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ABSTRACT

Specific genetic loci related to differences in brain anatomy have recently been discovered through efforts of the ENIGMA consortium and others. Yet different neuroanatomical traits may have different genetic determinants, and their discovery may offer a deeper understanding of genetic factors that shape the human brain. To investigate the degree of genetic contributions to variability in sulcal measures - the fissures within the brain's surface - 1009 healthy young adult Australian twins and siblings were imaged with high-resolution 4-tesla MRI. Length, mean depth, surface area and fold openings for 60 sulci were measured for both hemispheres. We calculated the heritability for each sulcal measure (average h^2~0.3-0.4); genetic correlations determined if there was an overlapping genetic profile across hemispheres for the same trait, or if lateralized genetic influences were statistically distinguishable. In cases with no hemisphere-specific genetic influences, the heritability of the bilateral average was computed for greater stability.

Index Terms— *Genetics, Heritability, Brain Imaging, Sulci, Shape*

1. INTRODUCTION

Understanding the mechanisms underlying brain anatomical variability is critical for neuroscience and psychiatry. Genetic drivers of brain differences are crucial to identify, for understanding risk factors for heritable brain diseases, as well as for evolutionary biology. Recently, large-scale consortia in the field of neuroimaging genetics including the Enhancing Neuro Imaging Genetics through Meta Analysis (ENIGMA) have identified 8 single nucleotide polymorphisms (SNPs) among the 10s of millions genotyped in the human DNA sequence, that have a significant, global impact on brain volume or the volumes of key brain substructures [1]. Studies are now underway successfully discovering common genetic variants associated with both brain structural variability and genetic risk for disease. However, there are many more features besides volume that can be reliably extracted from brain scans, and these may offer additional insights into the specific genetic programs that help shape the human brain.

"Sulcus-based morphometry" provides measures of the cortical fissures of the brain, that have been found to be associated with developmental maturation in adolescents [2], degenerative changes in the elderly [3], schizophrenia [4,5], bipolar disorders [6] and autism in children [7]; altered fissuration is also found in several neurogenetic disorders, such as Williams syndrome [8,9].

Sulcus-based morphometry also confirms the heritability of primary gyrification phenotypes in baboons [10]. To ensure that these analyses can be informative for more in-depth efforts to discover specific genetic variants that may drive brain changes and contribute to disease risk, it can be advantageous to identify (1) which sulcal traits are easily and reliably extracted, and (2) whether they exhibit significant heritability.

The relative influences of genetic and environmental factors on human traits can be estimated by modeling the known genetic relationship between individuals and relating it to observed correlations in measured traits; for example, here we use imaging in monozygotic (MZ) twin pairs - who typically share all their common genetic variants - and dizygotic (DZ) twin pairs, who share, on average, 50%. For a given cohort of participants, the heritability (h^2) is the

proportion of the observed variance in a trait (σ^2_p) that can be attributed to additive genetic factors (σ^2_g) : $h^2 = \sigma^2_g / \sigma^2_p$.

Here we investigated the heritability of a range of measures from 60 consistently occurring sulci per hemisphere of the brain, i.e. 120 sulci in total. We focused on four measures extracted from their parcellations: sulcal length, mean depth, surface area and a measure of fold opening. As genome wide screens require millions of statistical tests, and a strict multiple comparisons correction threshold, we should also ensure that the 480 measures we are testing are not identical in their genetic determination, and therefore redundant to measure. We therefore performed a bivariate heritability analysis for all left-right pairs of traits to determine if there were significant genetic correlations between hemispheres. If no uniquely lateralized genetic effects were detected, we proceeded to average the left and right hemisphere measures, to reduce the number of statistical tests and with the goal of improving the reliability of the measure.

2. MATERIAL AND METHODS

2.1 Participants and MRI Imaging

1009 right-handed participants [11] were studied, including 375 dizygotic (DZ) and 530 monozygotic (MZ) twins (one set of DZ triplets) and 104 siblings, with an average age of 23 ± 3 years [range: 18–30]. T1-weighted images were acquired with an inversion recovery rapid gradient echo sequence. Acquisition parameters were: inversion/repetition/echo time (TI/TR/TE) = 700/1500/3.35 ms; flip angle = 8 degrees; slice thickness = 0.9 mm, with an acquisition matrix of 256 × 256.

2.2 MRI image processing and sulci extraction

FreeSurfer (http://surfer.nmr.mgh.harvard.edu/) and quality controlled using ENIGMA protocols for outlier detection and visual inspection. BrainVISA (http://brainvisa.info) was run for sulcal extraction and identification. Morphologist 2013, an image processing pipeline included in BrainVISA, was used to quantify sulcal parameters (**Figure 1**). Briefly, the Morphologist 2013 segmentation pipeline computes a brain mask, classifies brain tissue into gray and white matter and CSF, and performs a gray/white surface identification, and a spherical triangulation of the external cortical surface of both hemispheres. However, to improve sulcal extraction and build on current protocols used by hundreds of collaborators within ENIGMA, quality controlled FreeSurfer outputs (*orig.mgz, ribbon.mgz and talairach.auto*) were directly imported into the pipeline to avoid re-computing several steps. Sulci were automatically labeled according to a predefined anatomical nomenclature of 60 sulcal labels per hemisphere [12,13].

2.3 Sulci descriptors and quality control

Analyzing the geometry of the cortex through sulcus-based morphometry allows us to quantify the geometry of a sulcus in terms of several distinct and complementary descriptors. The geometrical descriptors of each sulcus used in this work are illustrated in **Figure 1**; see [14] for details. We measured the length, mean depth, surface area and fold opening of all extracted sulci, for both hemispheres, ensuring the description of the sulcal shape across hemispheres and subjects.

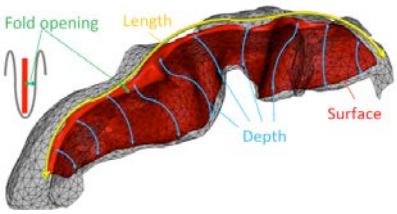

Figure 1: The four shape descriptors (fold opening, length, mean depth, and total surface area) analyzed for each sulcus.

The *length* of a sulcus is measured as the number of voxels on the junction between a sulcus and the hull of the brain. The *mean depth* corresponds to the average of the depth across all the voxels along the bottom of a sulcus (the depth of a voxel located at the bottom of a sulcus is defined as the geodesic distance along the sulcus to the brain hull). The *surface area* is the total area of the sulcal surface. The enclosed CSF volume divided by the sulcal surface area gives the *fold opening,* a gross approximation of the average width of the CSF in the fold.

To further quality control the extracted sulcal measures and identify subjects whose sulci were not optimally identified, we consider the surface, which takes into account the length and the depth, as more representative of sulcal shape across the population. After correcting the sulcal area for the total sulcal surface area of each subject, the *z*-score across subjects was computed on the corrected surface values. Sulci with an absolute *z*-score greater than 2.5 were discarded from further analysis **(Figure 2).**

2.4 Univariate and bivariate quantitative genetic analyses.

Variance components methods, implemented in the Sequential Oligogenic Linkage Analysis Routines (SOLAR) software package [15], were used for all genetic analyses. Heritability (h^2) is the proportion of total phenotypic variance accounted for by additive genetic factors, and is assessed by contrasting the observed phenotypic covariance matrix with the covariance matrix predicted by kinship. High heritability indicates that the covariance of a trait is greater among more closely related (genetically similar) individuals; here, for example, monozygotic twins as compared to dizygotic twins and siblings.

Prior to testing for the significance of heritability, sulcal descriptor values for each individual are adjusted for a series of covariates. We estimated the influence of specific variables (additive genetic variation, and covariates

including intracranial volume, sex, age, age^2, age × sex interaction, age^2 × sex interaction) to calculate heritability and its significance (*p*-value) for accounting for a component of each trait's variance within this population.

The significance threshold for heritability analysis of individual sulci was set to be $p \leq (0.05/m)$, where $m = 60$ (number of sulci) times 4 (number of measurements). This reduced the probability of Type 1 errors associated with multiple measurements.

Apart from work by the ENIGMA Laterality group [16] most prior ENIGMA studies [1,17,18,19] performed analyses on pooled bilateral measures, averaging data from left and right hemispheres. To formally test whether this is acceptable when evaluating sulcal parameters, we performed a bivariate genetic analysis to estimate the genetic correlation between left and right sulcal measures. Classical quantitative genetic models were used to partition the phenotypic correlation into the genetic ρ_G, and environmental ρ_E components. For two traits A and B this decomposition is:

$$\rho_p = \sqrt{h_A^2}\sqrt{h_B^2} \cdot \rho_G + \sqrt{1 - h_A^2}\sqrt{1 - h_B^2} \cdot \rho_E$$

where h^2_A and h^2_B denote the heritability for each lateralized estimate of the trait. The correlation is composed of an additive genetic (ρ_G) and a unique environmental effect, ρ_E, for each pair of traits. Just as with the univariate model, the bivariate phenotype of an individual is modeled as a linear function of kinship coefficients that express relatedness among all individuals within the cohort (MZ twins share all their additive genetic information and DZ twins and siblings share on average 50%). The significance of ρ_G and ρ_E were estimated from the likelihood ratio test when comparing the model to ones where the correlation components are constrained to be zero [15,20,21]. This estimates ρ_G and ρ_E and their standard errors. The significance of these coefficients is determined by a *z*-test of their difference from zero. If ρ_G differs significantly from zero then a significant proportion of the traits' covariance is influenced by shared genetic factors.

In this case, we tested another model where the genetic correlation factor ρ_G is fixed to 1. Fixing ρ_G to 1 suggests that the additive genetic components comprising the two traits are identical, and there is no detectable unique genetic composition for the individual traits. Once again, the log-likelihood of this model is compared to one where the parameters are freely optimized. If ρ_G is not found to significantly differ from 1, then we cannot reject the hypothesis that both heritable traits are driven by the same set of genetic factors. If ρ_G is significantly different from 0 and significantly different from 1, then the traits share a significant portion of their variance, exhibiting pleiotropy, but each is also partially driven by a unique set of genetic factors.

3. RESULTS AND DISCUSSION

3.1 Outlier detection

Figure 2 shows box plots of the sulcal surface for each of the 60 left hemisphere sulci, plotted as *z*-scores. We considered, as outliers, those sulci with abs(z-score) > 2.5. These were visually evaluated, and discarded from the heritability computation. Across subjects and hemispheres less than 4% of sulci had such an abnormal *z*-score; the anterior sub–central ramus showed the greatest number of outliers. **Figure 2a** shows an example of a well-identified central sulcus. **Figures 2b,c** show two subjects, identified by the *z*-score, with a mislabeled central sulcus (S.C.). Visually inspecting some randomly chosen outliers (~5%) confirms that the cut off on $|z|>2.5$ was able to correctly identify labeling errors.

3.2 Genetic analysis of sulcal phenotypes

Using a bivariate model we tested whether left and right hemisphere measures were genetically correlated. Furthermore, we determined whether the genetic correlations differed significantly from 1, or whether the same genetic factors were driving heritability in both hemispheres. We found that no pairs of lateralized measures had significantly different genetic contributions to the left or right hemisphere uniquely.

Globally, among the descriptors analyzed here, the fold opening shows the higher number of sulci with genetic correlation (**Figure 3**). The frontal lobe showed heritability for 4 descriptors, as did the perisylvian cortex in a pattern that has been shown to be heritable in prior studies [22]. Sulci with highly significant genetic correlation with the shape measures are: for the length, the *primary intermediate ramus of the intraparietal sulcus* (F.I.P.r.int, $p = 3x10^{-9}$) for depth and surface, the *Anterior sub-central ramus of the lateral fissure* (F.C.L.r.sc.ant, $p = 7x10^{-15}$), and for the fold opening of the *intraparietal sulcus* (F.I.P., $p = 2x10^{-28}$). From here onward, we therefore averaged all left and right measures and we computed h^2 on the bilateral average length, mean depth, surface area and fold opening.

Figure 4B shows the regional h^2 for the 4 descriptors for areas with significant heritability, after correcting for multiple testing across 240 sulcal descriptors. Each measure showed a pattern of heritability across the brain. The fold opening measurement was the descriptor with the most widely significant h^2 across the full brain.

The sulci showing the most highly significant estimation of h^2, for the 4 descriptors, are the *intermediate ramus of the intraparietal sulcus* for length ($p=2x10^{-17}$), mean depth ($p=2x10^{-17}$) and surface area ($p=2x10^{-13}$), and the *intraparietal sulcus* for fold opening ($p=2x10^{-19}$) (**Figure 4-A**). The highest h^2 was found to be: for length, the *primary intermediate ramus of the intraparietal sulcus* (F.I.P.r.int.1, $h^2 = 0.54$); for mean depth and surface area *the superior postcentral intraparietal superior sulcus* (F.I.P.Po.C.inf. $h^2=0.63$ and $h^2=0.61$); and for fold opening, the

intraparietal sulcus (F.I.P., h^2=0.68).

Table 1: Sulci with significant univariate h^2 with no significant genetic correlation between left and right.

	Depth	Surface	Fold Opening	Anatomical Regions
S.Or.			*S.Or.*	*Inferior-frontal lobe*
	S.Pe.C.sup	*S.Pe.C.sup*		*Pre-motor area*
	S.R.inf.			*Brodmann area 11*
			F.C.L.r.sc.post	*Lateral Sulcus*
			S.F.median.	*Medial Frontal sulcus*
			S.F.polar.tr	*Frontal lobe*
			S.Or.l.	*Frontal lobe*

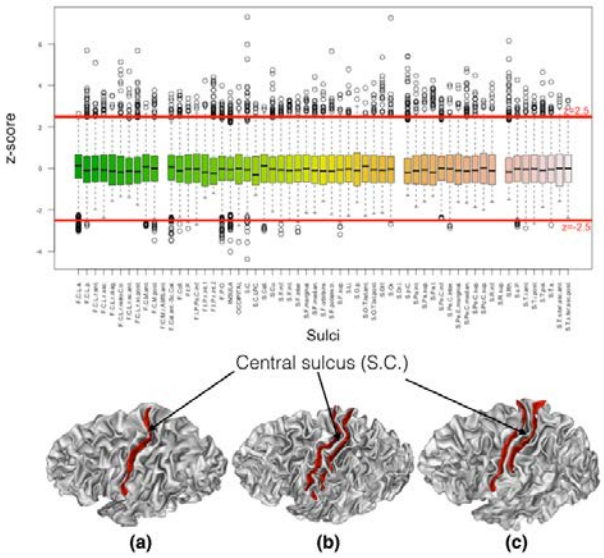

Figure 2: Top: boxplots for the *z*-scores across subjects. The sulci with |z|>2.5 were discarded from further analyze. Bottom: (a) a subject with a well-defined central sulcus (S.C.) and (b-c) two subjects whose S.C. was mislabeled.

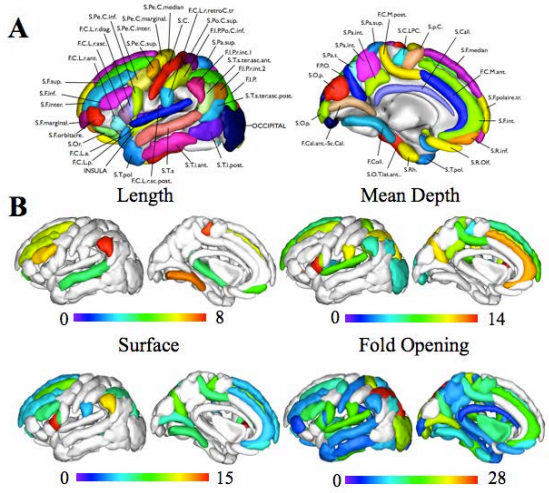

Figure 3: A) BrainVISA sulcal model B) Sulcal-based genetic correlations between left and right hemispheres (bivariate analysis). The $-\log_{10}$ of the *p*-value (corrected for multiple tests) per sulcus is mapped on the left hemisphere sulcal model used for automatic labeling [12].

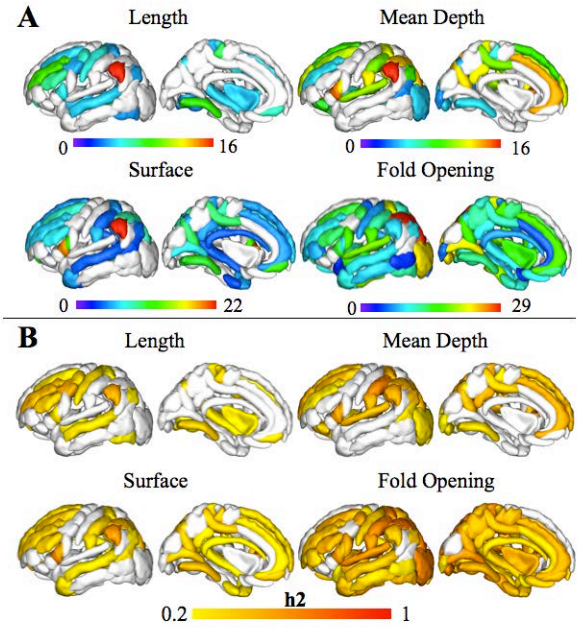

Figure 4: Heritability of bilaterally averaged sulcal measures (univariate analysis). **(a)** *p*-value (corrected for multiple tests) mapped on left hemisphere sulcal models [12]; **(b)** Heritability (h^2) for the 4 descriptors.

4. CONCLUSIONS

We investigated the heritability of length, mean depth, surface area, and fold opening for sulcal shape features in a large cohort of 1,009 young adults. A bivariate analysis of genetic correlation revealed minimal differences between the genetic correlation of the left and right hemisphere sulcal shape measures. The fold opening measure was globally more heritable than other measures of sulcal morphometry. This could reflect the cortical thickness of the surrounding cortex, which has previously been reported as highly heritable, especially in parietal, frontal and temporal lobes [23,24]. Univariate heritability analysis showed a unique pattern of heritability across the brain and descriptors, for the perisylvian cortex - in particular a ramus of the lateral fissure (F.C.L.r.asc.) that is localized in the Broca's area. On the contrary, regions such as the posterior lateral fissure (F.C.L.p) showed heritability only for sulcal depth and fold opening, while the superior temporal sulcus (S.T.s.) only for the length and the surface. Analysis of these 4 descriptors of the 60 sulci per hemisphere will open the way to the assessment of the pleiotropy of genetic effects on sulcal shape, and genetic influences on brain folding.

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