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Editorial

Imaging the genetics of brain structure and function

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ABSTRACT

Imaging genetics combines brain imaging and genetics to detect genetic variation in brain structure and function related to behavioral traits, including psychiatric endpoints, cognition, and affective regulation. This special issue features extensive reviews of the current state-of-the-art of the field and adds new findings from twin and candidate gene studies on functional MRI. Here we present a brief overview and discuss a number of desirable future developments which include more specific *a priori* hypotheses, more standardization of MRI measurements within and across laboratories, and larger sample sizes that allows testing of multiple genes and their interactions up to a scale that allows genetic whole genome association studies. Based on the overall tenet of the contributions to this special issue we predict that imaging genetics will increasingly impact on the classification systems for psychiatric disorders and the early detection and treatment of vulnerable individuals.

Biological Psychology, quite literally, deals with the connection between the body and the mind. In the past two decades two specific instances of body-mind connections have captured the imagination of many researchers in this broad field: (1) the strong role of genetic variation in individual differences in cognition and emotion, including susceptibility for cognitive and mood disorders, and (2) our ability to directly monitor the link between brain activity and cognitive and affective processes using functional MRI technology. This special issue deals with the inevitable synthesis of these two fields, the use of brain imaging in genetically informative designs. This new field of 'Imaging Genetics' is rapidly emerging. The search term 'brain imaging and (cognition or emotion)' yields 7574 hits in PubMed, and the term 'genetics and (cognition or emotion)' yield a similar huge amount of 7297 hits. However, a combination of all three terms also already yields a respectable 467 hits. The current special issue will add to this growing field. Five invited reviews summarize the ongoing work in imaging genetics from various different perspectives, focusing respectively on aging (Mattay et al., 2008), early development (Fossella et al., 2008), executive function (Greene et al., 2008), emotion regulation (Aleman et al., 2008), and neurobehavioral syndromes related to chromosomal aberrations (Bearden et al., 2008). These reviews allow readers who are new to imaging genetics to rapidly catch up, but they also present a succinct summary of the state-of-the-art for those already engaged in this research. The reviews are followed by seven empirical papers that add to the growing knowledge of how genetic variation affects brain structure and function (Blokland et al., 2008; Wolfensberger et al., 2008; Den Braber et al., 2008; Szeszko et al., 2008; Reuter et al., 2008; Canli et al., 2008; Puls et al., 2008). To provide focus, all papers employ MRI as the main imaging strategy.

1. Imaging genetics

What exactly is imaging genetics? In the opening review of this special issue four pioneers of this field have formulated an elegant answer to this question which we repeat ad verbatim: "*Imaging genetics is a form of genetic association analysis, in which the phenotype is not a disease, symptom complex or behavior but a measure of brain structure (volume), chemistry or function (physiological response of the brain during information processing). It is based on the assumption that brain structure, chemistry and function are closer to gene function than trait differences in overt behavior. The advantage of imaging genetics over traditional strategies for phenotyping brain function based on neuropsychological tests and personality inventories is that it makes possible a more direct measurement of the impact of the gene at the level of information processing and/or neurochemistry within discrete brain regions and/or networks in the context of specific informational load. In contrast, traditional behavioral measures or test scores are more complex, can be affected by the use of alternate task strategies, level of cooperation, etc. that can mask potential gene effects on the underlying neural substrates meant to be engaged by the tests.*" (Mattay et al., 2008).

It is important to note that imaging genetics does not aim to replace 'ordinary' genetic association studies at the level of complex symptom patterns and behavioral traits; it aims to complement these studies. By now, it is widely appreciated that the heritability of these traits derives from very many genes of small effect that are hard to detect, even with the huge improvements in our molecular genetic tool-kit. Most genetic variation surviving natural selection may code for very subtle changes in protein function that create minute changes in the development of neuronal networks. Variation in complex behavior

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emerges only at the end of a large chain of such small gene effects. The closer we place our measurements to the level of the neuronal circuitry, the larger the effect of a single gene may be. In keeping with this core idea behind imaging genetics, robust association between genetic variation and brain responses has emerged in studies that had only modest sample sizes (Munafò et al., 2007). These sample sizes would not have allowed detection of genetic effects on the emergent behavioral endpoints studied in psychiatric genetic studies.

2. Twin studies

The first fundamental question in imaging genetics is whether variation in structural and functional MRI traits represents genetic variation at all. A solid assessment of heritability of a trait can be achieved by twin family studies, that compare trait resemblance in genetically identical participants (monozygotic, MZ twins) with trait resemblance in participants that share only half of their genetic material (dizygotic, DZ twins or siblings). For brain structure, substantial heritability has been firmly established by twin studies, both at the level of large volumes (e.g. total gray matter, total cerebellar size) (Baaré et al., 2001; Posthuma et al., 2000) as well as regional volumes (e.g. frontal lobe) or even single voxels (Thompson et al., 2001; Hulshoff Pol et al., 2006; Schmitt et al., 2007). For functional MRI, there is a surprising lack of information on the reliability, temporal stability and heritability of the majority of BOLD activation phenotypes in current usage. A large-scale twin study in Australia is currently addressing this problem for the N-back working memory task and some first results are presented in this issue (Blokland et al., 2008). In keeping with genetic effects on differences in brain activation during this task, the within-pair resemblance of MZ twins was larger than that of DZ twins, but MZ resemblance was far from unity suggesting that non-genetic factors also play a large role.

These non-genetic factors partly consist of the errors in estimating brain activation from fMRI signal intensity. This measurement error is partly inherent in the complex data-acquisition and data-analytic strategies of this technique but may also reflect deviations from a crucial assumption in imaging genetics, namely that there is little variation in the exact localization of task-related brain activity across individuals. The standard approach is to aggregate the intensity of the BOLD signal across voxels in a predefined region-of-interest (ROI) that is based on anatomical criteria. This ROI approach works only if all individuals use the very same region for the same task. This may not be the case and (mild) variation may exist in brain areas used to do the same task across individuals, perhaps contingent on different cognitive strategies. An important methodological advance in future imaging genetics studies could be to incorporate "region of highest intensity" as an additional trait that could be influenced by genetic factors.

In addition to measurement error, the non-heritable part of fMRI variation reflects the influence of the many environmental factors that can influence brain function. These effects can be captured by a specific study design in twins, the discordant monozygotic twin design. To maximize detection of the effects of environmental risk factors, brain activation can be compared in MZ twin pairs that are strongly discordant for behavioral traits like ADHD, generalized anxiety, obsessive compulsive behavior, or major depression. In these pairs, one twin scores very high on symptoms of these disorders, whereas the co-twin scores very low on these symptoms. Because monozygotic twins are (nearly) always 100% identical at the DNA sequence level (Boomsma et al., 2002), their discordance at the phenotypic level must arise from differential exposure to environmental influences. Differences in brain activation linked to these behavioral traits between the high-risk twin and the low-risk co-twin, therefore, also reflect environmental effects on the brain, including epigenetic effects, rather than effects of variation in the DNA sequence.

Two papers in this special issue used the discordant MZ twin approach. Wolfensberger et al. (2008) examined emotional processing and brain activation during an encoding and recognition paradigm using emotionally salient words in a sample of monozygotic twin pairs at low or high risk for anxiety and depression. Performance data did not support the existence of a negative response bias in participants at high risk. At the neural level, however, increased left inferior frontal gyrus (LIFG) activation by negative words was found in high-risk participants during retrieval. Further evidence for differential effects of negative words on participants at low or high risk for anxiety and depression was found in the amygdala response during encoding. In keeping with studies showing larger reactivity to negative emotional faces in depressed patients, discordant high-risk twins showed larger right amygdala activation to negative words than their low-risk co-twin. A second paper compared monozygotic twins strongly discordant for obsessive-compulsive symptoms (Den Braber et al., 2008). Intrapair differences in performance and brain activation were recorded during a Tower of London planning paradigm. Again the evidence at the brain level was stronger than that at the behavioral level. Despite only mild evidence for impairment in performance, twins with obsessive-compulsive symptoms showed significantly decreased brain activation during planning in dorsolateral prefrontal cortex, thalamus pulvinar, and inferior parietal cortex. These findings are consistent with the hypothesis of disturbed cortico-striato-thalamo-cortical (CSTC) circuitry underlying obsessive-compulsive symptoms.

3. Candidate genes

Despite their elegance, twin studies have inherent limitations in that they do not identify the actual genes involved and the dominant approach in imaging genetics has been the candidate gene approach. In this approach an association is tested between measured variation in a gene and variation in brain structure and function. Table 1 gives a list of potential candidate genes for MRI

Table 1
A 'gene index' to papers in this special issue

Gene	Location	Gene implicated (in humans) in	Addressed in this issue by
Apolipoprotein E (APOE)	19q13.2	Dementia or age-related cognitive decline	Mattay et al. (2008)
Amyloid precursor protein (APP)	21q21	Dementia or age-related cognitive decline	Mattay et al. (2008)
Presenelin 1 (PSEN1)	14q24.3	Dementia or age-related cognitive decline	Mattay et al. (2008)
Presenelin 2 (PSEN2)	1q31-q42	Dementia or age-related cognitive decline	Mattay et al. (2008)
Sortilin-related receptor (SORL1)	11q23-q24	Dementia or age-related cognitive decline	Mattay et al. (2008)
Klotho	13q12	Dementia or age-related cognitive decline	Mattay et al. (2008)
Prion Protein gene (PRNP)	20pter-p12	Dementia or age-related cognitive decline	Mattay et al. (2008)

Table 1 (*Continued*)

Gene	Location	Gene implicated (in humans) in	Addressed in this issue by
Insulin-like growth factor (IGF-1)	15q26.3	Dementia or age-related cognitive decline	Mattay et al. (2008)
Death associated protein kinase 1 (DAPK1)	9q34.1	Dementia or age-related cognitive decline	Mattay et al. (2008)
Fragile X mental retardation 1 (FMR1)	Xq27.3	Mental retardation	Bearden et al. (2008)
Lim domain kinase 2 (LIMK1)	7q11.23	Visuospatial cognition	Bearden et al. (2008)
Neurofibromatosis type 1 (NF1)	17q11.2	Visuospatial cognition, executive function	Bearden et al. (2008)
D-amino acid oxidase activator (DAOA)	12q24	Bipolar disorder; schizophrenia	Bearden et al. (2008)
Dystrobrevin binding protein 1 (DTNBP1)	6p22.3	Bipolar disorder; schizophrenia	Bearden et al. (2008)
Multiple ankyrin repeat domain 3 (SHANK3)	22q13.3	Autism	Bearden et al. (2008)
Guanine binding protein, β1L (GNB1L)	22q11.2	Schizophrenia	Bearden et al. (2008)
Neuregulin (NRG1)	8p22-p11	Schizophrenia; bipolar disorder	Greene et al. (2008); Bearden et al. (2008)
Reelin (RELN)	7q22	Schizophrenia	Greene et al. (2008)
Dystrobrevin-bidning protein 1(DTNBP1)	6p22.3	Schizophrenia	Greene et al. (2008)
Disrupted in Schizophrenia 1 (*DISC1*)	1q42	Positive symptoms in schizophrenia, working memory, attention, frontal gray matter volume	Szeszko et al. (2008)
		Dementia or age-related cognitive decline	Mattay et al. (2008)
Brain derived neurotrophic factor (BDNF)	11p13	Dementia or age-related cognitive decline	Mattay et al. (2008)
Sonic hedgehog (SHH)	7q36	Anterior cingulate development	Fossella et al. (2008)
7-Dehydrocholesterol reductase (DHCR7)	11q12-q13	Anterior cingulate development cognitive deficits	Fossella et al. (2008)
Patched (PTCH1)	9q22.3	Anterior cingulate development	Fossella et al. (2008)
Zinc finger protein of cerebellum 2 (ZIC2)	13q32	Anterior cingulate development	Fossella et al. (2008)
Gli-Kruppel family member 2 (GLI2)	2q14	Anterior cingulate development	Fossella et al. (2008)
Tachykinin 1 (TAC1)	7q21-22	Depressive illness	Fossella et al. (2008)
Catechol-*O*-methyl transferase (COMT)	22q11.21-q11.23	Dementia or age-related cognitive decline	Mattay et al. (2008)
		Alcohol dependence or alcohol-related traits	Puls et al. (2008)
		Schizophrenia	Greene et al. (2008); Bearden et al. (2008)
		Working memory performance	Greene et al. (2008)
		Response inhibition	Greene et al. (2008)
		Emotion regulation	Aleman et al. (2008)
Dopamine B-hydroxylase (DBH)	9q34	Sustained attention	Greene et al. (2008)
		Working memory	Greene et al. (2008)
MonoAmine Oxidase A (MAOA)	Xp11.23	ACC activation during executive attention	Fossella et al. (2008); Greene et al. (2008)
		Amygdala/ventromedial activity or antisocial traits	Aleman et al. (2008)
Dopamine receptor D1 (DRD1)	5q35.1	Alcohol dependence or alcohol-related traits	Puls et al. (2008)
Dopamine receptor D2 (DRD2)	11q23	ACC activation during executive attention	Fossella et al. (2008)
Dopamine receptor D3 (DRD3)	3q13.3	Alcohol dependence or alcohol-related traits	Puls et al. (2008)
Dopamine receptor D4 (DRD4)	11p15.5	ACC activation during executive attention	Fossella et al. (2008)
		Response inhibition	Greene et al. (2008)
Dopamine transporter (DAT1, SLC6A3)	5p15.3	Alcohol dependence or alcohol-related traits	Puls et al. (2008)
		Attention deficits	Greene et al. (2008)
		Working memory	Greene et al. (2008)
		Schizophrenia	Greene et al. (2008)
		Response inhibition	Greene et al. (2008)
Tryptophane hydroxylase (TPH2)	12q21.1	Response inhibition	Greene et al. (2008)
		Executive control	Reuter et al. (2007)
		Amygdala/ventromedial activity	Canli et al. (2008); Aleman et al. (2008)
Serotonin transporter (5-HTT, SLC6A4)	17q11.1-q12	Dementia or age-related cognitive decline	Mattay et al. (2008)
		Anterior cingulate volume and activation	Fossella et al. (2008); Greene et al., 2008
		Amygdala/ventromedial activity or anxiety and depressive disorders	Aleman et al. (2008); Canli et al. (2008)
Serotonin 2a-receptor (5HT2a)	13q14-q21	Dementia or age-related cognitive decline	Mattay et al. (2008)
		sustained attention	Greene et al. (2008)
		Verbal memory performance	Reuter et al. (2008)
Serotonin receptor type 3 (HTR3A)	11q23.1-q23.2	Amygdala/ventromedial activity	Aleman et al. (2008)
Glutamate receptor, NMDA 1 (NR1, GRIN1)	9p34.3	Alcohol dependence or alcohol-related traits	Puls et al. (2008)
Glutamate receptor, NMDA 2 (NR2B,GRIN2B)	12p12	Alcohol dependence or alcohol-related traits	Puls et al. (2008)
		Attention deficits	Greene et al. (2008)
Glutamate transporter 1 (GLT1, SLC1A2)	11p13-p12	Alcohol dependence or alcohol-related traits	Puls et al. (2008)
Glutamate receptor 3 (GRM3)	7q21.1-q21.2	Alcohol dependence or alcohol-related traits	Puls et al. (2008)
		Performance on cognitive tests of prefrontal and hippocampal function	Mattay et al. (2008)
Glutamate receptor, metabotropic 7 (GRM7)	3p26.1-p25.2	Prefrontal working memory function	Blokland et al. (2008)
Choline transporter (CHT1, SLC5A7)	2q12	Depressive disorder	Aleman et al. (2008)
Cholinergic receptor α-pp 4 (CHRNA4)	20q13.2-q13.3	Sustained attention	Greene et al. (2008)
Cannabinoid receptor 1 (CNR1)	6q14-q15	Social reward responsivity	Aleman et al. (2008)
Kidney and brain expressed protein (KIBRA)	5q35.1	Memory performance and hippocampal Activation during memory retrieval	Mattay et al. (2008)
Period 2 (PER2)	2q37.3	Alcohol dependence or alcohol-related traits	Puls et al. (2008)

The table list genes that show polymorphisms that appear involved in individual differences in human cognition and emotion. The special issue papers that discuss this link are indicated in the final column.

studies, together with the traits on which they may exert an effect. Note that this list is not meant to be exhaustive; it simply summarizes all genes with brain or behavioral effects in humans that are mentioned in the various contributions to this special issue.

3.1. Genes influencing neurotransmission

The strategies to select these candidate genes for imaging genetics research have been multifold. The strongest candidate genes are those that are involved in neurotransmission and carry variants in the coding regions of the gene or in regions that influence the splicing or the expression of the gene. Such genetic variants influence the quality of proteins or their quantity and may have immediate consequences for neurotransmitter metabolism, release, reuptake, receptor density and affinity, or any of the multiple events in the signal cascade between receptor activation and neuronal effects, e.g. short-term ion-channel activity induced depolarization or hyperpolarization and second messenger induced intracellular events leading to long-term anatomical changes. Two prime examples of candidate genes for brain phenotypes are the gene for the catecholamine-*O*-methyltransferase enzyme (COMT) and the gene for the serotonin reuptake transporter (SLC6A4).

The discovery that the dopamine transporter is sparse in prefrontal cortex led to an appreciation of the role of COMT as the main determinant of dopamine availability in the prefrontal cortex. In humans, a functional single nucleotide polymorphism (SNP) in the gene for COMT has been identified where an evolutionarily recent methionine (met) for valine (val) substitution at codon 108/158 results in a thermolabile protein with 2–4 times lower activity. The met form of the protein is less efficient at degrading dopamine and associated with more dopamine in the synapse then the val allele. This variation in the COMT gene immediately connects to a number of conditions that may be characterized by changes in prefrontal dopamine signaling, including ADHD, schizophrenia and the enhanced decline of cognitive functions subserved by the prefrontal cortex with advancing age. Not surprisingly, a number of imaging genetics studies have shown associations between this variant and activation in the overlapping (prefrontal) brain areas engaged by sustained attention, working memory, and response inhibition (see the reviews by Greene et al., 2008 and Mattay et al., 2008).

A second replicated finding in imaging genetics is the stronger amygdala response to emotional faces in carriers of the short allele of the 5-HTTLPR polymorphism in the serotonin transporter gene. A recent meta-analysis showed that this locus can account for up to 10% of the interindividual variance in amygdala activation (Munafò et al., 2007). At the same time some evidence for publication bias against null findings was observed cautioning us not to get carried away as yet. There are many issues that remain to be resolved including (1) the exact role of the Lg subgroup in long allele carriers, who may resemble short allele carriers, (2) the predominance of right amygdala activation in many studies which may potentially reflect the peripheral-autonomic rather than the central nervous system component of arousal, and (3) whether the hyperactivity found in short allele carriers reflects a tonic increase in amygdala activation or a phasic increase (Aleman et al., 2008). The safest conclusion is that the 5-HTTLPR gene has proven worthy of our attention, but its exact role in emotional processing remains to be established.

This special issue adds a second promising candidate gene from the serotonergic pathway, the tryptophan hydroxylase 2 (TPH2) gene coding for the enzyme that regulates the synthesis of serotonin. Based on three earlier studies implicating TPH2 in variation in brain activation and performance of conflict monitoring, sustained attention, and stop-signal tasks Reuter et al. examined the effect of a G/T polymorphism at position −702 (SNP rs4570625) on performance and brain activation during an N-back task. Although behavioral data showed no performance differences between the genotype groups a significantly stronger activation was visible in areas BA6, BA46, and BA40 of the TT-genotype carriers in contrast to the GT and GG carriers. These results may indicate that TT carriers compensate deficits in executive control functions by increased brain activity. Interestingly, TT carriers do not only show this overactivity during 'frontal executive' tasks but also show higher levels of harm avoidance (Reuter et al., 2007) and greater amygdala reactivity to emotional faces (Canli et al., 2008) suggesting cross-domain effects of TPH2 on cognitive as well as emotional processing.

3.2. Multiple genes and epistasis

Increasingly the candidate gene approach has started to look at gene–gene interactions in an attempt to explain larger portions of the variance in MRI-based traits. Such effects may be additive or, more interestingly, interactive (also called epistatic effects). In this issue an example of an additive effect of genes in the same pathway is given by Canli et al. (2008). The variants in the two key serotonergic genes, 5-HTT and TPH2, showed an additive effect on BOLD activity in the putamen and the amygdala, specifically in response to facial expressions of negative emotions. Individuals who carried both the S-allele of the 5-HTTLPR and the T variant of the −702 G/T SNP in the TPH2 gene had increased bilateral activation of the putamen and increased left amygdala activation in response to pictures of fearful faces compared to a fixation baseline. This gene–gene additive effect is in keeping with the previously reported main effects of each of these genes on amygdala activation.

An example of an epistatic effect of two genes in different pathways is provided in this issue by Puls et al. (2008). Acting on the replicated finding of smaller hippocampal volumes in alcohol-dependent individuals, that holds up after correction for overall cerebral atrophy, they examined the interactive effects of six SNPs in a glutamate receptor gene (GRM3) and three SNPs in the COMT gene on hippocampal volume. Effects of 90 cross-gene allele combination were scrutinized, but to avoid the huge multiple testing issue, a novel method was imported from the area of machine learning. In the group of participants chronically exposed to alcohol, only specific allele combinations of these genes, part of the two major pathways implicated in alcohol dependence, caused reduced hippocampal volume. It is of note that all these epistatic effects would have been rendered non-significant by correction for multiple testing in a conventional approach to association.

3.3. Genes influencing brain morphogenesis and neurodevelopment

Neurotransmission is not the only source of functional candidate genes. Developmental trajectories in cognitive maturation in (early) childhood and cognitive decline in aging show considerable variation across individuals with a significant portion of this variance arising from genetic factors. Therefore, genes that influence brain morphogenesis and neurodevelopment also immediately qualify as candidate genes. For example, Fossella et al. (2008) have focused on the developmental biology of the executive attention network, specifically on the role of the anterior cingulate cortex (ACC). The developing ACC is positioned dorsally at midline, and clear effects of the hedgehog-, BMP-, and FGF-signaling pathways on neural migration in the frontal midline have been described. Functional mutations in these pathways cause the

ACC to fail normal development and may lead to strong phenotypes like holoprosencephaly. More subtle allelic variation in these pathways could still affect ACC structure and function in clinically relevant ways, and be detectable by brain imaging. This suggests to us to prioritize genes in these pathways as candidate genes for fMRI examinations of the ACC.

A further example of a 'developmental' candidate gene is provided in this issue by Szeszko et al. (2008) who studied the potential effect of variation in the *DISC1* gene on prefrontal gray matter volume. The *DISC1* gene has been implicated in neuronal migration during development of the cerebral cortex and is located at the chromosomal translocation in the 1q42 region that co-segregates with schizophrenia. A functional mutation in a SNP resulting in a phenylalanine (phe) substitution for leucine (leu) at position 607 was used to predict frontal gray matter volume in 25 health controls and 19 schizophrenia patients. Among schizophrenia patients and healthy controls, carriers of one or more phe alleles had significantly less gray matter in the superior frontal gyrus and ACC compared to participants with two leu alleles. Interestingly, among patients the phe allele carriers also had reported more severe hallucinations and a significant negative correlation was found between this severity and left superior frontal gray matter volume.

The above findings illustrate how genes involved in neurodevelopment are meaningful candidate genes for structural variation in the brain detectable by MRI, and that such variation may indeed be intermediate between genetic variation and clinical outcomes. As stated earlier, however, many clinical outcomes like ADHD, schizophrenia and autism can reflect very heterogeneous processes, i.e. there is a very real possibility that many different allele combinations involving different or even non-overlapping sets of genes may all give rise to the same cluster of symptoms. Any single candidate disease gene will have only a minor influence on brain structure and function of the total group of patients. Finding such genes may require studying homogeneous groups of participants or patients, such as participants coming from the same pedigree, or the same isolated population. A specific strategy is described by Bearden et al. (2008) in this issue. They review studies on genetic disorders like the 22q11.2 deletion syndrome, Williams syndrome, fragile X syndrome and neurofibromatosis, associated with neuroanatomical phenotypes resembling those seen in ADHD, schizophrenia and autism. The advantage is that the genetic abnormalities leading to these syndromes are well-described, literally attenuating the problem of "heterogeneity" of the observed neuroanatomical defects. A clear example is disruption of the FMR1 gene (fragile X mental retardation 1). Here, the loss of a single protein causes a neurodevelopmental cascade leading to structural and functional brain abnormalities paired to severe cognitive and emotional deficits. Another example is provided by Aleman et al. (2008). Abnormal structure and function of emotional brain circuits like the amygdala and the insula have been found in men with an extra X-chromosome (Klinefelter) and women with a missing X-chromosome (Turner). Specifically, deletion of a region at Xp11.3 in X0 women seems to induce an increase in amygdala volume compared to typical males (XY), whereas Klinefelter men (XXY) have smaller amygdala volumes than typical men. Women with a triple X-chromosome, in turn have the smallest amygdala. This makes a strong case for a role of an X-linked gene in determining the size of the amygdala.

3.4. Positional candidate genes

Apart from selection based on known protein function there are two alternative ways to nominate candidate genes. The first is to select genes which are preferentially expressed in the brain. Using post-mortem tissue, gene expression profiling can be used to show which genes are particularly up- or down-regulated in the structure of interest. This may be taken as a sign of importance of the gene in variation in the function of this structure (for an example on the anterior cingulated region, see Fossella et al., 2008). A final set of candidate genes derives from positional cloning. Whole genome searches using linkage or whole-genome association approaches can identify chromosomal regions for genes influencing behavioral traits. Existing annotation for these regions, deposited in public databases through large-scale projects like the Human Genome Project, can then be used to select genes in this chromosomal region as positional candidates for related brain-traits.

The potential impact of such a strategy was recently illustrated in a study aimed at finding functional polymorphisms for human memory performance (Papassotiropoulos et al., 2006). They started with a blind, genome wide screen for human memory performance including over 500,000 SNPs in 351 participants. Significant SNPs were subsequently genotyped in two independent replicate samples (of 256 participants and 424 participants each). Only one SNP (rs 17070145 yielding a common T→C substitution in the KIBRA gene) showed replication in each sample. Carriers of the KIBRA T-allele performed better in both a 5-min delay recall and a 24-h delay free recall condition. By genotyping fresh frozen brain tissue from 14 individuals they next showed that expression of the truncated transcript of the KIBRA gene was high in all memory-related brain regions (including the temporal lobe and the hippocampus), whereas the full-length transcript was almost undetectable. In a final phase, 15 carriers and 15 non-carriers of the T-allele of rs17070145 underwent a functional MRI protocol, which included tasks known to activate the hippocampus. It was found that non-carriers of the beneficial T allele showed increased activity in brain regions known to be involved in a network important for episodic memory retrieval. The study awaits replication, but it suggests that non-carriers of the T-allele need more activation in these areas to reach the same level of delayed recall performance (Papassotiropoulos et al., 2006).

4. Future developments

So far, the young field of imaging genetics has been largely exploratory. Compared to the candidate gene studies on behavioral traits in psychiatric genetics, candidate gene approaches in imaging genetics have been less strict when it comes to sample size and replication efforts, and directional hypotheses are often lacking. Together with potential publication biases this may lead to non-replicable findings that may unnecessarily lead us astray. The risk for false positives will only further increase when multiple genes and their interactions are tested simultaneously. We believe three improvements in future studies imaging genetics can avert this looming threat:

1) studies on candidate genes should test well-specified hypotheses,
2) fMRI protocols should aim for within-laboratory replication of associations across different tasks measuring the same construct, and for across-laboratory replication by using very similar tasks to those published before, and
3) an increase in sample sizes is needed up to a scale that allows genetic whole genome association studies.

4.1. Formulating hypotheses

While we would like to emphasize that replication is the coin of the realm in genetic association studies, what constitutes a

replication is not always clear. Most candidate gene studies already doing well in selecting tasks that tap into neural systems plausibly related to the trait under study, such as response inhibition in children with ADHD or emotional faces in anxiety disorders. However, the association tests are often two-sided, it is not specified upfront whether a dominant or additive effects is expected, and studies on the same gene sometimes test different loci within the gene. This goes against the advice of Collins and colleagues (NCI-NHGRI Working Group, 2007) who have argued that a replication must involve not just the gene, but the locus and allele. At the neural level it is usually only the presence of a difference in BOLD activation between genotype groups that is tested, rather than the direction and magnitude of the BOLD response. Also, ROIs are often only loosely specified up front . This imprecision is undesirable in the long run, and we should instead specify clear predictions before the experiment, such that a specific genotype is expected to cause a specific change in activation (i.e. increase or decrease) in a specific ROI or a network of ROIs that are likely to be functionally connected.

At the neural level such prediction is not a trivial problem because increased activation in a region can be interpreted to mean many different and even opposite things. Increased regional fMRI activation during a cognitive task can mean that participants effectively engage the required brain areas to perform the task in contrast to participants performing more poorly, or it can mean that participants who do not outperform others but require more activation have a reduced efficiency of the brain. Decreased regional fMRI activation in response to emotionally salient stimuli can mean that participants insufficiently engage this brain region during emotional processing, or it can mean that tonic activation was already high which then acts to reduce the amplitude of phasic responses. On top of this, the exact BOLD response may depend on the structural aspects of the ROI studied, in terms of the size of the neuronal population and the exact wiring (dendritic branching, balance excitatory/inhibitory synapses) which itself can be influenced by genetic variation. Nonetheless, scientific strength of imaging genetics findings will greatly increase if we formulate directional hypotheses about the effects of the mutant allele *a priori.*

4.2. Standardization

Another way to achieve more robust results is to test for genotype effects on activation of an ROI in multiple tasks that are assumed to tap the same psychological construct. For instance, a number of tasks are known to engage inhibitory control (e.g. Go-Nogo tasks, Stroop test, Simon task, Flanker task) and partly activate the same brain areas. Significant allelic effects on the activation of these areas during all these tasks would help increase our confidence that the gene's effect is real. For emotional processing, similar allelic effects would be expected on the amygdala and medial prefrontal areas by negatively valenced stimuli independent of their modality, i.e. negative words, angry or fearful faces, or aversive pictures (snakes/spiders, suffering, violence). Imaging protocols that aim for within-laboratory replication of genetic associations across different tasks measuring the same construct would make a stronger case.

As a variation on the previous we would also like to make a plea for more across-laboratory standardization of task protocols. Already excellent role models for such standardization exist in the field of structural imaging. For example the Alzheimer's Disease Neuroimaging Initiative (ADNI, www.adni-info.org) has developed uniform standards for acquiring longitudinal, multi-site MRI and PET data on the elderly, including patients with Alzheimer's disease, and they created a generally accessible repository for such standardized data. Similar initiatives would benefit fMRI studies in genetically informative participants. Although effect sizes of association to fMRI traits can be very large, an important advantage inherent in the imaging genetics strategy, smaller effect sizes may be missed by the typical current sample sizes In the field of genetics at large, meta-analyses have become a major instrument to help detect genes of smaller effect (Ioannidis et al., 2005). Power of these meta-analyses can be increased in two important ways: (1) reducing heterogeneity in the MRI phenotypes which we can achieve by more standardized protocols, perhaps simply by copying well-tested existing designs, and (2) increasing sample sizes.

4.3. Sample sizes

More is always better, but when testing multiple candidate genes in a single study an increase in the sample size is really needed. As a rule of thumb we propose to have at least 10 participants in the least filled cell when testing a single gene, i.e. there should be at least 10 homozygotes for the minor allele. For genes with a low minor allele frequency this may require large genotyped samples from which the specific genotypes can then be drawn; a strategy that has been successfully applied previously (Reuter et al., 2007). It should be noted that the number '10' reflects current practice for the least number of participants required in the smallest group of an fMRI study, rather than the outcome of formal power analysis. For multiple genes, sample sizes based on power analysis is strongly recommended (e.g. using *quanto* at http://hydra.usc.edu/GxE/). Even for two genes the results may be humbling. To achieve 80% power (at $\alpha = 0.05$) to detect the main effects of two genes with additive effect ($MAF_{gene1} = 0.25$; $MAF_{gene2} = 0.20$) a total sample size of 150 MRI-scanned participants may be needed if each of the genes accounts for at least 4% of the variance in the MRI trait. If the interaction accounts for an additional 2% of the variance, 80% power would be achieved at 350 participants only.

At some point in time, fMRI studies may grow large enough to allow genome-wide association (GWA) directly on brain imaging data. For structural MRI data the first GWA has been conducted by the Framingham Study (Seshadri et al., 2007). A total of 705 stroke- and dementia-free Framingham participants who underwent volumetric brain MRI and cognitive testing had genotypes for 70,987 autosomal SNPs (on the Affymetrix 100 K Human Gene Chip). A number of highly significant associations were found to measures of total and regional brain volumes as well as ventricular and white matter hyperintensity volume. Many SNPs were in or near genes not previously associated with brain volume (e.g. the CDH4 and CNTN5 genes). At the same time a number of genes previously associated with brain phenotypes were replicated at the $p < 10e^{-4}$ significance level (e.g. the NGFB, NRG1, SORL1, PRNP, NTRk2 genes). This study nicely illustrates the two uses of genome-wide SNP data, namely replication of the role of genes previously associated with brain function and structure and detection of unknown genes.

The Framingham study tested 'only' 70,987 SNPs. Dramatic reductions in the costs of genotyping have increased the scale of GWA studies to half a million and even a million SNPs per participant (e.g. the Illumina Human1M BeadChip). In addition, these chips use tagging SNPs so that the information on population-specific linkage disequilibrium freely available from the HapMap project can be used to impute 1.5–2 million more missing SNPs. A typical GWA study, therefore, may yield highly reliable genotypes for up to 2.5 million SNPs. In imaging genetics this leads to the situation where we have "*a universe of permutations among several million common genetic variants with*

thousands of voxels in both white and gray matter and a wide array of cognitive tasks that activate multiple brain systems" (Fossella et al., 2008). In this universe, correction strategies for multiple testing will become a huge issue. Association of 20.000 voxels with 500.000 SNP genotypes in 500–1000 participants will yield results of mind-boggling significance. Some of these may reflect true biology but we can be sure that many more will be totally meaningless. Rigid control for multiple testing, on the other hand, will destroy all true association. Innovative strategies to this problem such as those presented by Puls et al. (2008) in this issue are direly needed.

Fortunately, many fMRI studies already rigidly control type 1 errors and there is cause for some optimism. A recent paper by Meyer-Lindenberg et al. (2008) suggests that the false positives may already be reasonable contained by the current commonly used correction procedures in brain imaging studies, e.g. family-wise error (FWE) based on Gaussian Random Field or the False Discovery Rate. They selected 720 SNPs with null association to psychiatric disorders (492 of these were also not associated with a host of cognitive performance measures) and tested for association with (1) gray matter content of over 2 million voxels (2) BOLD response in 24,048 voxels in an N-back or emotional faces task in the whole brain or pre-selected ROIs. If genotype effects exceeded the corrected threshold for any voxel, that SNP was counted as a positive. The number of SNPs showing 'false' association in this study remained well below the acceptable experiment-wise error of 5%. This suggests that corrected *p*-values from imaging genetics studies often represent true association

5. In closing

Imaging genetics is, above all, exciting science. Who would not be tempted to understand genetic variation in brain function and structure? But it is not just fun. This field has large societal ramifications. Understanding genetic variation in brain function and structure may help evolve the current classification systems for psychiatric disorders from a symptom cluster based system only, towards a system that profiles individuals at the 'gene, brain, behavior' levels simultaneously. This will improve early detection of susceptible individuals and enable a more optimal choice of therapy (personalized medicine). Imaging genetics research can also help us to map the proteins associated with psychiatric disease. Such knowledge, it is hoped, can foster the development of novel treatments for these diseases and alleviate the often grave suffering they cause to many families world wide.

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