Imaging genomics

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The recent completion of a working draft of the human genome sequence promises to provide unprecedented opportunities to explore the genetic basis of individual differences in complex behaviours and vulnerability to neuropsychiatric illness. Functional neuroimaging, because of its unique ability to assay information processing at the level of brain within individuals, provides a powerful approach to such functional genomics. Recent fMRI studies have established important physiological links between functional genetic polymorphisms and robust differences in information processing within distinct brain regions and circuits that have been linked to the manifestation of various disease states such as Alzheimer's disease, schizophrenia and anxiety disorders. Importantly, all of these biological relationships have been revealed in relatively small samples of healthy volunteers and in the absence of observable differences at the level of behaviour, underscoring the power of a direct assay of brain physiology like fMRI in exploring the functional impact of genetic variation.

Identifying the biological mechanisms that contribute to complex cognitive and emotional behaviours is paramount to our understanding of how individual differences in these behaviours emerge and how such differences may confer vulnerability to psychiatric disease. Advances in both molecular genetics and non-invasive functional neuroimaging have begun to provide the tools necessary to explore these as well as other behaviourally relevant biological mechanisms. With completion of a rough draft of the reference human genome sequence[1,2], a major effort is underway to identify common variations in this sequence that impact on gene function (*i.e.* functional polymorphisms) and subsequently to understand how such functional variations alter human biology. Since approximately 70% of all genes are expressed in brain, many of these functional polymorphisms will effect how the brain processes information. Functional neuroimaging (*i.e.* PET, fMRI, EEG/MEG), because of its capacity to assay within individuals information processing in discreet brain circuits, has unique potential as a tool for characterizing functional genomics in brain. In this review, we: (i) describe the conceptual basis for, and potential of, this synthetic approach, referred to here as imaging genomics; (ii) propose several

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guiding principles for its implementation and advancement; and (iii) highlight recent studies that exemplify these principles.

Why study genes?

Genes represent the 'go' square on the Monopoly board of life. They are the biological toolbox with which one negotiates the environment. While most human behaviours cannot be explained by genes alone, and certainly much variance in aspects of brain information processing will not be genetically determined, variations in genetic sequence that impact gene function will contribute some variance to these more complex phenomena. This conclusion is implicit in the results of studies of twins that have revealed heritabilities of 40–70% for various aspects of cognition, temperament, and personality[3].

Genes have unparalleled potential impact on all levels of biology. In the context of disease states, particularly behavioural disorders, genes not only transcend phenomenological diagnosis, they represent mechanisms of disease. Moreover, genes offer the potential to identify at-risk individuals and biological pathways for the development of new treatments. In the case of psychiatric illness, genes appear to be the only consistent risk factors that have been identified across populations and the lions' share of susceptibility to major psychiatric disorders is accounted for by inheritance[4]. While the strategy for finding susceptibility genes for complex disorders, by traditional linkage and association methods, may seem relatively straightforward (albeit not easily achieved), developing a useful and comprehensive understanding of the mechanisms by which such genes increase biological risk is a much more daunting challenge. How many genes contribute to a particular complex behaviour or complex disease state? What genetic overlap exists across behaviours and diseases? How large are the effects of candidate genes on particular brain functions? And, perhaps most importantly, how does a gene affect brain information processing to increase risk for a disorder of behaviour?

The 'candidate gene association approach' has been a particularly popular strategy for attempting to answer these questions. Genetic association is a test of a relationship between a particular phenotype and a specific allele of a gene. This approach usually begins with selecting a biological aspect of a particular condition or disease, then identifying variants in genes thought to impact on the candidate biological process, and next searching for evidence that the frequency of a particular variant ('allele') is increased in populations having the disease or condition. A significant increase in allele frequency in the selected population is evidence of association. When a particular allele is significantly associated with a particular phenotype, it is potentially a causative factor in determining

that phenotype. There are caveats to the design and interpretation of genetic association studies, such as linkage disequilibrium with other loci and ancestral stratification, that are beyond the scope of this review and have been discussed at length elsewhere[5].

Imaging genomics is a form of genetic association analysis, where the phenotype is not a disease phenotype but a physiological response of the brain during specific information processing. The protocol for imaging genomics involves first identifying a meaningful variation in the DNA sequence within a candidate gene. For the variant to be meaningful, it should have an impact at the molecular and cellular level in gene or protein function (*i.e.* be a functional variation) and the distribution of such effects at the level of brain systems involved in specific forms of information processing should be predictable. For example, a genetic variation in the gene for the serotonin transporter that impacts the availability of synaptic serotonin would be expected to affect amygdala function because serotonin is important in amygdala physiology (see below). Finally, the contributions of abnormalities in these systems to complex behaviours and emergent phenomena, possibly including psychiatric syndromes, can then be understood from a more biological perspective. It is within the context of this 'candidate gene association approach' that imaging genomics provides an ideal opportunity to further our understanding of disease mechanisms.

Why functional neuroimaging?

Traditionally, the impact of genetic polymorphisms on human behaviour has been examined using indirect assays such as personality questionnaires and neuropsychological batteries. While a few such studies have reported significant associations between specific genetic polymorphisms and behaviours, their collective results have been weak and inconsistent[6]. This is not surprising given the considerable individual variability and subjectivity of such behavioural measures. Because such behavioural assays are vague and imprecise, it has been necessary to use very large samples, often exceeding several hundred subjects, to identify even small gene effects[7]. In addition, behavioural probes and neuropsychological tests allow for the use of alternative task strategies by different individuals that may obscure potential gene effects on the underlying neural substrates meant to be engaged by the tests.

Because the response of brain regions subserving specific cognitive and emotional processes may be more objectively measurable than the subjective experience of these same processes, functional genetic polymorphisms may have a more robust impact at the level of brain than at the level of behaviour. Thus, functional polymorphisms in genes

weakly related to behaviours and, in an extended fashion, psychiatric syndromes may be strongly related to the function of neural systems involved in processing cognitive and emotional information in brain. This is the underlying assumption of imaging genomics. The potential for marked differences at the neurobiological level in the absence of significant differences in behavioural measures underscores the need for a direct assay of brain function. Accordingly, imaging genomics provides a unique opportunity to explore and evaluate the functional impact of brain-relevant genetic polymorphisms potentially more incisively and with greater sensitivity than existing behavioural assays.

Functional neuroimaging techniques, especially those that are non-invasive like fMRI, typically require no more than a few minutes of subject participation to acquire substantial data sets, reflecting the acquisition of many hundreds of repeated measures of brain function within a single subject. This is analogous to the signal detection power of EEG and MEG approaches, which also have been used to identify physiological signals that are highly heritable[8]. Thus, these techniques, in contrast to their behavioural counterparts, may require considerably fewer subjects (tens *versus* hundreds) to identify significant gene effects on the response characteristics of the brain. Moreover, the efficiency of these techniques allows for the ability to investigate the specificity of gene effects by examining their influence on multiple functional systems (*e.g.* prefrontal, striatal, limbic) in a single subject in one experimental session. This capacity to assay rapidly differences in the brain responses of different information processing systems with enhanced power and sensitivity places functional neuroimaging at the forefront of available tools for the *in vivo* study of functional genetic variation.

Imaging genomics: three basic principles

Selection of candidate genes

Ideally, the application of functional neuroimaging techniques towards the study of genetic effects should start where studying gene effects on behaviour would also start (*i.e.* from well-defined functional polymorphisms, such as those reported for APOE, COMT, and 5-HTT, highlighted below). Because the genetic variation in such genes has been associated with specific physiological effects at the cellular level and their impact has been described in distinct brain regions and circuits, imaging paradigms can be developed to explore their effects on local information processing in both normal and impaired populations.

Short of well-defined functional polymorphisms, candidate genes with identified single nucleotide polymorphisms (SNPs) or other allele

variants in coding or promoter regions with likely functional implications (*e.g.* non-conservative amino acid substitution or missense mutation in a promoter consensus sequence) involving circumscribed neuro-anatomical systems would also be attractive substrates for imaging genomics. The investigation of genes and variations without well-established functional implications in brain, however, necessarily requires greater caution not only in the design of imaging tasks but also in the interpretation of differential brain responses.

Control for non-genetic factors

The contribution of single genes to the response characteristics of brain systems, while putatively more substantial than that to emergent behavioural phenomena, is still presumably small. Furthermore, typically large effects of age, gender and IQ as well as environmental factors such as illness, injury, or substance abuse on phenotypic variance can easily obscure these small potential gene effects. Since association studies in imaging genomics are susceptible to population stratification artefacts, as in any case-control association study, ethnic matching within genotype groups is also potentially critical. Thus, the identification and contribution of genetic variation to specific phenotypes should be limited to studies where other potential contributing and confounding factors are carefully matched across genotype groups. If the imaging protocol involves performance of a task, the groups should also be matched for level of performance or, at least, any variability in performance should be considered in the analysis and interpretation of the imaging data. This is because task performance and imaging responses are linked *pari passu*, and systematic differences in performance between genotype groups could either obscure a true gene effect or masquerade for one.

Task selection

The last 5 years have been witness to a tremendous proliferation of functional neuroimaging studies and, with them, behavioural tasks designed specifically for this experimental setting. Many of these are modified versions of classic behavioural and neuropsychological tests (*e.g.* the Wisconsin Card Sorting Task[9]) designed to tap neural systems critical to particular behaviours. More recent paradigms have emerged that focus on interactions of specific behaviours and disease states as these questions have become newly accessible with non-invasive imaging (*e.g.* the emotion Stroop and OCD[10]).

Because of the relatively small effects of single genes, even after having controlled for non-genetic and other confounder variables, imaging tasks must maximize sensitivity and inferential value. As the interpretation of potential gene effects depends on the validity of the information processing paradigm, it is best to select well-characterized paradigms that are effective at engaging circumscribed brain regions and systems, produce robust signals in every individual and show variance across individuals (see below). In short, imaging genomics studies are probably not the appropriate venue to design and test new functional tasks, and to do so might undermine their tremendous potential.

Imaging genomics: applications of the principles

The following three sections provide examples of how the application of the principles of imaging genomics outlined above can lead to insights about the biological mechanisms underlying complex behavioural traits. In each study, functional neuroimaging was employed to identify at the systems' level the effects of functional genetic polymorphisms that had been previously associated with alterations at the molecular and cellular level as well as specific behaviours and/or disease states. In addition, each study implemented rigorous controls for non-genetic factors such as age, gender, IQ and performance on the experimental task. They also capitalized on existing functional paradigms designed to explore physiological aspects of distinct neural systems.

Apolipoprotein E and memory systems

A common allelic variant of the apolipoprotein E (APOE) gene has been associated with late-onset familial Alzheimer's disease[11]. Specifically, the APOE ε4 allele has a dose-dependent effect on risk and age of onset for the disease[12]. PET studies have reported deficits in cortical resting glucose metabolism in cognitively normal middle-aged subjects with the APOE ε4 allele[13,14]. Despite these associations, the APOE genotype alone has not been a viable predictor of disease development in non-demented subjects[15].

Bookheimer *et al*[16] used fMRI during a challenging memory task to explore the genetic effect of the APOE ε4 allele on memory-related brain activity. In their landmark study, 16 subjects carrying the APOE ε4 allele and 14 subjects homozygous for the APOE ε3 allele, which is not associated with increased risk for Alzheimer's disease, were asked to memorize and recall unrelated word pairs, a demanding memory task previously used to identify damage to the medial temporal lobe memory system[17], while undergoing fMRI.

While all subjects were cognitively intact and performed the memory task equally well, the pattern of brain activation between the two groups was strikingly different. In comparison to subjects with the APOE ε3 allele, those with the high-risk APOE ε4 allele exhibited significantly greater activation (both magnitude and extent) in memory-related brain regions such as the prefrontal cortex and left hippocampus. Such relatively increased neural activity in those with the at-risk allele was interpreted by the authors as reflecting possible compensatory phenomena through the recruitment of additional cognitive resources in the face of greater task difficulty and demand. Interestingly, the magnitude of task-related brain activity was significantly correlated with subsequent memory decline. These data suggest that changes in cortical information processing during declarative memory are associated with the biological effects of APOE ε4 even if compensation is made at the level of observable behaviour (*i.e.* task performance). Thus, the authors concluded that observed differences in memory-related brain activity associated with variation in the APOE gene in the absence of behavioural impairments may provide a useful tool for predicting the course of cognitive decline.

Catechol-o-methyltransferase and the prefrontal cortex

Catechol-*o*-methyltransferase (COMT), a methylation enzyme that converts released dopamine to inactive 3-methoxytyramine, is believed to play an important role in prefrontal dopamine neurotransmission[18]. Because dopamine transporters are virtually absent at cortical synapses, dopamine regulation in the prefrontal cortex is uniquely coupled to inactivation mechanisms in postsynaptic neurons and glia, such as catabolism by COMT[19]. A common polymorphism (val[108/158]met) in the COMT gene has been associated with most of the human variation in enzyme activity, with the thermolabile met allele having one-fourth the activity of the thermostable val allele. Thus, the val[108/158]met COMT polymorphism may impact dopamine regulated prefrontal cortical activity during executive and working memory tasks that tax this functional circuitry and that are affected by variations in dopamine signalling[20]. In fact, this polymorphism has been linked to impairments in executive function and working memory in val carriers[21], suggesting that genetically driven alterations in COMT enzymatic activity and subsequent synaptic prefrontal dopamine concentrations may lead to diminished prefrontal function.

To assay directly the impact of the COMT val[108/158]met functional polymorphism on prefrontal physiology, Egan *et al*[21] used fMRI during the performance of a well-characterized working memory test (the n-

back task) that has been effective at engaging the dorsolateral prefrontal cortex in prior imaging studies[22,23]. The authors found that in two separate cohorts of healthy volunteers (n = 11–16), all matched for age, gender, education and task performance, the load of the high-activity val allele consistently predicted a relatively exaggerated prefrontal response during the working memory task.

These imaging results provide direct evidence that the effects of the COMT val[108/158]met polymorphism on executive tasks such as working memory may reflect alterations in prefrontal dopamine catabolism related to COMT enzymatic activity. Similar to the interpretation of Bookheimer *et al*[16] for the effects of the APOE polymorphism on task-related brain responses, these authors suggested that decreased concentrations of prefrontal dopamine associated with the high-activity val allele led to an inefficient and thus exaggerated cortical response, perhaps in an effort to maintain task performance or as a reflection of diminished prefrontal signal to noise. These data, much like those of Bookheimer and colleagues, also illustrate the ability of functional neuroimaging to reveal biological effects of genetic variation at the level of brain in the absence of significant behavioural differences.

The COMT fMRI results also illuminate other evidence that COMT val is a susceptibility allele for schizophrenia and possibly other psychoses[24,25]. Schizophrenia has long been known to involve abnormal prefrontal function, and COMT val inheritance appears to be a genetic mechanism contributing to this aspect of the disease. The study by Egan and co-workers also highlights the statistical power of imaging genomics. The small effect size of genotype on executive cognition in terms of working memory test performance, in which COMT genotype predicts approximately 3–4% of the variance, required several hundred subjects to achieve statistical significance. In contrast, powerful statistical differences were observed in imaging samples of less than 15 subjects.

5-HTT and the amygdala

A common polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) has been associated with alterations in both 5-HTT transcription and 5-HT uptake. Cultured lymphoblasts homozygous for the long (l) promoter allelic variant having increased levels of 5-HTT mRNA expression as well as 5-HT uptake in comparison to cells possessing either one or two copies of the short (s) promoter variant[26]. Similar differences in 5-HTT binding levels between the l/l and s/l or s/s genotypes have been detected in the human brain[27,28].

At the behavioural level, possession of either one or two copies of the s allele has been associated with abnormal levels of anxiety[26,29,30], fear[31]

and development of affective illness[32]. These differences most likely reflect relative differences in 5-HTT expression and subsequent levels of synaptic 5-HT, a potent modulator of emotional behaviour[33]. However, the relationship between the 5-HTT promoter polymorphism and subjective measures of emotion and personality has been weak and inconsistent[34–38], likely reflecting the vagueness and subjectivity of the behavioural measurements, but also casting doubt on the validity of the relationship[39].

In order to explore the neural underpinnings of the apparent relationship between the 5-HTT promoter polymorphism and emotional behaviour, Hariri *et al*[40] used fMRI to evaluate the response of the amygdala, a brain region critical to both normal and abnormal fear behaviour, in healthy volunteers. Because the response of the amygdala may be more objectively measurable than the subjective experience of emotionality, the investigators reasoned that the effects of the 5-HTT polymorphism might be more apparent at the level of amygdala biology than at the level of individual responses to questionnaires or ratings of emotional symptoms. Specifically, they hypothesized that subjects carrying the s allele, who presumably have relatively lower 5-HTT expression and higher synaptic 5-HT and who are typically more anxious and fearful, would exhibit a greater amygdala response than those homozygous for the l allele.

In their study, subjects from two independent cohorts (n = 14) were divided into two equal groups based on their 5-HTT genotype, with the groups matched for age, gender, IQ and task performance. During scanning, the subjects performed a perceptual processing task that required them to match the expression of fearful and angry human faces, stimuli that have consistently engaged the amygdala in imaging studies[41–43]. Consistent with their hypothesis, Hariri and colleagues found that subjects carrying the less efficient s allele of the 5-HTT promoter gene had an increased amygdala response to fearful stimuli in comparison to subjects homozygous for the l allele. This finding led the investigators to suggest that the increased anxiety and fear associated with individuals possessing the s allele may reflect the hyper-responsiveness of their amygdala to relevant environmental stimuli. These results are striking not only because they provide the first evidence for a genetically driven difference in the response of brain regions underlying emotional behaviour, but also because these differences at the neurobiological level were marked in a relatively small sample population in the absence of significant differences in behavioural measures. Moreover, as in the other examples, the results of this imaging genomics study help elucidate a potential biological mechanism for the genetic association of this polymorphism with vague psychiatric disturbances, namely various dimensions of anxiety and neuroticism.

Conclusions

The results of these studies underscore the power of a direct assay of brain function like fMRI to identify phenotypes in brain related to functional polymorphisms in genes likely important for human behaviour and neuropsychiatric illness. They also provide compelling evidence that the application of imaging genomics in light of the basic principles outlined above promises a unique opportunity to explore and evaluate the functional impact of brain-relevant genetic polymorphisms more rapidly and with greater sensitivity than existing behavioural assays. In turn, our appreciation of the biological mechanisms that contribute to complex behaviours and the variations in these mechanisms that lead to both individual differences and vulnerability to disease is likely to expand in a manner previously unattainable.

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