REDUCING STRUCTURAL VARIATION TO DETERMINE THE GENETICS OF WHITE MATTER INTEGRITY ACROSS HEMISPHERES – A DTI STUDY OF 100 TWINS

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ABSTRACT

Studies of cerebral asymmetry can open doors to understanding the functional specialization of each brain hemisphere, and how this is altered in disease. Here we examined hemispheric asymmetries in fiber architecture using diffusion tensor imaging (DTI) in 100 subjects, using high-dimensional fluid warping to disentangle shape differences from measures sensitive to myelination.

Confounding effects of purely structural asymmetries were reduced by using co-registered structural images to fluidly warp 3D maps of fiber characteristics (fractional and geodesic anisotropy) to a structurally symmetric minimal deformation template (MDT). We performed a quantitative genetic analysis on 100 subjects to determine whether the sources of the remaining signal asymmetries were primarily genetic or environmental. A twin design was used to identify the heritable features of fiber asymmetry in various regions of interest, to further assist in the discovery of genes influencing brain micro-architecture and brain lateralization. Genetic influences and left/right asymmetries were detected in the fiber architecture of the frontal lobes, with minor differences depending on the choice of registration template.

Index Terms— Diffusion Tensor Imaging (DTI), Genetics, Brain Asymmetry, Minimal Deformation Template, Fiber Architecture

1. INTRODUCTION

Asymmetries in brain structure and function have been a focus of neuroimaging studies for many years. Anatomical asymmetries can help understand the origins of lateralized cognitive functions or behavioral traits, such as language and handedness, affected by differences in the development of the two hemispheres [1]. Studies of brain asymmetry are also practically valuable as some theories posit that disorders such as schizophrenia, dyslexia, or hemiparesis, may arise from derailment in processes that lead to brain asymmetry. Deformation-based morphometry studies have used the theory of random Gaussian vector fields to detect statistical departures from the normal level of brain asymmetry [2].

Diffusion tensor imaging (DTI) is an MRI-based method to study water diffusion in tissue, and is particularly sensitive to neuronal myelination and white matter micro-architecture. It can also be used to study fiber connectivity within the brain. Various measures, including fractional and geodesic anisotropy, can be calculated from the local diffusion tensor; diffusion anisotropy is commonly used as an index of fiber integrity in each voxel.

The few DTI studies of fiber asymmetries have focused on specific tracts (e.g., the corticospinal tract, and the arcuate fasciculus involved in language processing). A recent DTI study in infants found significant asymmetries, with left greater than right fractional anisotropy (FA) in the frontal and temporal white matter [3], suggesting that these tracts may be more heavily myelinated in the left hemisphere [3].

Studies of white matter differences between brain hemispheres may be confounded by the vast structural asymmetries present. This is particularly true in frontal and occipital lobes, where the natural petalia (torquing) of the brain causes many right hemisphere structures to be shifted anterior to their left hemisphere counterparts [1]. Men may have greater anatomical asymmetries than women [1], making it advantageous to reduce these pronounced macro-structural differences when gauging the level of micro-structural asymmetry in a mixed sex population.

Twin studies have long been used to determine genetically and environmentally influenced human traits, but twin studies with DTI are rare. Monozygotic twins share all their genes while dizygotic twins share on average approximately half of their genes. Estimates of the

proportion of variance attributable to genes versus environment can hence be made in studies involving large groups of both these types of twins. Twin neuroimaging studies have been performed to determine the genetics of brain structure, such as cortical thickness, and gray and white matter volumes [4].

Here, we examined asymmetries (left/right hemisphere differences) in fiber architecture in a large twin population (N=100) using fractional and geodesic anisotropy (FA and GA) measures calculated from DTI. We aimed to adjust for the known structural differences between hemispheres by aligning brains to a symmetrized minimal deformation template created from the group data. We then determined whether genetic factors influenced the residual asymmetries.

2. METHODS

2.1. Image Acquisition

We acquired structural and diffusion tensor (DT) MRI scans from 100 subjects using a high magnetic field (4T) Bruker Medspec MRI scanner. Diffusion images were acquired using 30 gradients (3 with no diffusion sensitization (T2-weighted images) and 27 diffusion-weighted images uniformly distributed on the hemisphere). Parameters were: 23 cm FOV, TR/TE 6090/91.7ms, b-value =1132 s/mm^2, scan time: 3.05 minutes. Each 3D volume consisted of 21 5-mm thick axial slices with a 0.5mm gap and 1.8x1.8 mm^2 in-plane resolution. The subjects included 50 young adult (mean age 24.37 years, stdev 1.936) monozygotic (MZ) twins and 50 dizygotic (DZ) twins (25 pairs of each). All subjects were right-handed, and all twin pairs were of the same sex.

2.2. Creating the Minimal Deformation Template

T1-weighted structural MR images were edited to remove extracerebral tissues and linearly registered to a symmetrical template. This symmetrical template was created by averaging a high-resolution single subject average scan, the Colin27 [5], with the same image reflected along the midsagittal plane. This centered each subject's midline in the image volume. All subjects' images were linearly registered to the symmetrical template using FLIRT software (http://fsl.fmrib.ox.ac.uk/fsl/flirt) with 9-parameter (degrees of freedom) registration - as opposed to 12 to avoid shearing - and a correlation ratio cost function. The linearly registered images were then reflected over the midline. Four independent (one per pair) monozygotic twins and four independent dizygotic twin image volumes were randomly chosen along with their corresponding reflected images. These 16 image sets were then used to generate a minimal deformation target (MDT) using non-linear registration as described in [6,7]. The flipped images of the same brains were included in the construction of the MDT to ensure structural symmetry in the resulting image. All original 100 structural images were fluidly registered to the MDT using a 3D Navier-Stokes-based fluid warping technique enforcing diffeomorphic mappings, using least squares intensity differences as a cost function [6,7]. The 3D deformation fields obtained from each subject's MRI were retained.

For comparison, the same analysis was also performed with an MDT created only from images in their original orientation.

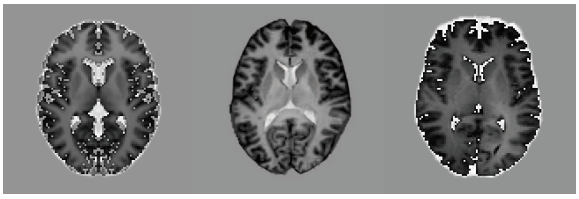

Figure 1: *Left:* Symmetric version of the Colin27 high-resolution MRI dataset used for linear alignment; *Center:* symmetrized MDT used for fluid registration; *Right:* non-symmetric MDT created from only non-flipped images.

2.3. Anisotropy Calculation and Registration

Diffusion tensors were computed from the diffusion weighted images and smoothed with Log-Euclidian tensor de-noising using MedINRIA software (http://www.sop.inria.fr/asclepios/software/MedINRIA). Extra-cerebral tissue was manually deleted from one directional component of the diffusion tensors creating a mask that was then applied to all components for that subject. Scalar images of anisotropy measures were created for each of the 100 subjects from the eigenvalues (λ_1, λ_2, λ_3) of the symmetric 3x3 diffusion tensor. These included the fractional anisotropy (FA), the geodesic anisotropy (GA) computed in the Log-Euclidean framework [8], and the hyperbolic tangent of the GA (tGA), which creates anisotropy maps with values comparable to the range of FA.

$$FA = \sqrt{\frac{3}{2}} \frac{\sqrt{(\lambda_1 - \bar{\lambda})^2 + (\lambda_2 - \bar{\lambda})^2 + (\lambda_3 - \bar{\lambda})^2}}{\sqrt{\lambda_1^2 + \lambda_3^2 + \lambda_3^2}} \qquad \bar{\lambda} = \frac{\lambda_1 + \lambda_3 + \lambda_3}{3} \quad (1)$$

$$GA(S) = \sqrt{Trace(\log S - \overline{\log SI})^2}$$
$$\overline{\log S} = \frac{Trace(\log S)}{3} \quad (2)$$

These images were individually aligned to the corresponding structural MR image already registered to the symmetric Colin27 template. Deformation fields obtained from the non-linear registration of the structural image to the MDT were then applied to the anisotropy measures.

2.4. Creating Asymmetry Maps

The mirror image of each aligned anisotropy map was then calculated, and the voxel-wise difference map between the original and flipped images was created. In this new map, the left side of the image represents the difference between the subjects' right and left hemispheres, with voxels in the other hemisphere having the opposite sign.

Voxel-wise Student's *t*-tests were used to determine significant differences between the original orientation of the image and its mirror image for all anisotropy measures examined. To eliminate the influence of intra-pair correlations on the degrees of freedom, the *t*-tests were run after selecting only one subject per twin pair. Non-parametric permutation based t-tests were conducted based on the correlation values found at each voxel to correct for multiple comparisons.

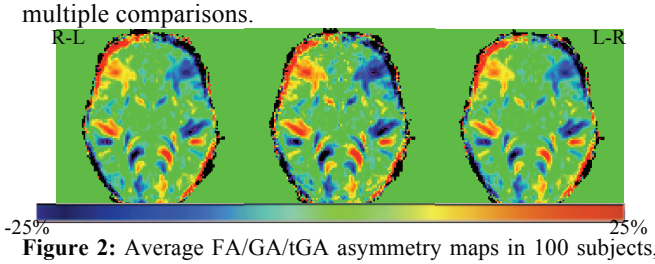

Figure 2: Average FA/GA/tGA asymmetry maps in 100 subjects, showing the percent differences. All anisotropy measures show similar patterns of asymmetry. R>L fiber asymmetry is particularly visible in frontal regions.

2.5. Genetic Analysis and Statistics

Voxel-wise maps of the intra-class correlations within monozygotic (MZ) and dizygotic (DZ) twins, r_{MZ} and r_{DZ} respectively, were derived as well as Falconer's heritability estimate [8] for the asymmetry in FA, GA and tGA.

Falconer's heritability: $h^2 = 2(r_{MZ} - r_{DZ})$ (3)

Average measures of the anisotropy difference were examined in certain regions of interest (ROIs). We determined the genetic contribution to the asymmetries in each lobe of the brain, and in the overall gray and white matter components. ROIs were traced for the four lobes (frontal, parietal, temporal, and occipital) in one hemisphere of the template MDT brain and were flipped to define the same ROI in the opposite hemisphere. This ensured consistency between hemispheres and reduced errors due to manual labeling. Gray and white matter ROIs were extracted automatically using BrainSuite2 software (http://www.loni.ucla.edu/Software/BrainSuite2).

Observed covariances for the average anisotropy value in each ROI between pairs of MZ and DZ twins were used to calculate the expected covariance using a univariate structural equation model (SEM) to determine additive genetic (A), shared environmental (C) and unique environmental (E) components of asymmetry [10]. The ACE genetic model was fitted for FA, GA, and tGA values in each lobe and for total gray and white matter regions using a maximum-likelihood estimate, with a χ^2 goodness of fit probability. Mx software (http://www.vcu.edu/mx/) was used.

Expected Covariance

$$\Sigma = \begin{bmatrix} a^2 + c^2 + e^2 & \alpha a^2 + c^2 \\ \alpha a^2 + c^2 & a^2 + c^2 + e^2 \end{bmatrix} \quad (4)$$

α =1 for MZ, 0.5 for DZ

3. RESULTS

Overall asymmetries were examined for FA, GA and tGA maps by averaging difference (original minus flipped) maps for subjects (Fig. 2). FA was lateralized in the temporal lobe (Fig. 4), consistent with findings in infants in [3].

Differences in the intra-class correlation of the MZ and DZ twins led to a heritability map (Figure 4). The cingulum shows high heritability for the FA, GA and tGA.

In the SEM, if $p<0.05$ for the χ^2 goodness of fit test, then the ACE genetic model is not necessarily a good fit to the data. Results for which this occurred included all ROIs for the GA (except those of the frontal lobe) and the parietal lobe tests for all anisotropies; these results are consistent with those in [11] and have been eliminated from Figure 5.

Figure 5 shows the relative proportion of genetic versus environmental variance for the FA and tGA asymmetry maps. 40-50% of the variance in asymmetry in the frontal and occipital lobes is due to genetic factors, and heritability measures are high. tGA appears to identify some genetic components of variance in the temporal lobe while FA measures do not. Figure 6 shows these results for all three anisotropy measures in the frontal lobe.

Figure 7 shows the effects of creating a symmetrical MDT from images in their original orientation and their flipped versions. The estimates of the genetic contributions to variance went up or down, by around 4%, depending on the template chosen; this is important to know in genetic studies so that the sources of variance are understood. FA asymmetries can arise due to inter-hemispheric differences in at least 3 factors: (1) anatomical shape and cortical patterning, (2) tract trajectories, and (3) signal differences between homologous tracts. If genes influence the first factor, then after adjusting for it, genetic proportions of the variance in the remaining signals may go up or down. Many heritability studies aim to find features to associate with polymorphisms (individual variations) in specific genes. For these studies to be interpreted correctly, it is important that the factors contributing to signal asymmetries are separated.

4. DISCUSSION & FUTURE WORK

Here we determined brain regions where white matter differs significantly between hemispheres, and we found a very high genetic involvement (40%-50%) in this asymmetry. The micro-architecture patterns of the frontal lobe were highly genetic. We reduced the impact of structural variations on heritability maps by creating a symmetrized minimal deformation template from all the individual data. Initial results appear promising, but it is clear that structural variations were not fully eliminated (Figure 2 – asymmetry variations occur at the edge of the brain). Further research could use a more refined MDT, including cortical surface matching where possible, to further adjust for anatomical shape and tract trajectory differences between hemispheres.

The ACE genetic model detected no genetic influences in the parietal lobes, which is intriguing; a larger sample may be needed to detect them. We are refining our definition of FA to avoid problems with the single-tensor model. Computing anisotropy from the full set of high angular resolution diffusion images (HARDI) using measures such as the recently proposed Tensor Distribution Function (TDF; [12]) should allow a more precise mapping of the genetic influences of fiber integrity.

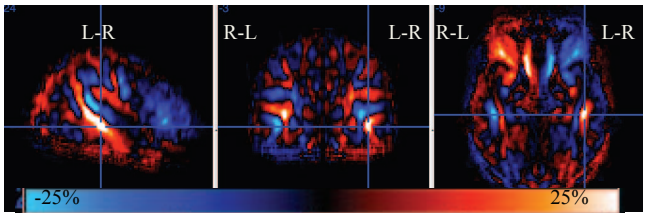

Figure 3 High degree of L>R FA asymmetry in the temporal lobe.

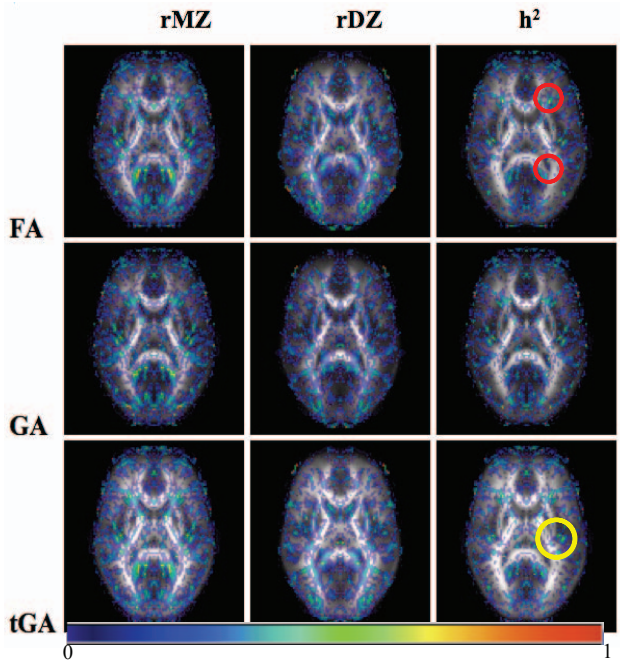

Figure 4 Differences in intra-class correlations for monozygotic (rMZ) and dizygotic (rDZ) twins are used to compute a heritability map (the proportion of the variance signal asymmetry that is attributable to genetic factors). Areas of high heritability include the cingulum (shown in red on the FA map) and regions associated with language processing (shown in yellow on the tGA map)

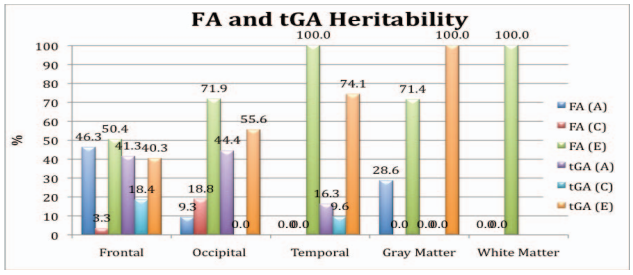

Figure 5 Genetic (A) and environmental (C) components are shown for the FA (left 3 bars) and tGA (right 3 bars), as a percentage of the overall variance. ACE values for each lobe in the two hemispheres were almost identical, average values are shown.

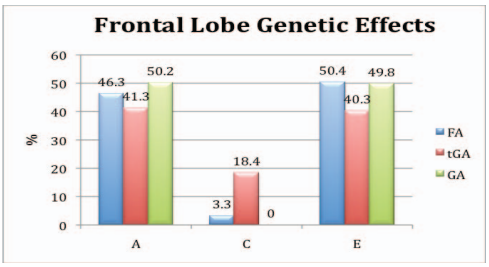

Figure 6 All anisotropy measures examined show high genetic heritability for frontal asymmetry.

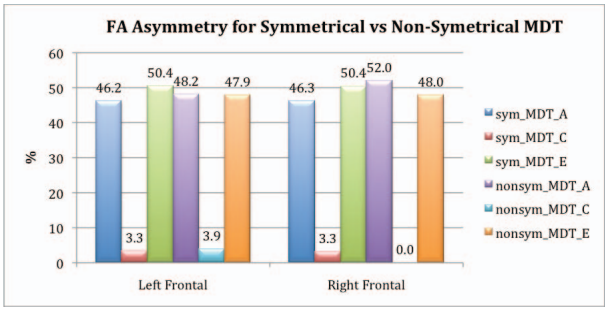

Figure 7 Comparisons between the symmetrical MDT created for this study and one from images of original orientation only reveal differences of ~ 4% in the contribution of variance to genetics.

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