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Meeting the Challenges of Neuroimaging Genetics

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Abstract As research encompassing neuroimaging and genetics gains momentum, extraordinary information will be uncovered on the genetic architecture of the human brain. However, there are significant challenges to be addressed first. Not the least of these challenges is to accomplish the sample size necessary to detect subtle genetic influences on the morphometry and function of the healthy brain. Aside from sample size, image acquisition and analysis methods need to be refined in order to ensure optimum sensitivity to genetic and complementary environmental influences. Then there is the vexing issue of interpreting the resulting data. We describe how researchers from the east coast of Australia and the west coast of America have embarked upon a collaboration to meet these challenges using data currently being collected from a large-scale twin study, and offer some opinions about future directions in the field.

Keywords Neuroimaging · Genetics · Heritability · High-angular resolution diffusion imaging (HARDI) · ACE modeling

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Evidence that genetic and environmental factors shape human brain structure and function has been accumulating for nearly two centuries. Our knowledge has broadened considerably since the early attempts to identify genetic variations in cranial capacity and brain weight. Modern neuroimaging technology offers an unprecedented opportunity to search for genetic and environmental influences among thousands of voxels in individual high resolution brain images. Moreover, the data acquired has the potential to enhance our understanding of human behaviour by serving as an intermediate phenotype. Like any emerging field, neuroimaging genetics faces its own share of challenges. In this article, we describe some of those challenges and provide examples of how we have attempted to address them in our own research with magnetic resonance imaging (MRI).

At the time of writing this article, reviews of MRI investigations of monozygotic (MZ) and dizygotic (DZ) twins have already concluded that brain macrostructure is significantly heritable (e.g., grey and white matter, lobar and whole brain volumes; see Peper et al., 2007; Schmitt et al., 2007) and that volumetric measures can serve as intermediate phenotypes for intellectual performance (IQ; e.g., Toga & Thompson, 2005). There is also evidence emerging from the first fMRI studies in MZ and DZ twins that task-related brain activity might be significantly heritable (e.g., Blokland et al., 2008; Matthews et al., 2007; Polk et al., 2007; cf. Côté et al., 2007). The quantitative twin model is a powerful approach for studying

the relative contributions of genetic (i.e., heritable) and environmental influences on variability in brain morphometry and function (Peper et al., 2007). This is because MZ twins share all (100%) of their genes, whereas DZ twins share, on average, half of their genes. MZ and DZ twins are also likely to share similar upbringings within families. When imaging phenotypes show greater correlations for MZ than DZ twin pairs, heritability is inferred. If the correlations between MZ twins are not twice the size of those between DZ twins, then, in addition to genes, common or shared environmental factors may also be involved. When MZ twin pairs show little resemblance on a given measure, environmental factors unique to one twin may be at play.

Sample size and replication

Although the results of these studies appear promising, the emerging field of neuroimaging genetics is yet to address the issue of *replicability*. This issue was problematic for early behavior genetic studies due, in part, to the small sample sizes employed. Results could not always be corroborated. A recent review of twin MRI studies indicates that heritability estimates for smaller brain structures tend to be low and show considerable variability across studies (Schmitt et al., 2007). This might also be due to the reliability of some methods used to define these structures, as a variety of methods have been employed across studies, including regions of interest (ROIs) defined manually or otherwise and voxel-based analyses. This is where replication will be informative because unreliability puts a ceiling on heritability estimates. MZ co-twins cannot be more similar than themselves. While it is possible that genetic polymorphisms might have a more robust impact at the level of the brain than at the level of behavior, the main limitation of small sample sizes is that they do not afford the power to test for the influence of common environment (Peper et al., 2007). Consequently, future neuroimaging studies will require large sample sizes to achieve sufficient power to detect true associations (see Glahn, Thompson & Blangero, 2007).

Several large-scale twin studies are currently underway in North America and in the Netherlands. Recently, we embarked on a 5-year large-scale neuroimaging study of healthy Australian twins and their siblings. In addition to investigating heritability, with a projected sample size of approximately 1150 at completion, polymorphisms that account for as little as 2.5% of the variance in brain morphometry and function will be detectable. The project will use genome wide association scans (GWAS) to identify genetic polymorphisms spread across the entire genome that influence measures of brain morphometry and function. GWA studies extend the allelic association (candidate gene) design to the level of the whole genome, and are 'hypothesis free' allowing the identification of previously unsuspected genes and pathways. Last year saw the publication of results of the first GWA studies for a range of diseases and quantitative phenotypes (Wellcome Trust Case Control Consortium, 2007). Results from GWA show that associated variants have small effect size (Odds ratio 1.1); large sample sizes are needed to have the power to detect variants of this effect size.

The Australian twin sample is unusual in that it is already well characterized psychometrically and undergoes repeat testing at regular intervals (Wright & Martin, 2004), thus providing important cognitive phenotypes such as IQ, language and working memory for correlating with the imaging measures being obtained. Moreover, the sample has already been employed to test the replicability of genetic effects on cognition reported by other groups around the world (e.g., Bates et al., 2007; Luciano et al., 2008). Of course, there might be cultural factors unique to the Australian study that could conceivably impact upon the imaging results, introducing environmental variability when comparing the sample with others around the world. For example, the measures of academic achievement being administered in the cognitive study reflect a predominantly state-based education curriculum. Consequently, multi-national replication studies of intermediate neuroimaging phenotypes will be especially important in the future.

A project of this size and scope would not be successful without the collaborative efforts of researchers located across the Pacific: images are acquired in Brisbane and analyzed in Los Angeles, leveraging the skills of the respective research groups as we describe below.

From macrostructure to microstructure

In addition to employing large samples, neuroimaging genetics studies will need to optimize the sensitivity of their image acquisitions and analyses to detect subtle effects. Most prior studies have examined macrostructural features such as gray and white matter volumes (Peper et al., 2007; Schmitt et al., 2007). Measures of white matter microstructure, for example, are likely to be more informative (Pfefferbaum et al., 2001). High angular resolution diffusion imaging (HARDI) provides measures of anisotropic water diffusion in brain tissue corresponding to patterns of myelination, assessed using a number of diffusion-sensitized gradients during image acquisition. The only twin study to date of white matter microstructure used a clinical sequence comprising 6 gradient directions (Pfefferbaum et al., 2001). This small sample study (15MZ, 18DZ twin pairs) used structural equation modeling (SEM)

to examine the heritability of the corpus callosum, deriving the proportion of trait variance due to additive genetic (A; i.e., heritable), common or shared environment (C), and unique or random environment (E). Whereas for volume estimates similar heritability was indicated for both the genu and splenium, the fractional anisotropy (FA) measure of fiber integrity showed a greater heritability for the splenium. This result is intriguing as it indicates interhemispheric connections in frontal cortical regions are influenced more by environmental factors, consistent with their myelination occurring relatively late in development (Gogtay et al., 2004).

Fiber integrity is now measured routinely with FA in clinical studies. However, more interesting information about genetic influences might be gleaned from the geometries of fiber pathways. This necessitates increasing the number of gradient directions during HARDI acquisition to enable reconstruction of orientation distribution functions (ODFs) giving the probability of directional water diffusion within each voxel. We recently calculated ODFs for a 30-direction spherical HARDI acquisition in a preliminary study of 22MZ and 23DZ twin pairs, after fluidly registering the 90 datasets to the ICBM atlas space (Fig. 1; Chiang et al., 2008).

Using the ACE structural model combined with permutation testing, we analyzed genetic influences on novel maps of generalized fiber anisotropy (GFA) and Jensen–Shannon divergence (JSD), the latter a measure of the regional divergence of directional water diffusion, or fiber complexity. We fitted four alternative models to the data (ACE, AE, CE, E), systematically testing the significance of A, C, and E effects. Figure 2 shows the covariance structure fitting for GFA and JSD maps in the 90 twins. Interestingly, for both GFA and JSD measures an AE model (i.e., additive genetic and unique environmental effects) was the best fit for more voxels than any other model, indicating that cortical white matter microstructure is generally more genetically than environmentally influenced.

While this approach will continue to provide important information about genetic contributions to microstructural features at a regional level, it might also be informative to examine genetic influences on fiber pathways or tracts. The extent to which specific tracts are influenced by genes, the environment, or their interaction, will be a key area of future research as they can also be linked to cognitive processes (e.g., the arcuate fasciculus and language). However, tractography studies of smaller fiber pathways will require many more gradient directions to be acquired in order to overcome problems related to partial voluming in voxels where fibers cross. Put simply, the goal is to achieve the highest possible angular resolution data within an acceptable scan time.

We recently implemented a 94-direction spherically distributed HARDI acquisition with 1.8mm^2 in-plane resolution in our twin sample, with a total scan time at 4 Tesla of 14.5 minutes. The results of preliminary analyses are promising. By comparing reconstructed subsets of increasing numbers of gradient images, we found signal-to-noise ratio (SNR) for measures including FA improved dramatically as the number of directions increased from 20 to 94. However, acquiring such high angular resolution data

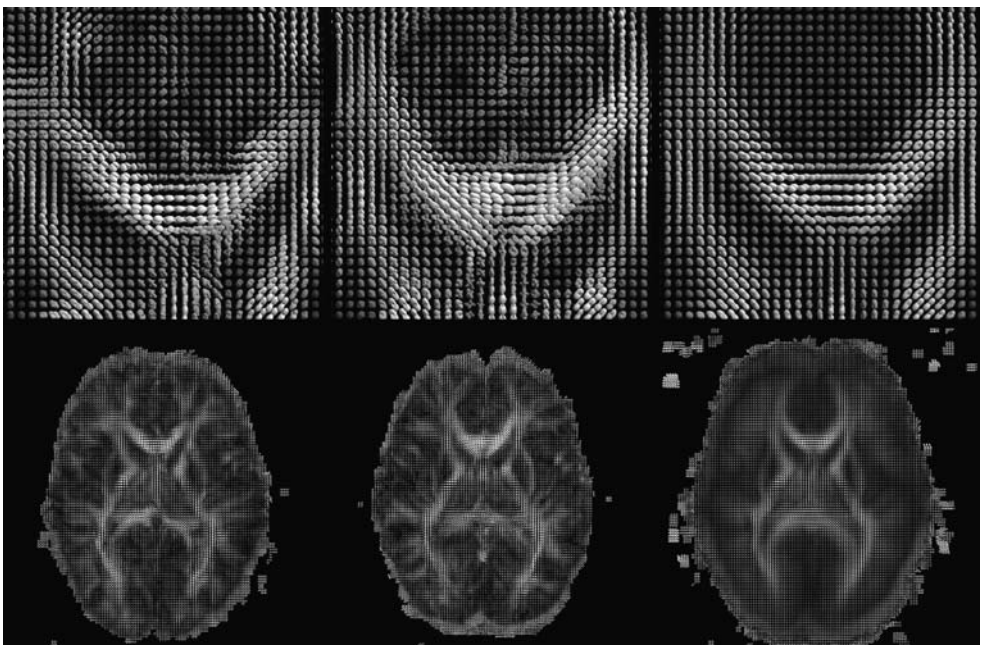

Fig. 1 Orientation distribution functions (ODFs) of brain white matter fiber pathways calculated from HARDI data from (*left*) a single subject, (*middle*) a second subject after data alignment to match the first subject, and (*right*) an average of 90 subjects. Zoomed views of each image are shown at top. Note that the average HARDI from 90 subjects retains significant shape characteristics

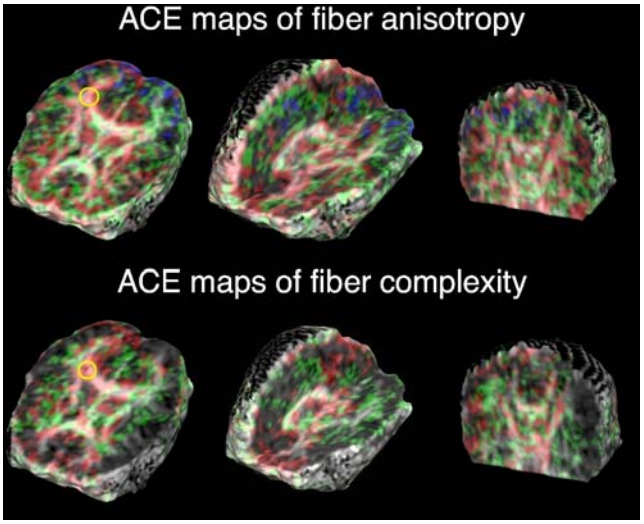

Fig. 2 Brain maps of white matter fiber integrity (GFA; *top*) and complexity (JSD; *bottom*) color-coded according to genetic or environmental effects. Voxels where the AE model fit best are coded red (indicating significant heritability), CE *green* and E *blue* (indicating common and unique environmental effects, respectively). Note that for both HARDI measures AE and CE models fit best. The cingulum AE model fit is *circled in yellow*

required a novel solution for visualizing the information. A viewer needed to be developed specifically for this purpose (Fig. 3). One of the first steps will be to fit ACE models for each fiber tract identified.

From structure to function

The extent to which individual differences in neural activity, as measured by fMRI, are influenced by genetic or environmental factors is largely unknown and will therefore be a focus for much future research. Researchers using fMRI already contend with a number of interpretive issues, not the least being the complex physiology of the blood oxygen level dependent (BOLD) response and the observation that some activation elicited by a task might be epiphenomenal, i.e., not related directly to the cognitive process being manipulated. Detecting genetic influences in this context will require careful attention to experimental design and interpretation.

To date, only three fMRI studies in relatively small samples of normal twins have been published using quite different tasks, different analysis methods and reporting quite different results (Côté et al., 2007; Matthews et al., 2007; Polk et al., 2007). Côté et al. (2007) investigated genetic and environmental influences on brain activation associated with the subjective experience of sadness. Despite the relatively large sample employed (47MZ, 57DZ pairs), they failed to find evidence for genetic *or* shared environment effects on patterns of brain activation; the results were best explained by unique environmental effects. In a smaller sample (13MZ, 11DZ pairs), Polk et al. (2007) found that visual cortical activity associated with processing of pictures of faces and houses was more similar in MZ than DZ twins, while processing of pictures of chairs and pseudowords did not show greater similarity for MZ twins. Matthews et al. (2007; 10MZ, 10DZ pairs) were able to detect a genetic effect, finding moderately heritable anterior cingulate cortex (ACC) activation during interference processing.

One issue that is already apparent from these studies concerns the selection of an appropriate measure of brain activation: Whereas Côté et al. (2007) used voxel counts and peak *Z*-scores within ROIs, Polk et al. (2007) used *t*-scores and ß-values, and Matthews et al. (2007) extracted percent BOLD signal from their ROIs. In a recent fMRI study of 60 pairs of twins (29MZ, 31DZ), we found that the former method suffers from restriction of range issues. Many of our sample (approximately 33%) had no suprathreshold activation within ROIs (at a liberal $p < .05$ threshold) and/or *Z*-scores had reached ceiling (Blokland et al., 2008). Polk et al. (2007) likewise found that two of their MZ twins failed to demonstrate suprathreshold activation within their ROIs, leading them to exclude the data from analysis. Another issue of note concerns analysis methods adopted to demonstrate genetic effects; whereas Côté et al. (2007) and Matthews et al. (2007) used the conventional ACE structural model, Polk et al. (2007) compared the similarity of the activation maps within MZ and DZ twin pairs by conducting statistical tests on the correlation coefficients. This latter method, while providing some information about the heritability of task-based brain activation, is limited in that it does not provide a measure of environmental effects.

Our fMRI study, the first to examine genetic and environmental influences on brain activation during perfor-

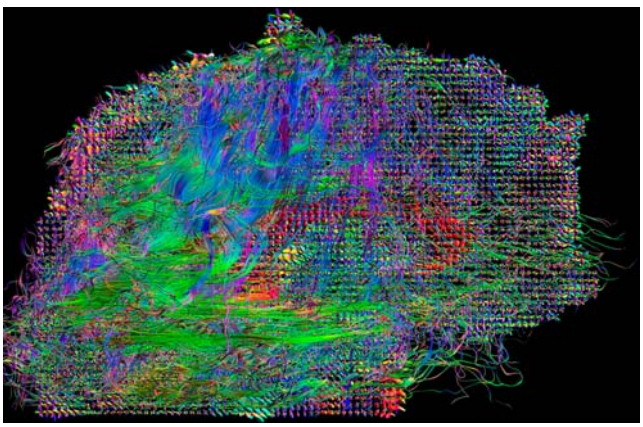

Fig. 3 Color-coded tractography through the orientation distribution functions (ODFs) of brain white matter fiber pathways from a single subject's HARDI acquisition

mance of an N-back working memory task, found MZ correlations in *a priori* defined frontal and parietal cortical ROIs for 2- > 0-back BOLD signal changes that were, on average, approximately twice those of the DZ pairs, although with non-significant heritability estimates (14–30%). We found no evidence for genetic influences on ACC activation. These results contrasted with stronger heritability estimates for the behavioral data (57–73%). While this result might be due to the small sample size we employed, it is worth noting that Matthews et al. (2007) reported significant heritability of ACC activation with far fewer subjects. Again, the replicability of these results will need to be addressed. We will revisit the N-back task when more of our cohort is imaged.

Beyond heredity

In addition to studies of heritability, candidate gene association studies in healthy non-family cohorts have begun to reveal single-nucleotide polymorphisms (SNPs) associated with alterations in brain structure and function. For example, healthy carriers of the apolipoprotein E epsilon-4 gene (ApoE e4; a risk factor for Alzheimer's disease) have smaller cortical structures and larger ventricular volumes than non-carriers (e.g., Chou et al., 2008). Variability in working memory performance and prefrontal cortical activation elicited during fMRI has also been associated with a polymorphism of the COMT (catechol-*O*-methyltransferase) gene, a risk factor for schizophrenia (Tan et al., 2007).

The search for phenotypes in neuroimaging studies has been largely informed by clinical investigations of genetic polymorphisms that are commonly overexpressed in specific patient populations. This approach, while useful, is limited in that (1) many disorders are polygenic and (2) it does not identify novel polymorphisms that might be associated with variability in normal brain morphometry and function. Recently, an alternative approach was adopted by Papassotiropoulos et al. (2006). These researchers first performed large-scale GWA studies to identify a memory-retrieval related SNP of the *KIBRA* gene, followed by gene expression studies to determine where in the brain the gene was expressed. Only then was functional MRI used with an appropriate task designed explicitly to target those brain structures, revealing differences in retrieval-related hippocampal activation according to allelic status. Constraining hypotheses in this manner is likely to be of considerable assistance in reducing false positive findings in neuroimaging genetics data.

While the results of these initial neuroimaging genetic studies are promising, it is worth noting that GWA in non-familial cohorts can be prone to false-positives due to differences in ancestry, and requires additional controls (the "stratification" problem; Freedman et al., 2004). Hence, large twin cohorts will play an important role in future neuroimaging research identifying QTLs.

Future prospects

Our review has highlighted several potential developments for future neuroimaging genetics studies. The first is that these studies will entail much larger sample sizes than the majority of investigations published to date. This will ensure sufficient power to test for the influence of environmental effects in addition to genetic ones. Acquiring data on this scale will require concerted and long-term efforts by behavioural geneticists and neuroimaging researchers. Second, while early results from fMRI studies are encouraging, evidence that genetic associations with brain activity are reproducible within cohorts is needed. This suggests that future studies will ultimately incorporate test-retest designs to demonstrate reliable genetic effects. We are currently acquiring this data with our N-back task in a subset of twins. Third, although candidate gene approaches have had some success identifying genetic contributions to altered brain morphology and function in neuropsychiatric disorders, future studies will be able to exploit their much larger samples to conduct GWA investigations, permitting previously unsuspected genes and pathways involved in normal morphology and function to be discovered. This will broaden both the perspective and relevance of the field of neuroimaging genetics. Finally, once genetic associations have been confirmed, it will be important to identify intermediate neuroimaging phenotypes for more specific cognitive processes. We are already aware that volumetric indices can serve as intermediate phenotypes for broad measures of cognition such as IQ (Toga & Thompson, 2005). Many more discoveries can be expected as neuroimaging methods are refined.

Given the data currently being acquired in large cohorts, changes across the lifespan could also be examined by extending these studies to longitudinal designs. This would address important questions about the stability of genetic or environmental influences on brain morphometry and function. One study has already sought to answer these questions using images acquired 4 years apart in 34MZ and 37DZ elderly twin pairs (Pfefferbaum et al., 2004). Heritability estimates of macrostructural measurements of the corpus callosum and lateral ventricles did not change over the period studied, but the volumes did show evidence of further environmental influences. Much further ahead, neuroimaging genetics may benefit from MRI contrast mechanisms based on gene expression (Westmeyer & Jasanoff, 2007). However, this research is currently in its infancy, and little work has been performed *in vivo*.

Conclusions

In this article, we described some of the challenges facing the emerging field of neuroimaging genetics and some of our own attempts to meet them. Our research demonstrates the feasibility of generating large-scale brain maps of genetic and environmental influences, using multi-modality imaging. To this end, the collaboration between researchers across the Pacific Rim has been invaluable. We look forward to future discoveries.

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References

Bates, T. C., Luciano, M., Castles, A., Coltheart, M., Wright, M. J., & Martin, N. G. (2007). Replication of reported linkages for dyslexia and spelling and suggestive evidence for novel regions on chromosomes 4 and 17. *European Journal of Human Genetics*, *15*, 194–203. doi:10.1038/sj.ejhg.5201739.

Blokland, G. A., McMahon, K. L., Hoffman, J., Zhu, G., Meredith, M., Martin, N. G., Thompson, P. M., de Zubicaray, G. I., Wright, M. J. (2008). Quantifying the heritability of task-related brain activation and performance during the N-back working memory task: a twin fMRI study. in press, doi:10.1016/j.biopsycho.2008.03.006

Chiang, M.-C., Barysheva, M., Lee, A. D., Madsen, S. K., Klunder, A. D., Toga, A. W., McMahon, K. L., de Zubicaray, G. I., Meredith, M., Wright, M. J., Srivastava, A., Balov, N., & Thompson, P. M. (2008). Mapping genetic influences on brain fiber architecture with high angular resolution diffusion imaging (HARDI), 5th IEEE Symposium on Biomedical Imaging (ISBI 2008), 871–874.

Chou, Y-Y., Lepore, N., de Zubicaray, G. I., Carmichael, O., Becker, J., Toga, A., & Thompson, P. M. (2008). Automated ventricular mapping with multi-atlas fluid image alignment reveals genetic effects in Alzheimer's disease. *NeuroImage*, *40*, 615–630.

Côté, C., Beauregard, M., Girard, A., Mensour, B., Mancini-Marie, A., & Perusse, D. (2007). Individual variation in neural correlates of sadness in children: a twin fMRI study. *Human Brain Mapping*, *28*, 482–487.

Freedman, M. L., Reich, D., Penney, K. L., McDonald, G. J., Mignault, A. A., Patterson, N., Gabriel, S. B., Topol, E. J., Smoller, J. W., Pato, C. N., Pato, M. T., Petryshen, T. L., Kolonel, L. N., Lander, E. S., Sklar, P., Henderson, B., Hirschhorn, J. N., & Altshuler, D. (2004). Assessing the impact of population stratification on genetic association studies. *Nature Genetics*, *36*, 388–393.

Glahn, D. C., Thompson, P. M., & Blangero, J. (2007). Neuroimaging endophenotypes: strategies for finding genes influencing brain structure and function. *Human Brain Mapping*, *28*, 488–501.

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaitiuzis, C., Nugent, T. F., Herman, D. H., Classen, L., Toga, A. W., Rapoport, J. L., & Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood and adolescence. *Proceedings of the National Academy of Sciences U S A*, *101*, 8174–8179.

Luciano, M., Lind, P., Deary, I., Payton, A., Posthuma, D., Butcher, L., Bochdanovits, Z., Whalley, L., Visscher, P., Harris, S., Polderman, T., Davis, O., Wright, M., Starr, J., de Geus, E., Bates, T., Montgomery, G., Boomsma, D., Martin, N., & Plomin, R. (2008). Testing replication of a 5-SNP set for general cognitive ability in six population samples. *European Journal of Human Genetics*. doi:10.1038/ejhg.2008.100.

Matthews, S. C., Simmons, A. N., Strigo, I., Jang, K., Stein, M. B., & Paulus, M. P. (2007). Heritability of anterior cingulate response to conflict: an fMRI study in female twins. *NeuroImage*, *38*, 223–227.

Papassotiropoulos, A., Stephan, D. A., Huentelman, M. J., Hoerndli, F. J., Craig, D. W., Pearson, J. V., Huynh, K. D., Brunner, F., Corneveaux, J., Osborne, D., Wollmer, M. A., Aerni, A., Coluccia, D., Hänggi, J., Mondadori, C. R., Buchmann, A., Reiman, E. M., Caselli, R. J., Henke, K., & de Quervain, D. J. (2006). Common Kibra alleles are associated with human memory performance. *Science*, *314*, 475–478.

Peper, J. S., Brouwer, R. M., Boomsma, D. I., Kahn, R. S., & Pol, H. E. H. (2007). Genetic influence on human brain structure: a review of brain imaging studies in twins. *Human Brain Mapping*, *28*, 464–473.

Pfefferbaum, A., Sullivan, E. V., & Carmelli, D. (2001). Genetic regulation of regional microstructure of the corpus callosum in late life. *Neuroreport*, *12*, 1677–1681.

Pfefferbaum, A., Sullivan, E. V., & Carmelli, D. (2004). Morphological changes in aging brain structures are differentially affected by time-linked environmental influences despite strong genetic stability. *Neurobiology of Aging*, *25*, 175–183.

Polk, T. A., Park, J., Smith, M. R., & Park, D. C. (2007). Nature versus nurture in ventral visual cortex: a functional magnetic resonance imaging study of twins. *Journal of Neuroscience*, *27*, 13921–13925.

Schmitt, J. E., Eyler, L. T., Giedd, J. N., Kremen, W. S., Kendler, K. S., & Neale, M. C. (2007). Review of twin and family studies on neuroanatomic phenotypes and typical neurodevelopment. *Twin Research and Human Genetics*, *10*, 683–694.

Tan, H. Y., Chen, Q., Goldberg, T. E., Mattay, V. S., Meyer-Lindenberg, A., Weinberger, D. R., & Callicott, J. H. (2007). Catechol-O-methyltransferase Val158Met modulation of prefrontal-parietal-striatal brain systems during arithmetic and temporal transformations in working memory. *Journal of Neuroscience*, *27*, 13393–13401.

Toga, A. W., & Thompson, P. M. (2005). Genetics of brain structure and intelligence. *Annual Review of Neuroscience*, *28*, 1–23.

Wellcome Trust Case Control Consortium (2007). Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*, *447*, 661–678.

Westmeyer, G. G., & Jasanoff, A. (2007). Genetically controlled MRI contrast mechanisms and their prospects in systems neuroscience research. *Magnetic Resonance Imaging*, *25*, 1004–1010.

Wright, M. J., & Martin, N. G. (2004). Brisbane adolescent twin study: outline of study methods and research projects. *Australian Journal of Psychology*, *56*, 65–78.