The genetic architecture of structural left–right asymmetry of the human brain

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Left–right hemispheric asymmetry is an important aspect of healthy brain organization for many functions including language, and it can be altered in cognitive and psychiatric disorders. No mechanism has yet been identified for establishing the human brain’s left–right axis. We performed multivariate genome-wide association scanning of cortical regional surface area and thickness asymmetries, and subcortical volume asymmetries, using data from 32,256 participants from the UK Biobank. There were 21 significant loci associated with different aspects of brain asymmetry, with functional enrichment involving microtubule-related genes and embryonic brain expression. These findings are consistent with a known role of the cytoskeleton in left–right axis determination in other organs of invertebrates and frogs. Genetic variants associated with brain asymmetry overlapped with those associated with autism, educational attainment and schizophrenia. Comparably large datasets will likely be required in future studies, to replicate and further clarify the associations of microtubule-related genes with variation in brain asymmetry, behavioural and psychiatric traits.

The human brain is characterized by various population-level asymmetries on its left–right axis1, including an overall ‘torque’ whereby the left hemisphere extends posteriorly and ventrally relative to the right, a left–right difference in frontal–occipital gradients of cortical thickness2, and hemispheric differences of morphology around the Sylvian fissure. Many brain functions are also lateralized, including hand motor control and language which show left-hemisphere dominance in roughly 85% of people. Altered brain or behavioural asymmetries have been reported in various cognitive and psychiatric disorders1–3, which suggests that population-typical asymmetries are linked to optimal human brain function.

Behavioural and anatomical brain asymmetries are already apparent in utero4–8, which indicates an early genetic-developmental programme of brain left–right axis formation9,10. Studies of visceral organ development (the heart, stomach, liver, etc.) have revealed that the generation of population-level asymmetry requires at least three important steps in the early embryo11,12: (1) the breaking of bilateral symmetry to create a left–right axis in a consistent orientation relative to the anterior–posterior and dorsal–ventral axes, (2) the triggering of different patterns of gene expression on the left and right sides of early embryonic structures and (3) the translation of asymmetric gene expression into lateralized morphology and organ placement.

In principle, establishing an embryonic left–right axis requires chirality at some level, that is, key biomolecules or cellular structures that exist in only one of two possible mirror forms. Life on Earth is based on L-form amino acids rather than the mirror D-form, and this chirality carries through to the macrostructure and movement of primary cilia13–16, which help to create the left–right axis of the visceral organs in embryos17. However, hemispheric dominances for language and hand motor control do not typically reverse in people with situs inversus of the viscera, that is, reversal of the visceral organs on the left–right axis, when caused by mutations in genes that encode primary ciliary components18–20. This observation indicates that there are distinct and possibly organ-intrinsic mechanisms at play in brain development, but such mechanisms remain unidentified. Therefore the genetic origins of human brain asymmetry remain unknown.

Post mortem studies that contrasted gene expression between the left and right sides of the embryonic central nervous system have identified molecular pathways that may be involved, including that the two sides may transition through developmental stages slightly out of synchrony with each other21–23. However, these studies were necessarily based on data from just handfuls of samples, because of limited availability arising from appropriate ethical, legal and practical concerns. An alternative approach to identify genetic influences on brain asymmetry is to relate genomic variation in large population datasets to variation in adult brain asymmetry. Only three loci have previously been reported at a genome-wide significant level to affect variation in adult human brain asymmetry, in studies targeted at single features of temporal lobe anatomy, and they did not yield broader insights into biological pathways24–26. Here we made use of the unprecedented sample size available through the 2020 release of the UK Biobank magnetic resonance imaging (MRI) data, in combination with genome-wide genotype data, to perform the first multivariate, brain-wide genetic analysis of human brain anatomical asymmetry. The results from this analysis also allowed us to test whether genetic polymorphisms that are associated with variation in brain asymmetry are also associated with neurodevelopmental disorders or other behavioural and psychological traits, using publicly available genome-wide association scan (GWAS) summary statistics for these traits.

Results

Heritabilities and genetic correlations of brain regional asymmetry measures. For each of 32,256 participants with post-quality-control MRI and genetic data (Methods), and each of 73 bilaterally...
paired regional measures of brain structure, we calculated hemispheric asymmetry indexes (AI) as \(( \text{left} - \text{right})/((\text{left} + \text{right})/2)\) (for 33 cortical surface area AIs, 33 cortical thickness AIs and 7 subcortical volume AIs; Supplementary Table 1). The measures were derived from cortical parcellation and subcortical segmentation of structural brain images (Methods). All but one of the regional mean AIs were significantly different from zero, indicating population-level asymmetries (Bonferroni-corrected \(P < 0.05\), Supplementary Fig. 1 and Supplementary Table 2), consistent with previous reports\(^6\). For example, some language-related regions showed greater average left than right surface areas, including superior temporal and supramarginal cortex, and pars opercularis.

GCTA\(^8\) software was used to estimate the single-nucleotide polymorphism (SNP)-based heritability \((h^2)\) for each AI, that is, the extent to which variance in each AI was linked to common genetic variation over the entire genome (Methods). Forty-two AIs showed significant SNP-based heritabilities (false discovery rate (FDR)-corrected \(P < 0.05\)), that is, 28 of the surface area AIs, 8 cortical thickness AIs and 6 subcortical volume AIs (Fig. 1a and Supplementary Table 3), ranging from 2.2% for the AI of entorhinal cortical thickness to 9.4% for the AI of superior temporal surface area. The overall pattern was consistent with previous, twin-based heritability analyses\(^8\).

SNP-based genetic correlation analysis (again using GCTA software) indicated overlapping genetic contributions to some of the AIs (Fig. 1b, Supplementary Fig. 2 and Supplementary Tables 4–10). Within a small number of cortical regions (Supplementary Table 10), surface area and thickness AIs had negative genetic correlations, which indicates that variants can have antagonistic effects on surface and thickness asymmetries of these regions.

**Multivariate genome-wide association analysis.** We performed a multivariate (mv)GWAS for 9,803,522 SNPs, using meta-canonical correlation analysis as implemented in MetaPhat\(^37\), with the 42 AIs shown to clump mvGWAS results on the basis of linkage disequilibrium (LD), and identify lead SNPs (maximally associated SNPs) at multivariate associations of lead variants separately with left and right hemisphere measures are presented in Supplementary Table 11.

In addition, five lead SNPs affecting cortical surface area AIs were also associated with AIs of subcortical volumes (Table 1 and Fig. 3), and the locus on 17q21 was associated with asymmetries of cortical surface area, thickness and subcortical volume (Table 1). The univariate associations of lead variants separately with left and right hemispheric measures are presented in Supplementary Table 11.

**Functional annotations of genomic loci associated with brain asymmetry.** FUMA\(^{38}\) software applied three strategies to annotate candidate SNPs to genes at significantly associated loci (Methods): physical position, expression quantitative trait locus (eQTL) information and chromatin interactions (Supplementary Table 12 and Supplementary Figs. 4 and 5). Here we summarize and cite notable information on each of the lead SNPs:

Ten of the loci had annotations involving cytoskeleton-related genes: On lp33, rs6658111 is close to AL3564581, a pseudogene of MTRM14 (myotubularin related protein 14). On 2p23.3, rs62130503 is intronic to MAPRE3 (microtubule associated protein RP/EB family member 3a), and rs12617392 is a brain eQTL\(^{39}\) of MAPRE3. Located between these two SNPs on 2p23.3 is also AGBL5, which is a post-translational modulator of tubulin\(^{40}\). On 2q34, rs368536282 is close to MAP2 (microtubule associated protein 2), a well-known dendrite-specific marker of neurons\(^41\), previously implicated in left-handed by GWAS analysis\(^42\). On 6p21.33, rs7781 is in the 3′ untranslated region (UTR) of TUBB (tubulin beta class 1). On 12q13.12, rs10783306 is close to the alpha tubulin gene TUBA1B. This variant is also in high LD with a handedness-associated variant, rs1168884 \((r^2 = 0.89)\). On 14q23.1, two lead variants for two independent genomic loci, rs160459 and rs201816193, showed evidence for cross-locus chromatin interaction via the promoters of nearby genes in foetal cortex\(^42\) (Supplementary Fig. 4). The former is near to DACT1, a locus which has been reported to associate with superior temporal sulcus depth\(^43\), while the latter is close to DAAM1, which modulates the reorganization of the actin cytoskeleton and the stabilization of microtubules\(^44\). Two lead variants on 16q24.3, rs72813426 and rs111398992, are in introns of SPIRE2 and the tubulin gene TUBB3, respectively, both of which are key proteins in cytoskeleton organization\(^45\). rs111398992 is also in high LD with a handedness-associated variant, rs4550447 \((r^2 = 0.94)\). On 17q21.31 there were five independent lead SNPs: rs35908989 is intronic to MAPT which encodes microtubule-associated protein tau, and rs55983816, rs35853889 and rs586039055 are brain eQTLs\(^{39,45,46}\) of MAPT, while rs80103986 is in high LD \((r^2 = 0.91)\) with handedness-associated variant rs55974014. On 19p13.3, rs11672092 is intronic to the tubulin gene TUBB4A, and in high LD with rs66479618 \((r^2 = 0.88)\), another handedness-associated SNPs\(^46\).

The 11 other loci did not have obvious microtubule-associated annotations, but most had annotations related to brain phenotypes or development: On 3q24, rs2279829 is a cortical eQTL\(^{47}\) of ZIC4, which is involved in visual and auditory pathway development\(^48\). rs9307052 is on q22.1 is in high LD \((r^2 = 0.99)\) with the handedness-associated variant rs286582824. On 5q15, rs869219775 is close to NR2F1, which is involved in neural activity during cortical patterning\(^49\). On 6q22.31–q22.32, rs9385385 is close to NCOA7, a nuclear receptor co-activator with its most abundant expression in the brain\(^50\). On 7p14.3, rs6947352 is intronic to BBB9, which causes Bardet–Biedl syndrome when mutated, involving retinopathy and intellectual disability\(^51,52\). On 9q22.33, rs911934 is located in a region having a chromatin interaction with TRIM14 in adult cortex\(^53\) (Supplementary Fig. 4), a gene which may activate Wnt/b-catenin signalling and affects mesodermal versus ectodermal differentiation of embryonic stem cells\(^54\). On 10p14, rs41298373 is a predicted deleterious missense coding variant in ITIH5, which was previously reported to affect planum temporale volumetric asymmetry\(^55\). Inter-alpha-trypsin inhibitor proteins are involved in extracellular matrix stabilization\(^56\). On 20p12.1, rs6135555 is in a region having a
chromatin interaction with the *FLRT3* promoter in neural progenitor cells\(^6\) (Supplementary Fig. 4), a gene which regulates axon guidance and excitatory synapse development\(^6\). On 21q22.3, rs7283026 is intronic to *COL18A1*, involved in neural tube closure and mutated in Knobloch syndrome\(^6\), which can include skull abnormalities. On 22q13.31, rs9615351 is an exonic variant of a gene involved in planar cell polarity, *CELSR1*\(^6\). On Xp22.33, rs12400461 is close to pseudogene *ASS1P4* and upstream of *MXRA5*; the latter encodes a matrix remodelling-associated protein and is implicated in autism\(^4\).

**Fig. 1 | SNP-based heritability and correlation analysis of regional brain asymmetry measures.** a, SNP-based heritability estimates for brain asymmetry measures. Only regions for which AIs were significantly heritable are indicated in colour. b, Genetic and phenotypic correlations between AIs. Phenotypic (upper right triangle) and genetic (lower left triangle) correlations between each pair of AIs. Only significantly heritable AIs that also have at least one significant phenotypic or genetic correlation after FDR correction are shown. The colours of the squares indicate the correlation coefficients according to the colour key, and their areas are proportional to the correlation coefficients.
To further link asymmetry-associated SNPs to genes, the MAGMA software was used to perform genome-wide gene-based association analysis65 based on the results from mvGWAS. There were 57 significant genes at Bonferroni-corrected \( P < 0.05 \) (Supplementary Fig. 6 and Supplementary Table 13). Five of these genes were previously associated with handedness46: MAP2, FAM13A, TUBA1B, TUBB3 and CRHR1. Forty-three of the 57 genes have been reported to associate with educational attainment52 and 15 with intelligence76 (Supplementary Table 14). For the proteins encoded by the 57 genes, there were 80 known or putative pairwise interactions in the STRING database65, compared with 8 interactions expected for a random set of this size from the whole proteome \( (P < 1 \times 10^{-16}) \). This observation supports the validity of our mvGWAS association findings, as random noise would not lead to such functional clustering. Microtubule-related genes (for example, MAP2, MAPT, SPIRE2 and TUBA1A) linked different clusters together in the largest protein interaction network (Fig. 4a).

We also used the genome-wide, gene-based \( P \) values for functional enrichment analysis using MAGMA65, in relation to 7,343 Gene Ontology ‘biological process’ sets defined within the MSigDB69 database. The gene set ‘regulation_of_microtubule_binding’ \( (P = 3.73 \times 10^{-10}) \) showed significant enrichment (adjusted \( P < 0.05 \), Bonferroni correction, Supplementary Table 15). Significant enrichment within various microtubule-related sets, such as ‘microtubule_cytoskeleton_organization’ \( (P = 2.19 \times 10^{-7}) \) and ‘microtubule_based_process’ \( (P = 2.36 \times 10^{-6}) \), was also found when using the list of single closest genes (Table 1) to the 27 lead SNPs (Supplementary Table 16). Enrichment in microtubule-related sets was not reported in a recent GWAS of bilaterally averaged cortical surface area and thickness measures in 51,665 individuals70, suggesting a particular involvement in hemispheric asymmetry rather than bilateral measures. We observed no statistically significant relation of our gene-based association \( P \) values with differential expression across cell types (Methods).

Testing our genome-wide, gene-based \( P \) values with respect to human gene expression data from the BrainSpan database, from either 29 age groups or 11 defined developmental stages, we found relatively higher mRNA expression of brain-asymmetry-associated genes during early-prenatal \( (P = 4.27 \times 10^{-3}) \) and early–mid-prenatal \( (P = 9.37 \times 10^{-4}) \) stages, from 9 \( (P = 1.84 \times 10^{-3}) \) to 24 \( (P = 7.36 \times 10^{-4}) \) post-conceptual weeks (FDR-corrected \( P \) values <0.05) (Fig. 4b,c and Supplementary Table 17). This is consistent with the fact that various anatomical asymmetries of the brain are already visible in utero8,19, and supports the existence of an early developmental mechanism for establishing the brain’s left–right axis14,32,72.

Genetic overlap of brain asymmetry with other traits. We next used iSECA software2 to perform genetic overlap analysis with our mvGWAS results in relation to GWAS summary statistics from neurodevelopmental disorders, behavioural and psychological traits which have been reported to associate phenotypically with aspects of structural and/or functional brain asymmetry: attention deficit hyperactivity disorder54–57, autism spectrum disorder15,58–61, educational attainment66,83,84, handedness85–87, intelligence62,88 and schizophrenia58–61. There was evidence for genetic overlap between brain asymmetries and autism \( (P = 0.005) \), educational attainment \( (P = 0.001) \) and schizophrenia \( (P = 0.002) \) which remained significant at Bonferroni-corrected \( P < 0.05 \) (Fig. 5, Supplementary Figs. 7 and 8 and Supplementary Table 18). In other words, SNPs that showed lower (more significant) association \( P \) values in our mvGWAS for brain asymmetry showed a statistically significant tendency to also show lower \( P \) values in previous, large-scale GWAS of autism, educational attainment and schizophrenia. Although we did not observe genetic overlap of brain asymmetry with handedness at a genome-wide level, we did note individual loci in common between these traits (above). In addition, we found no overlap between our mvGWAS results and those from a previous GWAS of intracranial volume in 32,438 participants64 (Supplementary Table 18, Supplementary Figs. 7 and 8), which again indicates that the genetic architecture of brain asymmetry is largely distinct from brain size.

Validation of lead SNPs associated with brain asymmetry. To achieve a reasonable level of genetic homogeneity in our mvGWAS, we had excluded any individuals not annotated as having ‘white British ancestry’ (through a combination of self-report and clustering based on principal components that capture major axes of ancestral diversity from the genotype data89 (Methods)). The UK Biobank includes additional participants who self-identify as being ‘white’, but who did not self-identify as British, or did not cluster genetically with the bulk of the ‘white British’ ancestry participants (Methods). After applying the same quality control criteria to these additional participants as in our discovery mvGWAS (except with respect to ancestry), and imposing the extra criterion that relatedness kinship coefficients should not be greater than 0.0442 with any participants from the discovery mvGWAS, data were available from 3,600 participants for an independent replication set. We tested each of the 27 lead SNPs from the mvGWAS in the replication set, using the same approach as the mvGWAS analysis, except that 40 genetic principal components were used as covariates to control for the greater degree of ancestral diversity in the replication set.

Ten of the 27 independent lead SNPs from the discovery mvGWAS showed association \( P \) values <0.05 on multivariate testing in
| Genomic locus | Lead SNP   | Position | Functional category | Effect allele | mvGWAS r value | mvGWAS P value | Nearest gene                                      | Central asymmetry indexesa |
|---------------|------------|----------|---------------------|---------------|----------------|----------------|--------------------------------------------------|---------------------------|
| 1             | rs6658111  | 1p33     | Intergenic          | G             | 0.37           | 9.75 × 10⁻¹¹  | AL356458.1                                      | Parahippocampal (SA), superior frontal (SA), parahippocampal (CT) |
| 2             | rs62130503 | 2p23.3   | NcRNA_intronicb     | T             | 0.05           | 1.22 × 10⁻¹⁰  | MAPRE3                                          | Thalamus (SUB), parahippocampal (SA) |
| 3             | rs12617392 | 2p23.3   | Intrinsic           | A             | 0.44           | 4.02 × 10⁻¹⁰  | CGREF1                                          | Inferior temporal (SA), caudal anterior cingulate (SA), isthmus of cingulate (SA) |
| 4             | rs2279829  | 3q24     | 3’ UTR²             | T             | 0.22           | 1.26 × 10⁻⁹   | ZIC4                                            | Isthmus of cingulate (CT), precuneus (SA), posterior cingulate (CT), fusiform (SA) |
| 5             | rs9307052² | 4q22.1   | Intrinsic           | T             | 0.11           | 2.27 × 10⁻⁸   | FAM13A                                          | Rostral anterior cingulate (CT), posterior cingulate (CT), medial orbitofrontal (SA) |
| 6             | rs869219775| 5q15     | Intergenic          | T             | 0.14           | 3.06 × 10⁻³   | NR2F1                                           | Inferior parietal (SA), transverse temporal (SA) |
| 7             | rs7781     | 6p21.33  | Downstream          | G             | 0.24           | 1.62 × 10⁻¹⁰  | TUBB                                            | Isthmus of cingulate (CT), rostral anterior cingulate (CT), pars triangularis (SA) |
| 8             | rs9385385  | 6q22.31  | ncRNA_intronic³     | T             | 0.45           | 1.37 × 10⁻⁸   | NCOA7                                           | Posterior cingulate (CT), pericalcarine (SA) |
| 9             | rs6947352  | 7p14.3   | Intrinsic           | A             | 0.31           | 4.38 × 10⁻³   | BBS9                                            | Banks of the superior temporal sulcus (SA) |
| 10            | rs911934   | 9q22.33  | Intergenic          | G             | 0.70           | 2.39 × 10⁻⁸   | GALNT12                                         | Inferior parietal (SA), isthmus of cingulate (SA), precuneus (SA), paracentral (SA), supramarginal (SA), entorhinal (CT) |
| 11            | rs41298373 | 10p14    | Exonic              | A             | 0.10           | 4.75 × 10⁻³⁸  | ITIH5                                           | Superior temporal (SA), parahippocampal (SA), fusiform (SA), inferior temporal (CT), transverse temporal (SA) |
| 12            | rs10783306³| 12q13.12 | Intergenic          | C             | 0.33           | 9.99 × 10⁻¹²  | TUBA1B                                          | Superior frontal (SA), entorhinal (SA), medial orbitofrontal (SA), pars triangularis (SA) |
| 13            | rs160459   | 14q23.1  | Intergenic          | C             | 0.46           | 4.98 × 10⁻¹²  | DACT1                                           | Banks of the superior temporal sulcus (SA), transverse temporal (SA), pericalcarine (SA) |
| 14            | rs201816193| 14q23.1  | Intergenic          | G             | 0.12           | 4.38 × 10⁻¹⁰  | DAAM1                                           | Isthmus of cingulate (SA), cuneus (SA) |
| 15            | rs72813426 | 16q24.3  | Intrinsic           | G             | 0.24           | 2.45 × 10⁻¹⁴  | SPIRE2                                          | Paracentral (SA), isthmus of cingulate (SA), middle temporal (SA) |
| 16            | rs111398992²| 16q24.3  | Intrinsic           | T             | 0.13           | 5.99 × 10⁻¹⁵  | TUBB3                                           | Isthmus of cingulate (CT), fusiform (SA), rostral anterior cingulate (CT), pericalcarine (SA) |

Continued
the replication set (Supplementary Table 19). The combined $P$ value of the remaining 17 lead SNPs was $P = 3.3 \times 10^{-4}$ in the replication set (calculated by Stouffer’s method), which we confirmed by permutation with respect to 10,000 repeat random samplings of 17 SNPs from the whole genome in the replication set (permutation-based $P = 4 \times 10^{-4}$). This indicates that the limited sample size of the replication set, compared with the discovery set, did not provide adequate power to replicate at the level of some individual SNPs, but that in combination there was evidence for replication. Moreover, among the 17 SNPs that showed $P > 0.05$ on multivariate testing in the replication set, some showed association $P < 0.05$ on univariate testing of the specific central traits identified for those SNPs in the discovery mvGWAS (Supplementary Table 19). It is also worth noting that 4 of these 17 SNPs (or SNPs in high linkage disequilibrium with them) have been reported to associate with left-handedness at a genome-wide significant level ($P < 5 \times 10^{-8}$) (see above for details), which is an additional form of validation with respect to a phenotype related to brain asymmetry. As also mentioned above, the high degree of functional clustering of genes identified through gene-based association testing is another form of support for the association results in the mvGWAS.

Discussion

Despite the importance of asymmetry as an organizing feature of the human brain, the early developmental processes which establish its left–right axis are unknown. In this study, we carried out multivariate GWAS analysis which identified 21 genetic loci associated with different aspects of adult brain anatomical asymmetry. Functional annotation implicated genes particularly involved in microtubule organization and prenatal brain development. Our study therefore sheds new light on the molecular genetic foundations of human brain asymmetry. In addition, at the genome-wide level, SNPs associated with brain asymmetry overlapped with those associated with educational attainment, autism and schizophrenia, while five specific loci that are associated with both brain asymmetry and handedness were identified.

Previous studies in invertebrates and frog embryos have shown that the cytoskeleton plays a role in determining cellular chirality and asymmetrical patterning of other organs. Cellular chirality refers to directional biases in cellular morphology, position, rotation or migration, which arise because of the inherent chirality of intracellular macromolecules such as those composing the cytoskeleton, and also manifest in terms of the intracellular distributions
of organelles\textsuperscript{103}. For example, during early cell divisions in \textit{Xenopus} (frog) embryos, the cytoskeleton has been reported to mediate asymmetric intracellular protein localization, as protein transport molecules move along cytoskeletal tracks within cells\textsuperscript{104}. Thus the cytoskeleton can provide a directionally consistent, organ-intrinsic bias during embryonic development that acts as a determinant of morphological asymmetry\textsuperscript{97,98,105}, arising from fundamental aspects of molecular and cellular biology.

As our study associated cytoskeletal and embryonically expressed genes with variation in adult human brain asymmetry, it is possible that these genes are involved in the establishment of left–right asymmetry during early brain development, through a mechanism involving cellular chirality. As mentioned in Main, at least some aspects of human brain asymmetry appear to be uncoupled from the developmental pathway that leads to left–right organization of the visceral organs, which involves cilia and the nodal pathway.

A cytoskeletal-mediated mechanism of brain asymmetry may therefore be organ intrinsic\textsuperscript{97,98,105}, that is, distinct from other pathways that establish broader aspects of body asymmetry. In this study, we identified genetic loci that are associated with 42 heritable aspects of brain asymmetry through a multivariate, brain-wide approach. A multivariate approach can boost statistical power while achieving data reduction, compared with separate univariate analyses of individual brain traits\textsuperscript{37}. A single set of genome-wide association results, pertaining simultaneously to multiple aspects of brain asymmetry, was then taken forward into functional annotation and downstream analyses, such as testing for genetic overlaps with other traits. The multivariate approach therefore helped to detect and interpret key aspects of the genetic architecture of brain asymmetry, without the noise inherent in repeat univariate testing. An important challenge remained to identify the particular brain traits that drove the multivariate
associations at each locus, which was achieved in MetaPhat by decomposing associations into sets of 'central' traits on the basis of the Bayesian information criterion and canonical correlation $P$ values.

A consequence of the multivariate approach is that it does not yield univariate association effect sizes, and therefore mvGWAS results cannot be used for standard genetic correlation analyses, such as is performed with LD score regression. Therefore, we used...
A cytoskeleton-based origin of brain asymmetry would fit this scenario, as the cytoskeleton is essential for various fundamental functions in cellular biology, beyond axis formation\textsuperscript{113,114}. Previous, twin- and family-based analyses\textsuperscript{15-16} have reported heritabilities up to roughly 25% for some of the same asymmetry measures that we analysed in the present study, with an overall similar regional pattern. Higher heritabilities were found particularly for regions that are important in the language system (for example, superior temporal cortex) and limbic system (for example, medial temporal and cingulate cortex). Twin-based heritability is often measured to be higher than SNP-based heritability, which may be expected because SNPs are just one class of genetic variation, and also because twin studies can overestimate heritability when certain assumptions are not fully met\textsuperscript{115}. As twin studies have not indicated effects of shared environment on brain asymmetries\textsuperscript{15}, early developmental randomness is likely to cause most variation\textsuperscript{116}.

We did not correct for handedness as a covariate in our genetic analyses, as it is generally not advisable to correct for covariates which are themselves partly heritable. This is because biased genetic effects can be measured with respect to the target trait\textsuperscript{117} (in this case, brain asymmetry). Handedness cannot therefore be treated safely as a confound variable when analysing brain asymmetry. We were interested in any genetic effects associated with brain asymmetry, regardless of whether they might also be shared with other traits such as handedness. Having identified genetic variants associated with brain asymmetry, we then queried post hoc whether they have been reported as significant in previous GWAS of handedness in over 1 million people\textsuperscript{44}. We did not observe a significant genetic overlap between structural brain asymmetry and handedness at the genome-wide level, which again may be due to the relatively low SNP-based heritabilities of these traits, in combination with limited statistical power in the present sample size for this kind of analysis. However, five individual SNPs associated with both brain asymmetry and handedness were identified, which suggests that a significant genome-wide overlap might be detected when using a larger dataset in the future.

The UK Biobank currently includes by far the largest single imaging–genetic dataset available to the scientific community. A limitation of the present study is the lack of a large, age-matched replication sample with comparable homogeneity of ancestry to the discovery mvGWAS analysis. We included an independent replication sample of 3,600 individuals from the UK Biobank, with greater diversity of ancestry than the 32,256 individuals of the primary

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**Fig. 5 | Genetic overlaps between brain asymmetries and other traits.** a–c. Heatmap plots illustrating pleiotropic effects between brain asymmetries and autism (a), educational attainment (b) and schizophrenia (c), based on per-SNP GWAS \( P \) values for these traits from previous studies (Methods), in relation to the mvGWAS \( P \) values from the present study of brain asymmetries.
Articles

Nature Human Behaviour

Studies of left–right differentiation of the embryonic mammalian brain's left–right axis.

Participants.

This study was conducted under UK Biobank application 16066, with C.F. as principal investigator. The UK Biobank is a general adult population cohort. The UK Biobank received ethical approval from the National Research Ethics Service Committee North West-Haydock (reference 11/NW/0382), and all of their procedures were performed in accordance with the World Medical Association guidelines. Informed consent was obtained for all participants. We used the brain imaging data released in February 2020, and data availability and processing (described below) resulted in a final sample of 32,256 participants of white British ancestry for the primary GWAS analysis, together with the structural MRI data and genotype data from the same participants. The age range of these participants was 45 to 81 years (mean 63.77 years). 15,288 were male and 16,968 were female. An independent replication sample of 2,680 individuals was also drawn from the UK Biobank, who self-identified as white, but not British, or did not cluster genetically with the bulk of the ‘white British’ ancestry participants (see below). The age range of these participants was 45 to 80 years (mean 62.89 years), 1,574 were male and 2,026 were female.

Genetic quality control.

We downloaded imputed SNP genotype data from the UK Biobank data portal. The first excluded subjects with a mismatch of their self-reported and genetically inferred sex, with putative sex chromosome aneuploidies, or who were outliers on the basis of heterozygosity (principle-component-corrected heterozygosity >0.19) and genotype missingness (missing rate >0.05) as calculated by Bycroft et al. The primary analyses were restricted to participants with ‘white British ancestry’, which was defined by Bycroft et al. In ‘white British ancestry’ participants, we used a combination of self-report and cluster analysis on the basis of the first six principal components which capture genetic ancestry from the genome-wide genotype data. We randomly excluded one from each pair of remaining individuals who had a kinship coefficient >0.0442, as defined by Bycroft et al. Next, QCTOOL (v.2.6.0) and PLINK (v.1.06) were used to perform genotype quality control: excluding SNPs with minor allele frequency <1%, Hardy–Weinberg equilibrium test P value <1 × 10⁻⁷ and imputation INFO score <0.7 (a measure of genotype imputation confidence). We also excluded multi-allelic SNPs because most of the downstream software (below) could not handle them. This resulted in 9,803,522 bi-allelic variants.

SNP-based heritability and genetic correlation analysis within the UK Biobank data.

From the primary dataset, 9,516,074 autosomal variants with minor allele frequencies >1%, INFO score >0.7 and Hardy–Weinberg equilibrium P >1 × 10⁻⁷ were used to build a genetic relationship matrix using GCTA (v.1.20.1.2). Specifically for analyses using GCTA, we further excluded one random participant from each pair having a kinship coefficient higher than 0.025 based on the calculated genetic relationship matrix (as this analysis is especially sensitive to higher levels of relatedness), resulting in 30,315 participants. Genome-based restricted maximum likelihood (GREML) analyses were performed to estimate the SNP-based heritability for each AI, controlling for the above-mentioned covariates, and applying FDR 0.05 across the 73 AIs to define significantly heritable AIs. Bivariate GREML analysis was used to estimate genetic correlations between pairs of AIs, separately for cortical surface area, cortical thickness and subcortical volume AIs, with FDR correction at 0.05 for multiple testing.

Multivariate genome-wide association analysis. In mgGWAS, a single association test is performed for each SNP in relation to multiple traits simultaneously. We used MetaPhat software to perform mgGWAS analysis across asymmetries for cortical surface area, cortical thickness and subcortical volume, including only the 42 AIs that had shown significant SNP-based heritability. MetaPhat performs meta-analysis and correlation analysis, and uses univariate and multivariate statistics as input from each separate AI, which were derived under an additive genetic model while controlling for the above-mentioned covariates, using RGENSE software (v1.2). Thus our mgGWAS tested effectively for association with 42 traits. This approach estimates the linear combination of traits that is maximally associated as assessed for each SNP, while ensuring that there is no correct false-positive rate. A total of 9,803,522 SNPs (see further above) were used for mgGWAS, spanning all autosomes and chromosome X. Statistically significant SNPs were considered as those with P < 5 × 10⁻⁸ in mgGWAS, which is a widely

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used threshold to account for multiple testing over the whole genome, in the context of LD in European-descent populations. MetaPhat also uses systematic criteria to define central traits which make the greatest contributions to significant multivariate associations, on the basis of an iterative process to optimize multivariate model properties with reference to canonical correlation analysis $P$ values and the Bayesian information criterion.

For the lead SNPs at genome-wide significant loci (see below for how these were defined), we also performed post hoc analysis in which we examined their separate left and right hemispheric associations, using traits corresponding to the central AIs that were involved in the multivariate associations (Supplementary Table 1), again using BGENIE, an additive genetic model and covariates as described above.

As a sensitivity analysis, we re-ran the mvGWAS after excluding from the primary dataset 886 participants who had lifetime diagnoses of neurological conditions that could potentially disrupt brain structure (Supplementary Table 20). The significant mvGWAS loci were minimally affected by this exclusion (Supplementary Fig. 9).

Identification of genomic risk loci and functional annotations. FUMA (version v1.36), an online platform for functional annotation of GWAS results, was applied to the results from mvGWAS. A multi-step process, using default parameters, was used to identify distinct, significantly associated genomic loci, and independent lead SNPs within those loci. Briefly, on the basis of the pre-calculated LD structure from the 1000 Genomes European reference panel, SNPs with genome-wide significant mvGWAS $P$ values $<5 	imes 10^{-8}$ that had LD $r^2 < 0.6$ with any other were identified. For each of these SNPs, other SNPs that had $r^2 > 0.86$ with them were included for further annotation (see below), and independent lead SNPs were also defined among them as having low LD ($r^2 < 0.1$) with any others. If LD blocks of significant SNPs are located within 250kb of each other (default parameter), they are merged into one genomic locus. Therefore, some genomic loci could include one or more independent lead SNPs (Table 1). The major histocompatibility complex region on chromosome 6 was excluded from this process by default, because of its especially complex and long-range LD structure. Functional annotations were applied by matching chromosome location, base-pair position, reference and alternate alleles to databases containing known functional annotations, which were ANNOVAR categories, Combined Annotation–Dependent Depletion scores, RegulomeDB scores and chromatin state.

1. ANNOVAR categories identify SNPs on the basis of their locations with respect to genes, such as exonic, intronic and intergenic, using Ensembl gene definitions.
2. Combined Annotation–Dependent Depletion scores predict deleteriousness, with scores higher than 12.37 suggesting potential pathogenicity.
3. RegulomeDB scores integrate regulatory information from eQTL and chromatin marks, and range from 1a to 5, with lower scores representing more importance for regulatory function.
4. Chromatin states show the accessibility of genomic regions, and were labelled by 15 categorical states on the basis of five chromatin marks for 127 epigenomes in the Roadmap Epigenomics Project, which were H3K36me3, H3K4me1, H3K4me3 and H3K9me3 for SNP, FUMA calculated the minimum chromatin state across 127 tissue/cell-type in the Roadmap Epigenomics Project. Categories 1–7 are considered as open chromatin states. We also used FUMA to annotate independent significant SNPs and their candidate SNPs according to previously reported phenotype associations ($P < 5 	imes 10^{-8}$) in the National Human Genome Research Institute–European Bioinformatics Institute catalogue.

For a significant mvGWAS association in the major histocompatibility complex region (Table 1), we took the most significant individual SNP, rs778112, as the single lead SNP to represent this locus, and annotated it manually.

SNP-to-gene mapping. SNP-to-gene mapping at significant mvGWAS loci was performed with the STRING database (v11.0), separately for each analysis.

Gene-based association analysis. Genome-wide gene-based association analysis was performed using mvGWAS summary statistics as input into MAGMA (v1.08), using default parameters implemented in FUMA (SNP-wide mean model). This process examines the joint association signals of all SNPs within a given gene (including 50 kb upstream to 50 kb downstream of the gene), while considering the LD between the SNPs. SNPs were mapped to 20,146 protein-coding genes on the basis of National Center for Biotechnology Information build 37.3 gene definitions, and each gene was represented by at least one SNP. We applied a Bonferroni correction for the number of tested genes ($P < 0.05/20,146$).

Gene-set enrichment analysis. We used MAGMA, again with default settings as implemented in FUMA, to test for enrichment of association within predefined gene sets. This process tests whether gene-based $P$ values among all 20,146 genes are lower for those genes within pre-defined functional sets than the rest of the genes in the genome, while correcting for other gene properties such as the number of SNPs. A total of 7,343 gene sets, defined according to Gene Ontology biological processes, were tested from MSigDB version 7.0. In the main text we report the gene sets with $P$ values that met Bonferroni correction for multiple testing ($P < 0.05/7,343$).

In addition, we used the list of single closest genes to the 27 lead SNPs arising from the mvGWAS (Table 1) as input for gene set enrichment analysis, using the same 7,343 Gene Ontology biological process gene sets, but now on the basis of the hypergeometric test as implemented in GENE2FUNC of FUMA, which is appropriate for gene lists.

Finally, we used the CELL TYPE function (as implemented within FUMA) to test whether lower gene-based association $P$ values for brain asymmetry were associated with differential expression levels across cell types, using Bonferroni correction within each separate analysis with respect to each cell-type expression dataset included in FUMA.

Protein–protein interaction network. We used the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; http://string-db.org) for protein network analysis, using as input the names of 57 genes identified through gene-based association analysis, as described above. The STRING dataset includes protein–protein interaction information from numerous sources, including experimental data, publications and computational prediction methods. Only links with medium confidence or higher (confidence score > 0.4; default parameter) were retained.

Developmental stage analysis. Using the gene-based association $P$ values for all 20,146 genes genome wide, we used MAGMA (default settings as implemented in FUMA) to examine whether lower gene-based $P$ values tended to be found for genes showing relatively higher expression in BrainSpan gene expression data from any particular ages compared with all other ages, separately for 29 different age groups ranging from 8 postconceptional weeks to 40 years old, and 11 defined developmental stages from early prenatal to middle adulthood. We corrected for multiple testing through a FDR of 0.05 (separately for the two analyses).

Genetic overlap of brain asymmetry with brain disorders, behavioural and cognitive traits. We applied the iSECA toolbox that can test for genetic overlap on the basis of per-SNP association $P$ values only (mvGWAS does not produce univariate beta coefficient effect size estimates that can be used in standard genetic correlation analysis). We tested for genetic overlap in relation to traits previously reported to associate phenotypically with different aspects of brain structural asymmetry (Main), using GWAS $P$ values from previously published, large-scale studies: educational attainment ($n = 1,131,881$), handedness ($n = 331,037$), intelligence ($n = 269,867$), autism spectrum disorder ($n = 46,350$), attention deficit hyperactivity disorder ($n = 55,374$) and schizophrenia ($n = 82,315$).

We also tested for genetic overlap in relation to brain intracranial volume ($n = 1,383$). After LD-based pruning and clumping using default parameters, iSECA tests for pleiotropy between two sets of GWAS results using an exact binomial statistical test at each of 12 $P$ value levels: $P \leq 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1$. The analysis compares the expected and observed overlap of
in the subsets of SNPs at these levels from two GWAS (144 combinations in total). In other words, iSECA iterates through each of the 12 P value levels and counts the number of overlapping variants between two GWAS at each P value threshold, and compares that number with the number expected under the null hypothesis of no genetic overlap, using the exact binomial test. iSECA then counts up the number of comparisons with evidence of overlap at a nominally significant level of P ≤ 0.05. To assess the significance level of overlap, we generated 1000 datasets through permutations (default parameter), which contained all the possible combinations for a pair of traits, and determined whether the number of levels with nominally significant genetic overlap was significantly more than expected by chance. Finally, Bonferroni correction < 0.05 was applied for multiple testing of seven traits. Additionally, iSECA generated Q–Q plots for asymmetry mvGWAS P values conditioned on the other trait P values (for example, P ≤ 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0) to visualize whether there is an excess of pleiotropic SNPs, which should be visible as a leftward shift of the curve as the P value threshold becomes tighter (Supplementary Fig. 8).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data Availability
The primary data used in this study are available via the UK Biobank, https://www.ukbiobank.ac.uk. Other publicly available data sources and applications are cited in the Methods section. The GWAS summary statistics are made available online within the GWAS catalogue https://www.ebi.ac.uk/gwas/.

Code availability
This study used openly available software and code, specifically GCTA (https://csgenomics.com/software/gcta/#GREML), BGENIE (https://jmarchini.org/bgenie/), MetaPhat (https://sourceforge.net/projects/meta-phenome-association-tracer/), FUMA (https://fuma.cglab.nl/), MAGMA (https://cigc.cnrc.rinsoftware/magma, also implemented in FUMA) and iSECA (https://web.archive.org/web/20190326171900/https://neurogenetics.qmri.berghof.eu/iSECA/).

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Our web collection on statistics for biologists contains articles on many of the points above.

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Publicly available software and versions are stated in the Methods section together with the relevant citations.

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The primary data used in this study are available via the UK Biobank website www.ukbiobank.ac.uk. Other publicly available data sources and applications are cited in the Methods section.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size: Sample size of >30,000 participants was determined by data availability. This range of sample size has been successfully used in many genome-wide association scan studies of diverse human phenotypes.

Data exclusions: Brain imaging phenotypes were excluded at > 6 SD from the mean to reduce the chance of including spurious datapoints or extreme points that could bias statistical testing. Single nucleotide polymorphisms were excluded when they had population frequencies below 1%, as statistical testing can be unreliable below this frequency.

Replication: We included a replication sample of >3000 individuals as described in the paper.

Randomization: Not relevant, this was an observational study.

Blinding: Not relevant.

Reporting for specific materials, systems and methods

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|----------------------------------|---------|
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| ■ | Human research participants |
| ■ | Clinical data |
| ■ | Dual use research of concern |
| ■ | ChIP-seq |
| ■ | Flow cytometry |
| ■ | MRI-based neuroimaging |

Human research participants

Policy information about studies involving human research participants

Population characteristics: The Uk Biobank dataset has been extensively described before. We include demographic information in the paper for the specific subset used in our analysis (i.e. those with post-quality-control brain imaging and genetic data).

Recruitment: The Uk Biobank dataset has been extensively described before.

Ethics oversight: National Research Ethics Service Committee North West-Haydock (reference 11/NW/0382)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Magnetic resonance imaging

Experimental design

Design type: Structural T1 MRI

Design specifications: 1 structural scan per subject

Behavioral performance measures: Not relevant
**Acquisition**

| Description                  | Details                                                                 |
|------------------------------|-------------------------------------------------------------------------|
| Imaging type(s)              | Structural T1                                                          |
| Field strength               | 3T                                                                      |
| Sequence & imaging parameters| Siemens Skyra 3T and 32-channel RF receive head coil.                     |
|                              | http://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=2367                 |
| Area of acquisition          | Whole brain                                                            |
| Diffusion MRI                | [ ] Used                                                                |
|                              | [x] Not used                                                           |

**Preprocessing**

| Description                  | Details                                                                 |
|------------------------------|-------------------------------------------------------------------------|
| Preprocessing software       | http://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=2367                 |
| Normalization                | http://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=2367                 |
| Normalization template       | http://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=2367                 |
| Noise and artifact removal   | http://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=2367                 |
| Volume censoring             | http://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=2367                 |

**Statistical modeling & inference**

| Description                  | Details                                                                 |
|------------------------------|-------------------------------------------------------------------------|
| Model type and settings      | Meta-canonical correlation analysis as implemented in MetaPhat: https://www.frontiersin.org/articles/10.3389/fgene.2020.00431/full |
| Effect(s) tested             | Genetic effects on brain regional asymmetry measures, as described in the paper. |
| Specify type of analysis     | [ ] Whole brain   [x] ROI-based   [ ] Both                           |
| Anatomical location(s)       | Freesurfer Desikan atlas.                                               |
| Statistic type for inference | Region-based measures                                                   |
| Correction                   | The paper includes various different analyses and the multiple testing approach for each is described explicitly in the methods, either Bonferroni or FDR. |

**Models & analysis**

| Description                  | Details                                                                 |
|------------------------------|-------------------------------------------------------------------------|
| Involved in the study        | [x] Functional and/or effective connectivity                           |
|                              | [x] Graph analysis                                                      |
|                              | [ ] Multivariate modeling or predictive analysis                        |
| Multivariate modeling and predictive analysis | Meta-canonical correlation analysis was used to test for gene-brain associations as implemented in MetaPhat: https://www.frontiersin.org/articles/10.3389/fgene.2020.00431/full Covariates are defined in the Methods section. |