Shared heritability of human face and brain shape

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Evidence from model organisms and clinical genetics suggests coordination between the developing brain and face, but the role of this link in common genetic variation remains unknown. We performed a multivariate genome-wide association study of cortical surface morphology in 19,644 individuals of European ancestry, identifying 472 genomic loci influencing brain shape, of which 76 are also linked to face shape. Shared loci include transcription factors involved in craniofacial development, as well as members of signaling pathways implicated in brain–face cross-talk. Brain shape heritability is equivalently enriched near regulatory regions active in either forebrain organoids or facial progenitors. However, we do not detect significant overlap between shared brain–face genome-wide association study signals and variants affecting behavioral–cognitive traits. These results suggest that early in embryogenesis, the face and brain mutually shape each other through both structural effects and paracrine signaling, but this interplay may not impact later brain development associated with cognitive function.

The human cerebral cortex forms the outer layer of gray matter of the brain and underpins cognitive function. It is characterized by complex folding patterns varying between species and individuals1,2. Family- and twin-based studies indicate substantial heritability of brain shape3, and a recent genome-wide association study (GWAS) found that brain shape is highly polygenic with genetic correlations to a broad range of neuropsychiatric disorders and behavioral–cognitive phenotypes4. These studies focused on predefined, univariate measures of brain shape, such as total or regional surface area, extracted from structural magnetic resonance imaging (MRI) scans5, which cannot capture morphological complexities of the cortical surface. We recently developed a data-driven approach to phenotyping complex, multidimensional traits; this multivariate approach, when applied to facial surface images, revealed numerous loci with no previously known role in human face shape variation6. Here, we implemented this approach to discover associations between common genetic variants and brain shape, using MRI data from middle-aged participants in the UK Biobank (UKB) who were free of disease diagnosis.

In addition to sharing complex morphologies, the development of the brain and face is highly integrated due to shared developmental lineage, spatial proximity and signaling cross-talk between both structures7. Early in embryonic development, the rostral end of the ectodermally derived neural tube gives rise to the forebrain, which in turn gives rise to the cerebrum that encompasses the cerebral cortex8. Just before forebrain formation, a subset of neuroepithelial cells within the neural folds give rise to facial progenitor cells called cranial neural crest cells (CNCCs)9. Following specification, CNCCs undergo an epithelial-to-mesenchymal transition and migrate ventrally10, giving rise to most of the craniofacial skeleton and connective tissue11. Early brain growth rates can modulate both positioning and outgrowth of the facial prominences12–14, as well as induce flexion and bone deposition of CNCC-derived basi-cranial bones15–17 and neurocranial sutures18–20, respectively. Finally, paracrine factors secreted by either the developing forebrain21–23 or CNCCs24–26 modulate the facial or brain development, respectively.

These physical and molecular interactions have been detailed by studies in developing chick and mouse embryos, but are also supported by widespread co-occurrence of neurodevelopmental and craniofacial malformations in rare human syndromes27. This phenomenon was noticed by DeMyer et al.24 in 1964, who coined the phrase ‘the face predicts the brain’ to describe correlations between the severity of brain and face malformations in patients with holoprosencephaly. While in some cases this co-occurrence may be caused by pleiotropic gene functions, a number of human syndromes have been mapped to genes functioning in brain–face cross-talk through paracrine signaling28–31. Nonetheless, close developmental links between face and brain are underappreciated;

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whether and how they extend to common human genetic variation influencing brain and face shape is unknown.

**Results**

Multivariate genome-wide association study of brain shape. We adapted our previously published data-driven phenotyping approach1 to brain shape, as measured by MRI scans of 19,644 individuals in the UKB. Participants included were of primarily European ancestry, such that results do not pertain to cross-population differences in brain shape. We focused on the mid-cortical surface (midway between the white–gray matter interface and the pial surface with the cerebrospinal fluid, as extracted using FreeSurfer2), which we refer to as brain shape. Using mid-cortical surfaces represented by a mesh of three-dimensional (3D) vertices, the method segments brain shape in a global-to-local manner, yielding brain segments at different hierarchical levels of scale. Within each segment, principal-component analysis (PCA) is used to describe effects in multivariate shape space explaining between-individual variation, and canonical correlation analysis (CCA) is used to define, for each variant, the linear combination of principal components (PCs) maximally associated with SNP dosage. Unsurprisingly, a GWAS of left and right hemispheres from the same individuals showed highly concordant results (Supplementary Fig. 1); therefore, we performed subsequent analyses using left–right hemisphere averaged data.

Applying this pipeline to UKB MRI data defined 285 hierarchical segments (Fig. 1 and Supplementary Table 1), decomposing brain shape into different levels of detail, from larger brain segments with integrated variation, to smaller brain segments with local effects. Each hierarchical level is a partition of its parent; the first level consisted of the entire brain, while the second and third levels segmented the whole brain into halves and quadrants, respectively, and the final, ninth level resulted in numerous smaller segments (Fig. 1b). Many smaller segments from the seventh hierarchical level onwards were discarded due to small surface areas, resulting in fewer total segments than the 511 (29−1) expected. Nevertheless, the ninth hierarchical level yielded a substantial number (74) of retained segments; a tenth level would contribute few additional segments (Supplementary Fig. 2). The segmentation broadly agreed with the commonly used Desikan–Killiany3, Destrieux4 and Glasser5 brain atlases (Supplementary Fig. 3). Before GWAS, we adjusted for covariates including total brain volume, height, body mass index (BMI), sex and population structure, as well as performing standard SNP filtering and quality control (Methods). Applying linkage disequilibrium score regression (LDSC)-based heritability estimation to each segment’s GWAS (see Methods and Supplementary Note for details on extension to multivariate traits) yielded inter-cept values close to 1 (range across segments 0.987–1.007, mean 1.001; Supplementary Table 1), indicating minimal confounding by population structure or cryptic relatedness. In total, we conducted 285 multivariate GWASs using CCA, each corresponding to one segment. Around 38,630 SNPs showed genome-wide significant (P < 5 × 10−8) associations with brain shape in at least one segment; of these, 23,413 reached study-wide significance (P < 2.07 × 10−10 correcting for the number of effective GWASs, estimated by permutation; Methods) in at least one segment. Collapsing these SNPs into independent signals based on linkage disequilibrium (LD) and distance yielded 472 and 242 loci reaching genome-wide and study-wide significance, respectively (Supplementary Table 2). Most of the 472 loci showed effects on multiple segments (305/472, 65%), and many showed effects on multiple quadrants (158/472, 33%; Fig. 1 and Supplementary Table 2), consistent with global-to-local effects at multiple levels of brain shape. Masking of associations from progressively higher hierarchical levels revealed that segments from higher levels contributed a substantial fraction of associations; for example, segments beyond the first three levels contributed 169 and 55 loci reaching genome-wide and study-wide significance, respectively (Extended Data Fig. 1). Associations between the 472 loci and brain shape were depleted from the frontal lobe segments (except for the most anterior orbitofrontal cortex) and enriched in the occipital and temporal lobe segments (Supplementary Fig. 4), mostly in agreement with point-wise heritability estimates (Extended Data Fig. 2).

We assessed the overlap between the 472 loci and previous GWAS results of brain surface areas or subcortical volumes6–8. The 472 loci recapitulated 27–78% of the associations reported in previous studies; the highest overlap of 78% was reported in a recent study of univariate brain surface area1, the phenotype most comparable to the shape measures studied here (Table 1). Of the 472 loci, 121 overlapped with those reported in previous studies on brain surface area or subcortical volume, while 351 represent previously undescribed associations with brain morphology. To assess the reproducibility of the 472 loci on the same shape measures, we analyzed MRI data from the Adolescent Brain Cognitive Development (ABCD) study9. Of the 472 loci, 466 were tested for replication (Methods). At a false discovery rate (FDR) of 5%, we replicated at least one associated segment for 305 of 466 (65.4%) loci, and 2,645 of 3,586 (73.8%) locus–segment combinations (Supplementary Table 3). We observed consistent rates when subdividing based on the hierarchical level of the segments being replicated, albeit with a slight decrease in replication rate at higher levels (Extended Data Fig. 3). These replication rates are notable given the substantial age difference of the ABCD cohort (9–10 years versus 40–70 years in the UKB). The high reproducibility of GWAS results between the two cohorts suggests that, despite the known continued growth and morphological changes of the brain throughout adolescence and into adulthood10, many of the observed associations with brain shape originate during development and are maintained throughout life.

We next used functional mapping and annotation of GWAS (FUMA)11 and the genomic regions enrichment of annotations tool (GREAT)12 to identify pathways enriched among genes near the 472 loci, as well as curated gene panels used to guide disease diagnoses13 to identify disease associations (Methods). As expected, we found strong enrichment for brain-specific processes (neurogenesis, axonogenesis, neuron differentiation, nervous system development and neuron projection guidance), morphogenesis-related processes (anatomical structure morphogenesis and animal organ morphogenesis) and neurodevelopmental disorders (intellectual disability, malformations of cortical development and ciliopathies). We also observed a weak enrichment of terms related to formation and closure of the neural tube, suggesting that early developmental events impact adult brain shape. Surprisingly, we also observed strong enrichment of terms related specifically to CNCC development and migration, as well as weaker enrichment of broader terms encompassing skeletal system development, chondrogenesis and osteogenesis (Supplementary Data 1). Furthermore, we found strong and weak enrichments for craniosynostosis (premature closer of the cranial bone sutures) and clefting gene panels, respectively. These enrichments suggest a link between variation in brain shape and craniofacial skeletal development.

Loci affecting both brain and face shape. To more directly test for sharing of genetic effects between brain and face shape, we intersected the 472 loci described in this study with 203 loci previously associated with face shape in individuals of European ancestry through a similar, open-ended phenotyping approach1. Thirty-seven of the loci for brain shape were linked (r2 > 0.2) to at least one of the face shape loci, significantly above random expectation (P = 2.03 × 10−12, odds ratio = 10.6) and greater than the overlap with other traits that have similar numbers of genome-wide significant associations in the NHGRI-EBI GWAS Catalog1 (Extended Data Fig. 4). Identifying signals showing a genome-wide significant
association with one of brain or face shape and a suggestive ($P < 5 \times 10^{-7}$) association with the other resulted in 76 brain–face shared loci (Fig. 2a).

Genes near the 76 brain–face shared loci were strongly enriched for disease associations, including 'skeletal disorders' and 'hearing and ear disorders', consistent with the contribution of CNCCs to...
Table 1 | Overlap between previous GWAS results of brain surface areas or subcortical volumes with GWAS results of brain shape in this study

| Study                  | Number of loci tested | Number of lead SNPs with $P < 5 \times 10^{-8}$ | Number of proxy SNPs with $P < 5 \times 10^{-8}$ | Overlap (%) |
|-----------------------|-----------------------|-----------------------------------------------|-----------------------------------------------|-------------|
| Subcortical combined  | 65                    | 15                                            | 18                                            | 27.6        |
| Grasby et al.         | 301                   | 195                                           | 236                                           | 78.4        |
| Zhao et al.           | 494                   | 212                                           | 273                                           | 55          |

Subcortical combined refers to a combined set of loci from four studies of subcortical volume measures.

Phenotypically, these highlighted loci largely affect brain shape in the frontal and temporal lobes, and face shape in the forehead and nose, as exemplified by PAX3 and ALX1 (Fig. 2c), consistent with the physical proximity of the frontonasal prominence and the forebrain during development. Phenotypic effects distinct from this pattern include effects of variants near BMP4 and DLX6 on jaw and chin morphology, consistent with their known roles in mandibular development57,58, and effects of variants near PTCH1 on occipital lobe morphology (Fig. 2c). Together, these results suggest that both cell-intrinsic mechanisms and paracrine signaling pathways contribute to the substantial number of loci with shared associations with brain and face shape.

Genome-wide sharing of signals with neuropsychiatric disorders and behavioral–cognitive traits. We next asked whether the brain–face overlap among genome-wide significant loci held across the genome, also considering GWASs of neuropsychiatric disorders and behavioral–cognitive traits. LDSC can estimate genetic correlations between univariate traits using signed summary statistics59. However, this approach is not applicable to unsigned statistics yielded by CCA. We therefore applied an alternative method of assessing genome-wide sharing of signals between two GWASs, summarizing SNP $P$ values within approximately independent LD blocks and computing Spearman correlations between the two summarized profiles (Methods). When applied to pairs of univariate GWAS results, the Spearman correlation method was largely concordant with, albeit generally smaller in magnitude than, unsigned estimates of LDSC-estimated genetic correlations (Extended Data Fig. 5), indicating that it is a conservative, robust measure for quantifying genome-wide sharing of GWAS signals.

We first assessed sharing of association signals between 63 face segments and 285 brain segments (Supplementary Table 5). All four main facial quadrants, representing shape variation within the forehead, nose, lower face (mandible and cheeks) and philtrum, respectively, showed the most sharing with frontal lobe segments, particularly the most anterior portions such as the rostral prefrontal cortex, and the least sharing with parietal lobe segments (Fig. 3a). Furthermore, among the facial quadrants, the forehead and nose showed more sharing with frontal lobe segments than the philtrum and lower face. These genome-wide correlations are consistent with the phenotypic effects of top brain–face shared loci (Fig. 2c and Supplementary Fig. 5).

We next assessed sharing of signals with other brain-related traits. We used publicly available genome-wide summary statistics for a range of neuropsychiatric disorders, behavioral–cognitive traits and subcortical brain volumes from studies other than UKB, since our Spearman correlation measure does not control for sample overlap (Supplementary Table 6). As approximate negative controls, we used four immune-related diseases shown to have minimal genetic correlation with schizophrenia and bipolar disorder60. Subcortical volumes showed the most sharing with brain shape in the corresponding regions, but the magnitude of these correlations was relatively low (on par with sharing between brain and face shape), indicating that our multivariate GWAS approach detects effects beyond those resulting from changes in relative subcortical volume (Fig. 3b). We found that disorders with primarily developmental etiology showed substantial sharing with brain shape in regions previously linked to these disorders. For instance, schizophrenia and attention deficit hyperactivity disorder (ADHD) showed sharing with shape variation in the primary auditory and prefrontal cortex regions, respectively. In contrast, we did not observe this association for Alzheimer’s disease, caused by plaque buildup and neurodegeneration much later in life. Behavioral–cognitive traits such as intelligence, neuroticism and worry showed broader patterns of sharing with brain shape, reflecting the involvement of distributed cortical regions in these traits61–66 (Fig. 3b).

Interactions between face and brain can be architectural, with the forebrain acting as a structural support for facial development, and facial skeletal structures flexing to accommodate early brain growth4. However, these interactions can also involve paracrine signaling, with fibroblast growth factor (FGF), Hedgehog and bone morphogenetic protein (BMP) pathways known to mediate the signaling from the developing brain to the face20–22. Interestingly, morphogenetic protein (BMP) pathways known to mediate the signaling, with fibroblast growth factor (FGF), Hedgehog and bone (associated with CHAR syndrome51). Consistent with the primary factors (TFs) involved in neural crest formation and/or craniofacial development50, we next manually scanned mouse models (Supplementary Table 4). We observed that many of the shared brain–face loci included genes encoding transcription factors (TFs) involved in neural crest formation and/or craniofacial skeletal development. Some of those TFs (for example, DLX5/6, SOX9, ZEB2, ZIC2, ZIC3 and TCF4) have known functions in both neural crest and brain development, and this pleiotropy may account for the shared brain–face genetic signals. However, other shared brain–face signals are associated with TFs thought to function primarily during neural crest rather than brain development, and whose mutation causes specific craniofacial defects; those TFs include ALX1 and ALX4 (associated with frontonasal dysplasias4–10), TWIST1 (associated with Saethre–Chotzen syndrome4–10), PAX3 (associated with Waardenburg syndrome4) and TFAP2B (associated with CHAR syndrome4). Consistent with the primary role of these TFs in facial development, transcriptome analysis showed high expression in in vitro-derived human CNCCs and their chondrocyte derivatives4, but low or no expression in either glia or neurons of human forebrain organoids spanning a range of developmental stages (Fig. 2b). These observations suggest that genetic variants affecting key craniofacial TFs have a greater than previously appreciated impact on brain shape.

Interactions between face and brain can be architectural, with the forebrain acting as a structural support for facial development, and facial skeletal structures flexing to accommodate early brain growth4. However, these interactions can also involve paracrine signaling, with fibroblast growth factor (FGF), Hedgehog and bone morphogenetic protein (BMP) pathways known to mediate the signaling from the developing brain to the face20–22. Interestingly, genes encoding members of all three pathways, FGF (FGF13, FGF18 and SPRY2), Hedgehog (PTCH1 and BMP2 and BMP4) are among the shared brain–face loci. For example, mutations in PTCH1, encoding the receptor for the sonic hedgehog ligand, cause holoprosencephaly6, a congenital, structural forebrain anomaly with associated craniofacial malformations. Conversely, CNCCs secrete anti-BMP signaling molecules that modulate forebrain development4,6,10; expression of these BMP antagonists is dependent on the SIX family of TFs, whose perturbation in CNCCs leads to both craniofacial malformations and secondary pre-otic brain defects4–10. SIX1 and SIX4 are also among the 76 brain–face shared loci (Fig. 2a). Furthermore, genes linked to other signal -
Sharing between brain shape and the immune diseases was generally lower than with neuropsychiatric disorders, behavioral–cognitive traits or subcortical volumes, but reached significance for type 1 diabetes (T1D) and rheumatoid arthritis (RA; Fig. 3c). This overlap may be because these immune traits have genetic correlation with brain-related traits other than those tested previously (schizophrenia and bipolar disorder), as suggested by a significant genetic correlation between RA and intelligence (Extended Data Fig. 6).

Finally, we compared the degree to which face shape shares signals with neuropsychiatric disorders, behavioral–cognitive traits and subcortical volumes. Brain shape shares significant (5% FDR) signal with most neuropsychiatric traits, as well as all behavioral–cognitive and subcortical volume traits analyzed. In contrast, face shape does not show significant sharing with any of the neuropsychiatric disorders or behavioral–cognitive traits, and significant but weaker sharing with the subcortical volume measures (Fig. 3c). To confirm these patterns using univariate approaches, we performed a GWAS on the most heritable individual PCs of full brain or face shape and computed genetic correlations using LDSC. Although genetic correlation estimates were noisy due to low heritability of univariate shape GWAS, they agreed with our Spearman correlation measure, finding nonzero genetic correlations between both brain and face shape and subcortical volumes, and between brain shape and both autism spectrum disorder and bipolar disorder (Extended Data Fig. 7). Thus, the substantial sharing of signals between brain and face shape (Fig. 3a) appears to be mostly independent of

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**Fig. 2 | Loci affecting both brain and face shape.** a. Miami plot of GWAS results for brain (top) and face (bottom) shape. For each SNP, \( P \) values aggregated across all brain or face segments were plotted. All 76 loci reaching genome-wide significance (\( P < 5 \times 10^{-8} \)) in one study and genome-wide suggestive significance (\( P < 5 \times 10^{-7} \)) in the other are highlighted by unfilled circles. Right-tailed, one-sided \( P \) values were computed based on CCA chi-squared statistics; exact \( P \) values are available in Supplementary Table 4. Loci near candidate genes highlighted in the text and in b and c are labeled, generally on the side where they show greater significance of association. b. Expression (in transcripts per million, TPM) of candidate genes near brain–face shared loci in CNCCs of different passages, representing different stages of maturation, from early (postnatal day (P) 1) to late (P4) and their chondrocyte (Chond. D9) derivatives52 (left), and 3D forebrain organoids at various stages of differentiation53 (right), further sorted into glial or neuronal lineages or profiled as whole organoids. c. Regional phenotypic effects of four candidate loci, showing effects of linked SNPs on brain (left) or face (right) shape. Segments shown are of hierarchical level v; \( -\log_{10}(P) \) values are normalized to the maximum at each locus. Full face and brain images from all 76 brain–face shared loci corresponding to all hierarchical levels can be found online (Data Availability).
neuropsychiatric disorder risk and behavioral–cognitive traits, perhaps because mutual influences of face and brain shape on each other involve phenotypic effects on brain shape distinct from those influencing neuropsychiatric disorder risk and behavioral–cognitive traits.

**Cell types influencing brain and face shape.** Our results thus far suggest that a substantial fraction of brain shape variation is underpinned by face shape, but that these observed effects are largely independent of effects shared between brain shape and other cognitive traits. To test this idea further, we sought to identify the cell types most enriched for heritability of brain shape, face shape and other cognitive traits. Partitioning heritability into cell-type-specific functional annotations via stratified LD score regression (S-LDSC) can prioritize trait-relevant cell types, but was developed for univariate traits; we thus sought to extend the theoretical framework of S-LDSC to multivariate traits such as our brain and face shape GWAS. We demonstrated that when applying unstratified
Partitioned heritability enrichments based on cell-type-specific regulatory annotations. Heritability enrichment z-scores, as estimated by S-LDSC, of multivariate shape for the first seven face segments (a), multivariate shape for the first seven brain segments (b), excluding segment 4 which had low heritability, neuropsychiatric disorders (c), behavioral–cognitive traits (d) and subcortical volume measures (e). Heritability enrichments were estimated for annotations based on open chromatin (based on ATAC-seq), regulatory regions (based on ChIP-seq) of multiple histone modifications, or a combination of the two. Annotations for the indicated samples, representing in vitro-derived cell types, primary tissues, or a combination of both (see Methods for source papers), were added to the S-LDSC baseline model, and the resulting z-score was scaled by column to visualize relative enrichments between traits. 5% FDR based on unscaled z-scores. hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell.

Fig. 4 | Partitioned heritability enrichments based on cell-type-specific regulatory annotations.
In summary, CNCCs secrete BMP antagonists that modulate forebrain development by blocking BMP and FGF production in the anterior neural ridge. BMP antagonist production in CNCCs is regulated by the SIX family TFs, with SIX1/SIX4 lying near a shared...
brain–face GWAS signal (Fig. 2a). In the reverse direction, studies in chick embryos have shown that Fgf, Shh and BMP ligands are secreted by the forebrain and regulate the formation of the frontonasal ectodermal zone, a signaling center that in turn patterns the frontonasal prominence of the developing face20–22,26. Notably, our study implicates all three of these signaling pathways, nominating specific ligands and receptors whose modulation may be associated with the brain–face cross-talk. Furthermore, our study nominates other pathways, such as Wnt and transforming growth factor beta, for roles in paracrine brain–face signaling. Altogether, we uncovered common genetic variants yielding numerous candidate molecular players whose diverse mechanistic roles in mediating brain–face interactions during development can be examined in future studies.

Relationships of facial shape with cognitive and personality traits have fascinated humans since ancient times, from the ancient Greeks, who introduced ‘physiognomy’ to describe a practice of assessing one’s personality from facial appearance77, through the Vedic traditions of Samudrika Shastra7 and to the Chinese art of face reading7. The concept of physiognomy was revived in the 18th century by Johan Kaspar Lavater, and later led to a related pseudo-scientific theory, phrenology, popularized by Franz Josef Gall. Both theories have a troubled history, as they have been used to justify racial discrimination and eugenic theories80,81. While the original formation of physiognomy has been debunked, modern studies have found correlations between facial width-to-height ratios and aggressive tendencies82, with regrettable renewed efforts in using machine learning approaches to detect such correlations raising serious ethical concerns83–85. Our results argue that while the ancient human intuition of a close relationship between the face and brain has genetic support at the morphological level, there does not appear to be genetic evidence for the supposed predictive value of face shape in behavioral–cognitive traits, which formed the core of physiognomy and related theories.

Online content
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pressure and the first 20 genetic PCs. Furthermore, the following imaging-specific covariates were collected to control for during statistical testing: genetic unrelatedness. This resulted in 9,705,931 filtered SNPs for GWAS analysis on 19,670 unrelated individuals of European descent.

Note for more details on filtering of SNPs and individuals based on ancestry and genomic data from the UKB, which consisted of the version 3 (March 2018) UK Biobank data preprocessing.

The centroid size) of all left and right hemispheres pooled together. We computed the position of table/coil in scanner coordinates, date of attending -coordinate for all of the 29,759 3D vertices on the surface of the right hemisphere. We performed a generalized Procrustes superimposition, thus eliminating differences in position, orientation and scale (measured by centroid size) of all left and right hemispheres pooled together. We computed the symmetric brain component as the vertex-wise averaged brain surface of paired and superimposed left and right hemispheres. This resulted in a final discovery dataset of 19,644 participants containing preprocessed MRI imaging data on the mid-cortical-symmetrized surface, 9,705,931 imputed SNPs and 54 covariates.

Adolescent Brain Cognitive Development Study data preprocessing. The ABCD Study (https://abcdstudy.org/about/) is a longitudinal study following brain development and health through adolescence. A total of 11,411 MRI scans with additional information on sex and age were available from the data release of April 2019 and, of those, 11,393 images were processed successfully using the four-step image preprocessing described above.

In total, 10,627 individuals from the ABCD dataset provided genetic data on 517,274 SNP variants. These were imputed via the Odyssey pipeline using the SHAPEIT4 (ref. 4) and IMPUTES (ref. 5) workflows to phase and impute, respectively. The Haplotype Reference Consortium panel was used for imputation. Quality control before phasing and imputation included using the Imputation preparation program by the McCarthy Group (https://www.well.ox.ac.uk/~wrayner/tools/) to check and fix strand, alleles, position and reference/alternative problems, as well as removing ambiguous A/T and G/C SNPs with minor allele frequencies greater than 0.4. See Supplementary Note for more details on phasing, imputation and ancestry-based selection. These steps resulted in a final replication dataset of 4,470 individuals with preprocessed MRI imaging data, representing brain shape, 15.3 million imputed SNPs and 7 covariates (sex, age and the first 5 genetic PCs). The minimum and maximum ages of participants in this final replication dataset were 8.9 years and 11 years, respectively, with a mean age of 9.9 years. Approximately 46.5% were women and 53.5% were men.

Auxiliary trait genome-wide association study summary statistics. We collected publicly available genome-wide summary statistics for 26 auxiliary traits encompassing neuro-psychiatric disorders, cognitive traits and subcortical volume measures with limited genetic correlation with schizophrenia or bipolar disorder. In Supplementary Table 6, we provide links to relevant publications and URLs for these summary statistics.

Point-wise SNP–phenotype associations. For each of the 29,759 vertices of the averaged mid-cortical 3D surfaces in the UKB, we computed a multivariate (x, y and z coordinate per vertex), narrow-sense heritability from common SNP variants using a linear mixed model (LMM).

Global-to-local segmentation of the mid-cortical surface. The UKB served as the discovery cohort using a data-driven global-to-local segmentation of brain shape similar to previous work on face shape1,2. First, the superimposed and symmetrized mid-cortical surfaces were corrected using a partial least-squares regression (PLSR) with the previously described superimposed mid-cortical surface1 as the overlap of brain segments at each of the eight levels from our global-to-local segmentation. We investigated overlap of brain atlases with global-to-local segmentation.

Overlap of brain atlases with global-to-local segmentation. We investigated the overlap of brain segments at each of the eight levels of our global-to-local segmentation with brain regions from three commonly used brain atlases (Desikan-Killiany (34 distinct gyral-based regions)1, Destrieux (74 distinct gyral- and sulcal-based regions)2 and the Glasser (180 distinct multimodal-based regions)3). See Supplementary Note for details on computing overlap between our brain segments and brain atlases.

Global-to-local multivariate genome-wide discovery. The global-to-local phenotyping partitioned brain shape into overlapping (across different hierarchical levels) and nonoverlapping (within a single hierarchical level) segments, each of which was represented by a different subset of mid-cortical surface vertices and spanned by multiple dimensions of variation (PCs). See the Supplementary Note for details of the CCA-based approach used to discover SNP–phenotype associations.

A significance threshold of \( P \leq 10^{-4} \) was used to declare ‘genome-wide significance’, which corresponds to a Bonferroni correction for 1 million independent tests in a European-ancestry cohort1. Due to 285 multivariate GWAS runs, the multiple comparisons burden was magnified. Therefore, we also determined a more stringent threshold for declaring ‘study-wide significance’, which accounts for the effective number of independent tests. In a first instance, the number of eigenvalues larger than one of a pairwise multivariate correlation (RV coefficient) matrix (285 x 285)1, determined a total of 210 independent tests. In a second instance, following the procedure by Kanai et al.1, we obtained an empirical estimate of the number of independent tests using the 472 lead SNPs representing the genome-wide significant independent loci, to keep the estimations computationally tractable. See the Supplementary Note for details on empirical estimation of the number of independent tests.

Peak detection, overlap and annotations. We observed 38,630 SNPs and 23,413 3SNPs at the level of genome-wide and study-wide significance, respectively. These were clumped into 472 (genome-wide) and 243 (study-wide) independent loci in three steps (Supplementary Note).
To study functional enrichment for genes near the 472 genome-wide lead SNPs, we performed Gene Ontology (GO) analysis using GREAT<sup>43</sup> (v4.0.4) and FUMA<sup>41</sup> (v1.3.6) with default settings. GO terms that were significant by both binomial and hypergeometric tests (FDR q value <0.05) across three or two windows were reported as strongly and weakly enriched, respectively.

In determining overlap between lead SNPs from different GWASs, we used a similar strategy: two lead SNPs tag the same genetic locus if they are within 10 kb of each other or if they are within 1 Mb of each other and with r<sup>2</sup> > 0.2. To quantify the overlap between the 472 brain locus and other studies from the NHGRI-EBI GWAS Catalog, we defined LD blocks of 0.2 around the 472 loci using PLINK v1.9, and then calculated the OR and P value for the overlap between these blocks and any given GWAS using betools v2.27.1 with the fisher function.

In determining brain–face shared loci, we first considered the 472 genome-wide lead SNPs from the brain GWAS and looked for any SNP within 10 kb or 1.7 Mb with r<sup>2</sup> > 0.2 of these lead SNPs with any other SNPs from the 1,725 genome-wide lead SNPs from the brain GWAS. We manually identified candidate genes in the vicinity of the 76 brain–face shared loci. For each locus, we first considered all genes within 500 kb of the lead SNP. We primarily relied on evidence for involvement of these genes in a human craniofacial or neurodevelopmental syndrome, or for evidence of craniofacial or neurodevelopmental defects in knockouts of their orthologs in mice. We also considered associations with GO terms related to craniofacial development, neurodevelopment or skeletal system development. In some cases (that is, SOX9, where enhancer–promoter interactions over 1 Mb have been described<sup>27</sup>), we extended the window to within 750 kb of the lead SNP.

Adolescent Brain Cognitive Development Study replication testing. The ABCD Study data were used for replication, with the UKB discovery cohort used as a phenotyping reference. First, after generalized Procrustes superimposition, the superimposed and symmetrized mid-cortical shapes were corrected for sex, age and the first five genetic PCs, augmented with centroid size to eliminate allometric values were quantified using kallisto<sup>118</sup> with sequence-biased statistics of a multivariate GWAS, albeit with a small correction to the resulting z-scores. When quantifying sharing of signals between pairs of GWAS, we used a Spearman correlation between two vectors of LD-block organized association P values. First, genome-wide SNPs were selected to overlap with the HapMap SNPs<sup>119</sup>, and SNPs within the 1 Mb overall significance region were removed. Second, we organized SNPs within 1,705 blocks in the human genome that can be treated as approximately independent in individuals of European ancestry<sup>119</sup>. For every LD block, we computed the mean SNP −log(P value), and then computed a rank-based Spearman correlation using the averaged value, and coefficient z-scores was provided in Supplementary Data 2. When quantifying heritability enrichments with brain–face shared loci removed, we removed all SNPs within approximately the same independent LD block<sup>27</sup> as any of the 76 brain–face shared loci and recomputed LD scores.

Expression analyses of candidate genes at brain–face overlapping loci. Gene expression levels (log<sub>2</sub>(TPM) values) for 3D forebrain organoids and purified neuronal and glial lineages were obtained from TREX<sup>120</sup> (accession GSE132403). Raw RNA-sequencing reads from CNCCs at passages 1–4, as well as day 9 chordrocytes derived from P1 CNCCs, were obtained from Long et al.<sup>120</sup> (GSE145327), and TPM values were quantified using kallisto<sup>118</sup> with sequence-biased bias correction.

Linkage disequilibrium score regression SNP heritability for multivariate traits. In the Supplementary Note, we show that when applying LDSC to summary statistics of a multivariate GWAS, albeit with a small correction to the resulting z<sup>2</sup> statistics, the heritability estimated by the LDSC slope is equal to \( \frac{1}{2} \text{trace} (\Sigma_{G}^{\top} \Sigma_{G}^{-1}) \), which is a D-dimensional generalization of heritability for genetic and phenotypic covariance matrices centered on the lead SNP. For the multivariate trait are either genetically or phenotypically uncorrelated, this expression simplifies to the average SNP heritability across dimensions. Similarly, when applying S-LDSC, enrichments for partitioned average heritability are obtained. We further show that \( \frac{1}{2} \text{trace} (\Sigma_{G}^{\top} \Sigma_{G}^{-1}) \) is an appropriate multivariate generalization of heritability since it satisfies the following four properties: (1) invariance to units of measurement, (2) coordinate-free, (3) linear in \( \Sigma_{G} \), and (4) maximum \( \Sigma_{G} = \Sigma_{G} = 0.5 \Sigma_{G} \) with a value of \( 0.5 \Sigma_{G} \) for strong pairwise LD.

Thus, for brain and face shape, we applied LDSC and S-LDSC using published software (https://github.com/bulik/l.dsc/wiki) to corrected z<sup>2</sup> statistics from GWAS data of each brain or face segment. We used unmodified z<sup>2</sup> values for the univariate traits analyzed (including indicated cases where we performed individual, univariate GWAS analysis for each brain and face shape PC). While using unmodified z<sup>2</sup> values results in a small bias, we used unmodified statistics for consistency with previous studies. We limited S-LDSC analyses to traits with SNP-heritability z>1.4, as in the work of Finucaine et al.<sup>27</sup>

Functional annotations for stratified linkage disequilibrium score regression. We downloaded a range of publicly available cell-type and sample-specific annotations representing open chromatin and/or active regulatory regions. Specifically, we obtained data on open chromatin (all ATAC–seq peaks) from brain organoids<sup>68</sup>, fetal brain tissue<sup>68</sup> and CNCCs and derived chondrocytes<sup>69</sup>. ATAC–seq reads from Long et al. were mapped to hg19 with bowtie2 (ref. 18) with default settings, and peaks were called using MACS2 (ref. 18) with default settings. Annotations for active regulatory regions (based on a range of epigenomic marks) were obtained from CNCCs<sup>70</sup>, embryonic craniofacial tissues<sup>70</sup>, fetal and adult brain tissue and broad groupings of cell types<sup>59</sup>. For CNCCs<sup>70</sup>, we combined all regions annotated as enhancers (weak, intermediate and strong) or promoters (weak and strong) and embryonic craniofacial tissues, we combined all regions with the following annotations from the 25-state chromHMM model: Enh, TexReg, PromD1, PromD2, PromU and IsaA. For fetal and adult brain tissue, we combined all regions with the following annotations from the 15-state chromHMM model: 1. IsaA, 2. IsaAFlnk, 7. Enh and 6. EnhG. Each annotation was added to the baseline LD model from Finucaine et al. The resulting S-LDSC output (heritability fold-enrichment magnitude and significance and coefficient z-scores) is provided in Supplementary Data 2. When quantifying heritability enrichments with brain–face shared loci removed, we removed all SNPs within approximately the same independent LD block<sup>27</sup> as any of the 76 brain–face shared loci and recomputed LD scores.

Quantifying sharing of signals between pairs of GWAS. To assess the extent to which genome-wide profiles of association were shared between a pair of GWAS, we computed a Spearman correlation between two vectors of LD-block organized association P values. First, genome-wide SNPs were selected to overlap with the HapMap SNPs<sup>119</sup>, and SNPs within the overall significance region were removed. Second, we organized SNPs within 1,705 blocks in the human genome that can be treated as approximately independent in individuals of European ancestry<sup>119</sup>. For every LD block, we computed the mean SNP −log(P value), and then computed a rank-based Spearman correlation using the averaged association value (n=1,725) for each LD block. A standard error of the Spearman correlation was estimated using statistical resampling with 100 bootstrap cycles with replacement from the 1,725 LD blocks.

Ethics statement. This study was conducted in compliance with the principles of the Declaration of Helsinki, the principles of Good Clinical Practice and in accordance with all applicable regulatory requirements. Local ethics review and approval for this study (S63179) was performed and obtained from the ethical committee for research of the University Hospital UZ Leuven and the University KU Leuven.

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

Data availability. All the data and detailed information for the UKB, including genetic markers, covariates and MRI images are available to bona fide researchers via the UKB data access process (http://www.ukbiobank.ac.uk/register-apply/). All the data and detailed information for the ABCD Study, including genetic markers, covariates and MRI images are also available to bona fide researchers through the ABCD data depository (https://nda.nih.gov/abcd/request-access/; controlled access due to highly identifiable facial scans and brain MRIs linked to genotyped data).

Relevant data and materials from the facial GWAS study are available online (https://figshare.com/s/36673614). Relevant data and summary statistics are available from the NHGRI-EBI GWAS catalog (study accession GCST90007181). Furthermore, relevant files generated from the face and brain GWAS summary statistics as input to (S-)LDSC regression and Spearman correlations are available on FigShare (Supplementary Table 7). Full brain GWAS summary statistics are available from the GWAS catalog under prepublished/controlled access due to highly identifiable facial scans and brain MRIs linked to genotyped data).

Additional information or code associated with this announcement is available in the Nature Research Communicating Summary linked to this article.
All relevant additional data related to this work are provided in the FigShare repository for this work (https://doi.org/10.6084/m9.figshare.c.5089841.v1). This includes additional figures, input files and updated implementations, listed in Supplementary Table 7.

Code availability
MATLAB implementations of the hierarchical spectral clustering to obtain phenotypic shape segmentations are available from a previous publication (https://doi.org/10.6084/m9.figshare.7649024.v1). Updated implementations used in this work are provided in Supplementary Table 7. The statistical analyses in this work were based on functions of the statistical toolbox in MATLAB (Methods). Other materials and software used are available online. No other custom software packages were used.

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**Competing interests**
The authors declare no competing interests.

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Extended Data Fig. 1 | Number of additional brain shape loci contributed by hierarchical levels. For all genome-wide (left) or study-wide (right) significant associations, associations with all segments in hierarchical levels up to the indicated number were masked, and the number of remaining associations was assessed.
Extended Data Fig. 2 | Point-wise SNP heritability estimates across the mid-cortical surface. Colors represent the total SNP heritability (computed by a linear mixed model approach, see Methods) at each point on the mid-cortical surface, represented by a set of three-dimensional coordinates in each individual.
Extended Data Fig. 3 | Replication rates in the ABCD cohort by hierarchical level. Only segments in the indicated hierarchical level were considered, and all loci (left) or locus-segment pairs (right) reaching genome-wide significance in those segments were tested for replication in the ABCD cohort at a 5% FDR.
Extended Data Fig. 4 | Overlap between genome-wide significant brain shape loci and genome-wide significant loci from 430 other studies. GWAS hits (number on x-axis) for other studies were obtained from the NCBI-EBI GWAS Catalog, and P-values (left, y-axis) and odds ratios (right, y-axis) for significance of overlap with regions in LD (> 0.2) with brain shape loci were computed using bedtools’ fisher function (see Methods). Note that relative to other traits with equivalent numbers of GWAS hits, face shape shows overlap with brain shape loci greater in both significance and magnitude.
Extended Data Fig. 5 | Comparison of LDSC genetic correlations and Spearman correlation between pairs of univariate traits. Each point represents a pair of univariate traits (of all those considered in this study, see Methods), while the x- and y-axes indicate the absolute value of the LDSC-estimated genetic correlation and the estimated genome-wide sharing of effects by the Spearman correlation method. Point colors and shapes indicate significance ($P < 0.05$) from LDSC or the Spearman correlation method, respectively. Exact p-values are provided in Supplementary Table 6.
Extended Data Fig. 6 | Genetic correlations between RA (rheumatoid arthritis) and univariate brain-related traits. Points (center of error bars) represent estimated genetic correlations. Error bars represent 95% confidence intervals. *, 5% FDR.
Extended Data Fig. 7 | Genetic correlations between the most heritable brain (top two rows) or face (bottom two rows) shape PCs and other traits. Points (center of error bars) represent estimated genetic correlations ($r_g$) between the top ten shape PCs (for segment 1, the full brain or face) with heritability z-score > 3 and each of the indicated univariate traits using LD score regression. Error bars represent 95% confidence intervals. *, 5% FDR for indicated PC; +, 10% FDR.
Extended Data Fig. 8 | SNP heritability of individual face shape PCs and multivariate face shape estimated by LDSC. Points (center of error bars) represent estimated SNP heritability of each PC. Error bars represent 95% confidence intervals. The red line represents the mean heritability of all 70 PCs, and the blue line indicates the heritability obtained by applying LDSC to corrected $\chi^2$ statistics from the multivariate CCA GWAS using all 70 PCs.
Extended Data Fig. 9 | Partitioned heritability enrichments for brain shape with respect to stage- and cell-type-specific brain organoid open chromatin. S-LDSC coefficient Z-scores and heritability fold-enrichment for annotations corresponding to the indicated cell-type and differentiation day were computed as described in Methods. Regression lines represent the linear best fit with intercept and organoid differentiation day as dependent variable, and grey areas represent 95% confidence intervals. P-values are from a two-tailed F-test.
Extended Data Fig. 10 | Partitioned heritability enrichments for brain shape with respect to open chromatin in CNCCs or early glial organoid cells, with or without 76 brain-face shared loci. S-LDSC Z-scores were calculated using full brain shape as the trait and the most enriched craniofacial (top) or brain organoid (bottom) ATAC-seq dataset as annotations. Z-scores were re-estimated (blue) after removing all SNPs in the same approximately independent LD block as one of the 76 brain-face shared loci (see Methods for details).
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

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

Software and code

Policy information about availability of computer code

- Data collection: No software was used for data collection as part of this study.
- Data analysis: Matlab implementations of the hierarchical spectral clustering to obtain phenotypic shape segmentations are available from a previous publication (https://doi.org/10.6084/m9.figshare.7649024.v1). Updated implementations used in this work are provided (https://doi.org/10.6084/m9.figshare.c.5089841.v1). The statistical analyses in this work were based on functions of the statistical toolbox in Matlab as mentioned throughout the Methods. Other materials and external software used mentioned throughout the methods, are all available online (see URL section). The following versions of software were used: SHAPEIT4, IMPUTE5, plink 1.9, bowtie2, MACS2, bedtools v2.27.1, kallisto v0.44.0, FreeSurfer v6.0.0

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

- All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
  - Accession codes, unique identifiers, or web links for publicly available datasets
  - A list of figures that have associated raw data
  - A description of any restrictions on data availability

All the data and detailed information for the UK Biobank, including genetic markers, covariates and MRI images are available to bona fide researchers via the UK Biobank data access process (see http://www.ukbiobank.ac.uk/register-apply/).
All the data and detailed information for the ABCD study, including genetic markers, covariates and MRI images are also available to bona fide researchers through
the ABCD data depository (https://nda.nih.gov/abcd/request-access)

Relevant data and materials from the facial GWAS study are available online (https://doi.org/10.6084/m9.figshare.c.4667261). The full facial GWAS summary statistics are available on the NHGRI-EBI GWAS catalog (study accession GCST90007181). Furthermore, relevant files generated from the face and brain GWAS summary statistics as input to (S-)LDSC regression and spearman correlations are available on FigShare, see Supplementary Table 8. The full brain GWAS summary statistics are available on the GWAS catalog (study accession GCST90012882).

All relevant additional data related to this work are provided in the FigShare repository for this work (https://doi.org/10.6084/m9.figshare.c.5089841.v1). This includes additional figures, input files and updated implementations, listed in Supplementary Table 8.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
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- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

| Sample size | No statistical method was used to predetermine sample size. Sample sizes were determined to be sufficient based on results of previous GWAS of brain phenotypes with similar sample sizes. Sample size was maximized based on data availability in the UK Biobank, after excluding samples that failed image processing, or had outliers with respect to covariates, or had non-European ancestry. |
| Data exclusions | MRI images were excluded if they failed any steps of the surface reconstruction and segmentation pipeline, as described in detail in Methods. Individuals with extreme outlier values for certain covariates were excluded, as described in Methods. Individuals of primarily non-European descent as well as related individuals were excluded, as described in Methods. These exclusionary measures were determined prior to performing GWAS analysis. |
| Replication | Effects of the 472 genome-wide significant loci for brain shape were subject to a single replication analysis using MRI images from the ABCD cohort. Of the 472 loci, 466 were available for testing in the ABCD cohort after imputation and filtering. Of these 466, 305 (65.4%) replicated at least one associated segment at 5% FDR. |
| Randomization | MRI images were assigned into groups based on SNP genotypes. Images were adjusted for sex, age, height, weight, diastolic and systolic blood pressures, and 10 principal components representing ancestry components. |
| Blinding | Investigators were not blinded to group allocation. While individual genotypes had to be accessed to perform quality control and filtering, the group allocation was based on individual genotypes and so could not be changed. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
| --- | --- |
| n/a | n/a |
| Involved in the study | Involved in the study |
| Antibodies | ChIP-seq |
| Eukaryotic cell lines | Flow cytometry |
| Palaeontology and archaeology | MRI-based neuroimaging |
| Animals and other organisms | |
| Human research participants | |
| Clinical data | |
| Dual use research of concern | |

Human research participants

Policy information about studies involving human research participants

| Population characteristics | The UK Biobank project (UKB) is a large dataset of about 500,000 British volunteers with informed consent containing genetics, non-imaging variables and brain imaging data acquired using a fixed protocol |
| Recruitment | Participants were recruited by the UK Biobank. Selection bias in the UK Biobank has been observed to favor healthy, European-ancestry individuals. |
### Ethics oversight

This study was conducted in compliance with the principles of the Declaration of Helsinki, the principles of GCP and in accordance with all applicable regulatory requirements. Local ethics review and approval for this study (S63179) was performed and obtained from the ethical committee for research of the University Hospital UZ Leuven and the University KU Leuven. Collection of the data in the UK Biobank was governed by the Ethics and Governance Council of the UK Biobank.

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          | Resting state               |
|----------------------|-----------------------------|
| Design specifications| [https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf](https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf) |
| Behavioral performance measures | Not applicable |

#### Acquisition

| Imaging type(s)       | T1-weighted structural imaging |
|-----------------------|-------------------------------|
| Field strength        | 3T                            |
| Sequence & imaging parameters | page 8 in [https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf](https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf) |
| Area of acquisition   | Whole brain scan              |
| Diffusion MRI         | Used                          |

#### Preprocessing

| Preprocessing software| Standard T1 preprocessing steps are described on page 12-13 in [https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf](https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf). Followed by Freesurfer recon-all and ciftify as described in the methods |
|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Normalization         | page 12-13 in [https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf](https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf)                                                                 |
| Normalization template| T1 preprocessing involved the MNI152 template (page 12-13 in [https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf](https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf)). Ciftify output used is based on the low resolution Conte69 cortical surface template for left and right hemisphere as described in the methods. |
| Noise and artifact removal| Freesurfer embedded noise and artifact removal. Additional imaging covariates, volumetric scaling from T1 head image to standard space, XYZ-position of brain mask in scanner co-ordinates, Z-position of table/coil in scanner co-ordinates, date of attending assessment center, and assessment center were used to correct the brain surface data using partial least square regression. |
| Volume censoring      | Not applicable                                                             |

#### Statistical modeling & inference

| Model type and settings       | Multivariate shape analysis                                                  |
|-------------------------------|-------------------------------------------------------------------------------|
| Effect(s) tested              | Fixed effects of SNP genotypes on multivariate shape variables                |
| Specify type of analysis:     | □ Whole brain □ ROI-based □ Both                                             |
| Anatomical location(s)        | Hierarchical data-driven shape segmentation as described in the methods and applied elsewhere on 3D facial shapes |
| Statistic type for inference  | Surface-based and not voxel-based multivariate shape variables, subjected to association with SNP genotypes using canonical correlation analysis |
| Correction                    | Correction of multivariate shape variables for covariates was performed using partial least squares regression. Correction for multiple testing was performed based on permutations, followed by an adjusted study-wide p-value threshold by division of the less stringent genome-wide threshold by the effective number of tests. |
For each of the 285 brain segments separately, the group of 3D surface vertices in a segment were subjected to a new GPA. As such, a multivariate shape-space for each brain segment was constructed independently of the other segments and its relative positioning within the full brain hemisphere. Subsequently, after GPA, each segment’s shape-space was spanned by a multivariate orthogonal basis using PCA on the pooled x, y and z coordinates of the collection of superimposed vertices in that segment. Finally, we retained enough PCs to explain up to 80% of the total shape variation within each segment. Associations of multivariate shape spaces with SNP genotypes were tested using canonical correlation analysis.