Genetic connectivity – correlated genetic control of cortical thickness, brain volume and white-matter.

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Abstract
MRI and DWI measures of brain volume, cortical thickness and white-matter integrity are commonly used in imaging genetics studies, yet the underlying genetic relationship between these measures is not well understood. Here we use structural equation modeling (SEM) in a twin design to test the genetic correlation between these common imaging measures. MRI and DWI data from 442 subjects (mean age: 23.5 years +/- 2.1 SD; 151 women; 98 MZ pairs, 123 DZ pairs) were obtained and processed using standardized ENIGMA protocols. We found significant genetic association between several WM tracts and subcortical volume ROIs, notably the thalamus and pallidum. Correlation between cortical thickness and volume and WM was low. Cortical surface area was, however, highly correlated with FA in several WM regions and all of the subcortical volume regions. These results may be useful for future gene targeting studies and give insight into the genetics underlying common imaging measures.

1 Aims

Understanding the degree to which different brain imaging measures are linked by shared genetic influences is paramount in explaining normal brain development, anatomy and pathology; not to mention of great utility for experimental design in the field of imaging genetics (Winkler et al. 2009). The complex structures of the brain are under strong genetic control (Blokland et al 2012), but the underlying genetic archi-

tecture across multiple brain measures (and across different imaging modalities) is still largely unknown. Understanding the genetic relationship between different brain measures will allow for the understanding of biologically meaningful endophenotypes for genetic analysis, and how they are interconnected. The endophenotype in imaging genetic studies is a quantitative imaging measurement that indexes changes in a behavior or illness. In looking for genes that influence these endophenotypes, it would be advantages to select imaging measures that are genetically unique, yet discovering genes that help shape multiple brain regions is also of extreme importance.

Twin and family studies afford the ability to estimate the common genetic influence underlying any two traits (called the genetic correlation or r_g). For example, in 2009, Panizzon et al., showed that surface area and cortical thickness are influenced by separate genetic influences— suggesting that surface or thickness measures would be more advantageous in gene discovery studies than volume measures, which contains genetic and phenotypic aspects of both. A corollary of this work is to examine measure across modalities and possibly to develop a trans-modality endophenotype for gene discovery.

Diffusion weighted imaging (DWI) is commonly acquired alongside standard T1-weighted structural MRI scans and has the potential to describe white matter (WM) integrity across the full brain. The relationship between DWI metrics and their manifestation in structural imaging is complex and largely unstudied. WM tracts imaged in DWI project from and pass through many of the subcortical regions commonly studied in imaging genetics. They furthermore develop in concert with cortical and subcortical gray matter as neural pruning and fiber organization occurs (Casey et al., 2005). The genetic control of this development is not yet understood.

In the present study, we examined 442 healthy, young adult twins to estimate the genetic correlation between 109 brain ROIs (21 measures of WM fractional anisotropy [FA], 15 of subcortical volume, 72 of cortical thickness, + intracranial volume) measured following standardized protocols developed by the ENIGMA (Enhancing Neuroimaging Genetics through Meta-Analysis) Consortium. The ENIGMA Consortium is a massive multi-site imaging genetics collaboration between 185 laboratories across 33 countries, which recently published a genome wide association study of subcortical volume, using MRI and genotyping summary statistics from 30,717 individuals (Hibar et al., 2015), and plan to conduct similar studies of DWI and cortical measures.

Here we hope to elucidate the genetic relationship of white matter, subcortical volumetrics, and cortical thickness and surface area. Given the developmental and physical link between white matter and cortical thickness, surface area, and subcortical volumetrics we hypothesize that the underlying genetic determinants of each of those measures will show some overlap with those measuring white matter integrity.

2 Methods

Bivariate genetic correlations were computed between ROIs in three imaging measures: subcortical volume, cortical thickness and DTI fractional anisotropy as described in the ENIGMA protocols.

2.1 Subject Information

A total of 442 subjects (mean age: 23.5 years +/- 2.1 SD; 151 women; 98 MZ pairs, 123 DZ pairs) were included in the present study; all subjects underwent structural T1-weighted brain MRI and diffusion tensor imaging (DTI) scans. All subjects were of European ancestry from 221 families. Subjects were recruited as part of a large-scale 5-year twin study examining healthy young adult Australians using structural and functional MRI and DTI (de Zubicaray et al., 2008).

2.2 Image Acquisition

Structural and diffusion-weighted whole-brain MRI scans were acquired for every subject (4T Bruker Medspec). T1-weighted images were acquired with an inversion recovery rapid gradient echo sequence (TI/TR/TE = 700/1500/3.35 ms; flip angle=8°; slice thickness = 0.9 mm, with a 256^3 acquisition matrix).

Diffusion-weighted images were acquired using single-shot echo planar imaging with a twice-refocused spin echo sequence to reduce eddy-current induced distortions. A 3-minute, 30-gradient acquisition was designed to optimize signal-to-noise ratio for diffusion tensor estimation (58). Imaging parameters were: TR/TE=6090/91.7 ms, FOV=23 cm, with a 128x128 acquisition matrix. Each 3D volume consisted of 55 2-mm thick axial slices and 1.8x1.8 mm^2 in-plane resolution. 105 images were acquired per subject: 11 with no diffusion sensitization (i.e., T2-weighted $b_0$images) and 94 diffusion-weighted (DW) images (b = 1149 s/mm^2) with gradient directions uniformly distributed on the hemisphere.

2.3 Image Preprocessing

All images were processed as described by the publically available ENIGMA image analysis proctocols (http://enigma.ini.usc.edu/protocols/ imaging-protocols/)

2.4 Establishing Zygosity, Genotyping and Imputation

Standard PCR methods and genotyping was used to establish zygostiy objectively by typing nine independent DNA microsatellite polymorphisms (polymorphism information content > 0.7).

Blood group (ABO, MNS, and Rh), was used to verify results along with phenotypic data (hair, skin, and eye color), providing a probablilty of accurate zygosity classification > 99.99%. Standard manufacturer protocols were used on the Human610-Quad BeadChip (Illumina) to analyze genomic DNA samples (Infinium HD Assay; Super Protocol Guide; Rev. A, May 2008). Genotypes were imputed by mapping to HapMap (Release 22, Build 36) with MACH (http://www.sph.umich.edu/csg/abecasis/MACH/index.html).

2.5 Cross-twin cross-trait analysis

To identify common genetic or environmental factors modulating cortical thickness, subcortical volume and DTI FA measures, we used a “cross-twin cross-trait" analysis (Neale et al., 1992). Covariance matrices for the phenotypes—MRI and DWI measures—were computed between the monozygotic twins (MZ) who share all the same genes, and the dizygotic twins (DZ) who on average share half of their genetic polymorphisms.

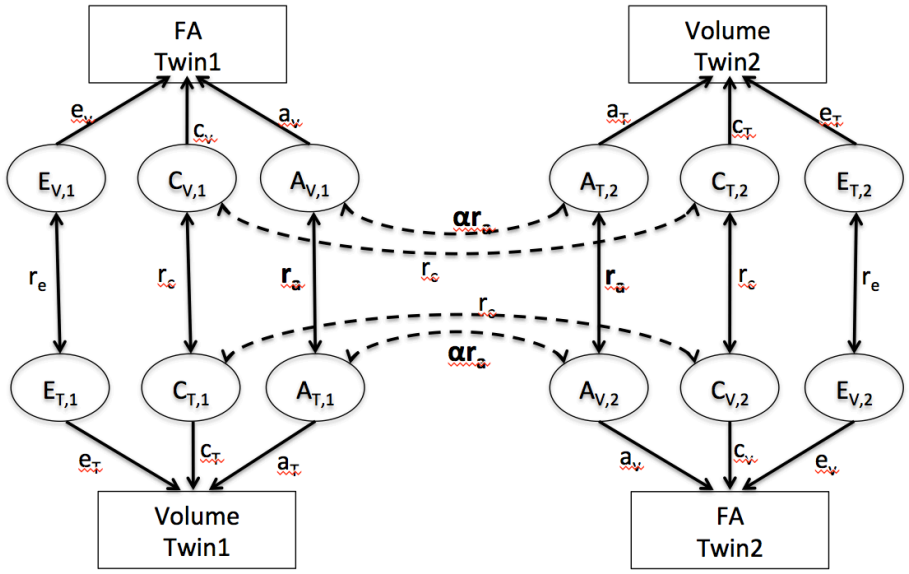
Using OpenMx software (http://openmx.psyc.virginia.edu/), covariance matricies were entered into a multivariate structural equation modeling (SEM) to fit the relative contributions of additive genetic (A), shared environmental (C), and unique environmental (E) components to the population variances and covariances of the observed variables. The E component also contains experimental measurement error as it assumed to be independent between both twins in a pair.

In multivariate SEM, it is expected that there are common genetic and environmental factors affecting various phenotypes. We may estimate the variance of the common genetic and environmental components from the total population variance by calculating the difference between the co-variances between the MZ and DZ twins within the same individual (cross-trait within individual) and also between one phenotype in one twin with the other phenotype in the second twin (cross-twin cross-trait). With this multivariate SEM, we then get r_A and r_C, which denote the additive genetic and shared environmental influences on the correlations between the two phenotypes, respectively.

The cross-trait within-individual correlation (the correlation between two phenotypes, FA and volume for example, in twin 1 or in twin 2) is split into the additive genetic, and shared and unique environmental components (e.g., $A_{V,i}$, $C_{V,i}$, and $E_{V,i}$ for each

ROI value), and the correlation coefficients between $A_{V,i}$ and $A_{T,i}$, $C_{V,i}$ and $C_{T,i}$, and $E_{V,i}$ and $E_{T,i}$, are indicated by r_a, r_c, and r_e, respectively. The cross-trait cross-twin correlation is shown as $A_{V,i}$ and $A_{T,j}$, and $C_{V,i}$ and $C_{T,j}$ for the FA value in twin *i* and the volume value in twin *j*, where *i, j* = 1 or 2, and $i \neq j$. Because the unique environmental factors between subjects independent, there is no r_e term for $E_{V,i}$ and $E_{T,j}$.

Using the path diagram, we derive the covariance across the two phenotypes within the same subject, (or separately in the two subjects,) by multiplication of the path coefficients for the closed paths (Figure 1).



For example, covariance between the FA values in *twin 1* and the volume in *twin 2* is equal to $a_V r_a a_T + c_V r_c c_T$ for MZ twins, and $a_V \frac{1}{2} r_a a_T + c_V r_c c_T$ for DZ twins. Paths connecting the same phenotype are identical to a univariate SEM model (Jahanshad et al,. 2010). MZ twins have a correlation coefficient of 1 for A1 and A2, while DZ twins have 0.5. By definition for the shared environment, C1 and C2 is always a correlation coefficient of 1. E1 and E2 are assumed to have no correlation.

It is common in twin studies to test whether the observed measures are best modeled using a combination of additive genetic, shared, and unshared environmental factors, or whether only one or two of these factors is sufficient to explain the observed pattern of inter-twin correlations. More details on model selection are described by Ja-

hanshad et al. 2012. Here we used the full set of path coefficients in each test as they achieved significance.

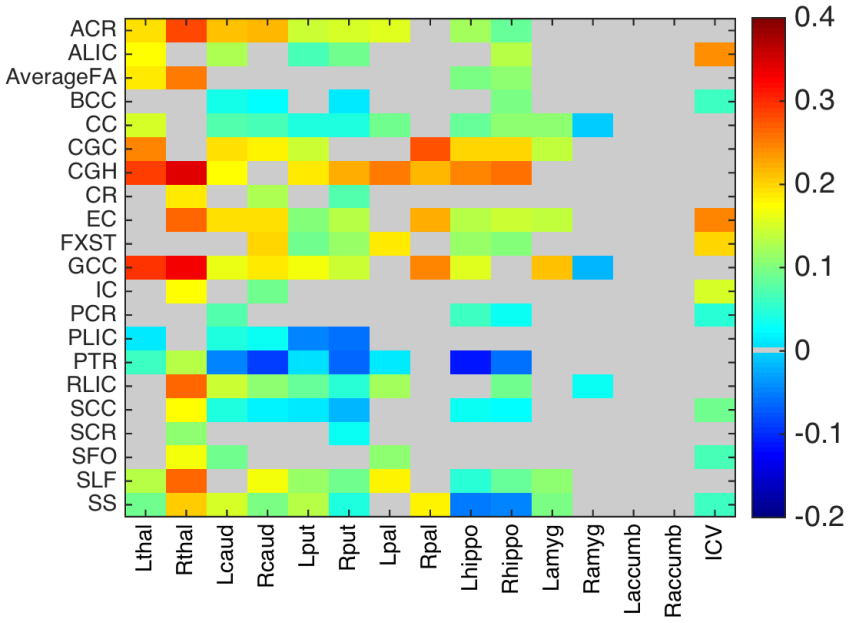
2.6 Multiple Comparisons Correction

For determining the best overall model for the SEM cross-twin cross-trait analysis, we use the standard Benjamini & Hochberg (1995) FDR procedure.

3 Results

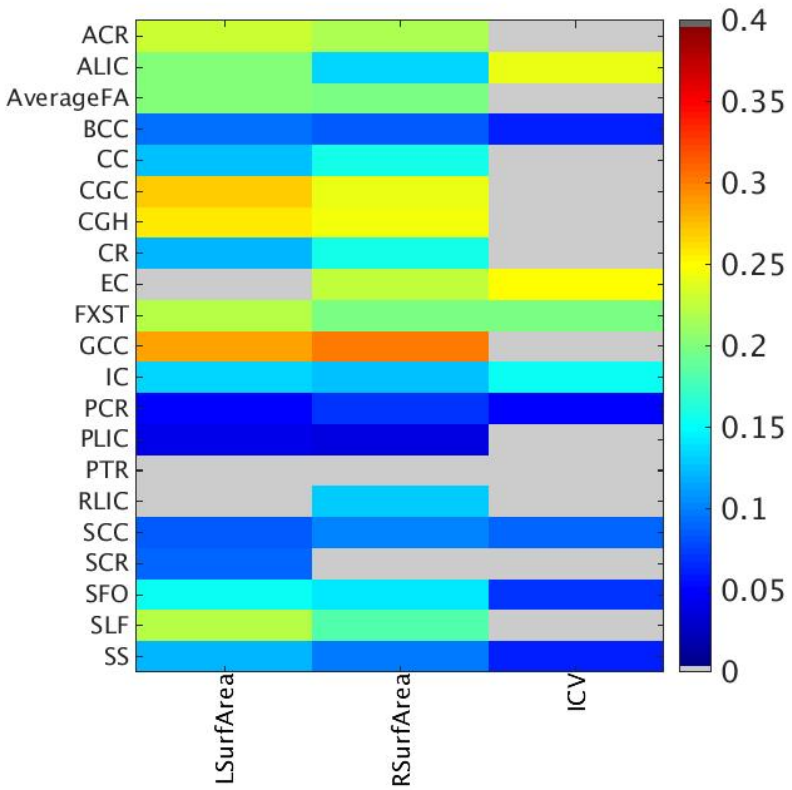
Bivariate genetic correlations between subcortical volume and fractional anisotropy are shown in Figure 2.

Fig. 2. Genetic correlations between FA in DTI white-matter tracts and subcortical volume ROIs. Colored elements indicate significant associations after FDR correction. Warmer colors indicate higher genetic correlation (r_g) values.



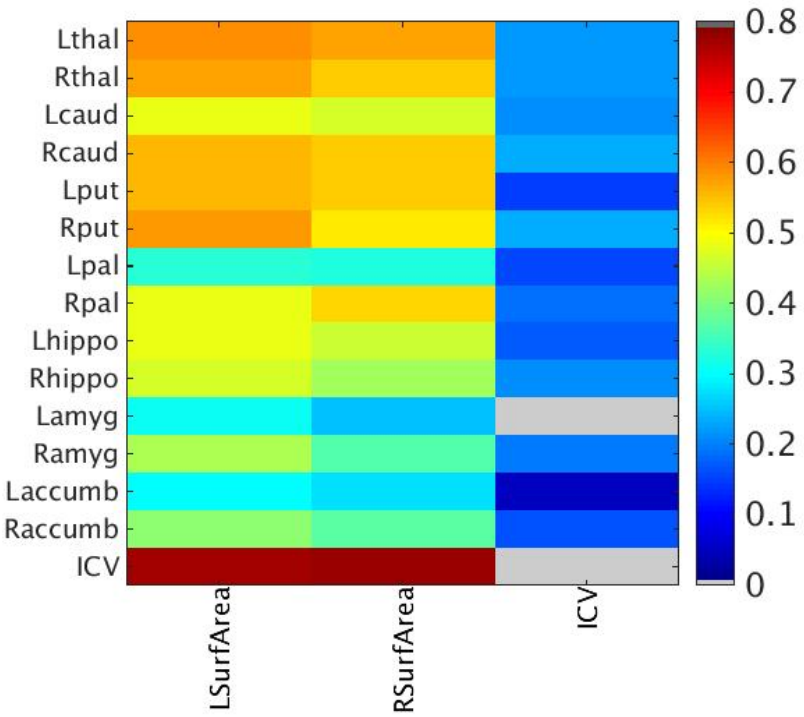
For tests of genetic correlation between CT & FA, the only significant correlations were of left and right hemisphere surface, ICV and various DTI tracts listed below in Figure 3.

Fig. 3. Genetic correlations between FA in DTI white-matter tracts, cortical surface area and ICV. Colored elements indicate significant associations after FDR correction. Warmer colors indicate higher genetic correlation (r_g) values.



Similarly in tests of genetic correlation between CT and volume, only surface area and ICV were significant, listed in Figure 4 .

Fig. 4. Genetic correlations between subcortical volume ROIs and cortical surface area measures. Colored elements indicate significant associations after FDR correction. Warmer colors indicate higher genetic correlation (r_g) values.



4 Discussion

Here we used cross-trait structural equation modeling in a twin design to study the common genetic influence between three categories of common MRI & DWI brain measures: cortical thickness, subcortical volume and FA in WM tracts. We found significant genetic association between several WM tracts and subcortical volume ROIs, notably the thalamus and pallidum, two regions highly connected to WM networks. Association between cortical thickness and both volume ROIs and WM was largely absent, however, cortical surface area for each hemisphere was highly correlated with FA in several white matter tracts and all of the subcortical volume measures. These results may be useful for future gene targeting studies and spur further investigation towards the genetic control of the anatomy underlying common imaging measures.

5 References

Benjamini Y & Hochberg Y (1995) Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. J Royal Stat Soc Ser B 1995;57(1):289–300.

Casey BJ, Tottenham N, Liston C, Durston S. Imaging the developing brain: what have we learned about cognitive development? Trends Cogn Sci. 2005 Mar;9(3):104-10. Review.

de Zubicaray GI*, et al.* (2008) Meeting the Challenges of Neuroimaging Genetics. *Brain Imaging Behav* 2(4):258-263.

Hibar DP, Stein JL, Renteria ME, Arias-Vasquez A, Desrivières S (2015) Common genetic variants influence human subcortical brain structures. Nature. Apr 9;520(7546):224-9.

Jahanshad N*, et al.* (2010) Genetic influences on brain asymmetry: a DTI study of 374 twins and siblings. *Neuroimage* 52(2):455-469.

Jahanshad N, Kohannim O, Hibar DP, Stein JL, McMahon KL, de Zubicaray GI, Medland SE, Montgomery GW, Whitfield JB, Martin NG, Wright MJ, Toga AW, Thompson PM. (2012) Brain structure in healthy adults is related to serum transferrin and the H63D polymorphism in the HFE gene. Proc Natl Acad Sci U S A. Apr 3;109(14):E851-9.

Neale MC, Cardon LR, & North Atlantic Treaty Organization. Scientific Affairs Division. (1992) *Methodology for genetic studies of twins and families* (Kluwer Academic Publishers, Dordrecht ; Boston) pp xxv, 496 p.

Rijsdijk FV & Sham PC (2002) Analytic approaches to twin data using structural equation models. *Brief Bioinform* 3(2):119-133.

6. Acknowledgments

This work was supported by a Consortium grant (U54 EB020403) from the NIH Institutes contributing to the BD2K Initiative, including the NBIB.