in LD with the three lead signals and looked for nonsynonymous SNPs or eQTLs to identify possible functional variants explaining the associations with head circumference. First, we used SNP Annotation and Proxy search (SNAP) developed by the Broad Institute to select all SNPs in LD ($r^2 > 0.50$) with our three lead signals. We used the 1000 Genomes Project Pilot 1 data set as the SNP data set for rs7980687 and rs1042725 and the HapMap Release 22 data set as the

SNP data set for rs11655470 ($r^2 > 0.50$). Next, we evaluated whether these SNPs were nonsynonymous using the dbSNP search engine from NCBI. To evaluate whether there were *cis* eQTLs in LD with our lead signals, we searched publicly available eQTL databases through the NCBI Genotype-Tissue Expression (GTEx) eQTL Browser and the eqtl.uchicago.edu genome browser. In total, these browsers search nine databases for eQTLs. Only *cis* associations (defined as genes within 1 Mb) that reached the *P*-value threshold for significance used in the original papers describing the gene expression data sets were considered (**Supplementary Table 10**). The statistics behind the eQTL analysis and calculation of the threshold for declaring significance of the associations are described in the published and validated eQTL data sets[20–22].

38. Krestyaninova, M. *et al.* A System for Information Management in BioMedical Studies–SIMBioMS. *Bioinformatics* **25**, 2768–2769 (2009).
39. Devlin, B. & Roeder, K. Genomic control for association studies. *Biometrics* **55**, 997–1004 (1999).
40. Higgins, J.P., Thompson, S.G., Deeks, J.J. & Altman, D.G. Measuring inconsistency in meta-analyses. *Br. Med. J.* **327**, 557–560 (2003).
41. Verburg, B.O. *et al.* New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. *Ultrasound Obstet. Gynecol.* **31**, 388–396 (2008).
42. 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).
43. Ikram, M.A. *et al.* Brain tissue volumes in the general elderly population. The Rotterdam Scan Study. *Neurobiol. Aging* **29**, 882–890 (2008).

doi:10.1038/ng.2238