Review

The future of fMRI and genetics research

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Abstract

I provide a brief and subjective view of where the field of imaging genetics is heading. After recapitulating early debates between imagers and geneticists revolving around the topic of candidate gene studies, I point out the importance of genome-wide significant, rare and common variants. I propose that the next stages will be dominated by large-scale multi-site studies that will enable the examination of rare-high penetrance variants and methodological developments that will be required to properly assess the effects of pleiotropy, epistasis, and gene-by environment interactions. The incorporation of new sources of biological information such as whole genome sequencing, proteomic, lipidomic and expression profiles and cellular models derived from induced pluripotent stem cells opens new vistas for imaging genetics in a translational enterprise that is ultimately hoped to improve and create therapeutic options for psychiatric disorders.

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Introduction

"Imaging genetics" is a research approach in which genetic information and fMRI data in the same subjects are combined to define neuro-mechanisms linked to genetic variation (Hariri and Weinberger, 2003). Pioneered a little over 10 years ago, this approach has already gathered its share of ups and downs, controversies and debate. All in all, the field of imaging genetics has seen massive growth. In preparing a special issue of Neuroimage on the topic in 2010 (Pezawas and Meyer-Lindenberg, 2010), Lukas Pezawas and I were overwhelmed at the number of submissions and the literature that already had to be covered by meta-analyses and -according to Pubmed and citation statistics- that growth continues unabated.

Of imagers and geneticists

On the face of it, neuroimagers and geneticists have much in common. Both have a tendency of generating high-volume data sets characterizing important aspects of human biology. Consequently, both have statistical and conceptual problems with handling that complexity without generating too many false positives or false negatives (more on that later). On the practical side, both groups of scientists have a tendency to show up at the Dean’s office year by year explaining why they urgently need exactly that brand-new and more expensive equipment to continue their work that they cannot do with the machines bought last year. Both disciplines have also enjoyed tremendous growth and scientific success, which is one reason why the Dean’s office may be inclined to grant their requests. Specifically as seen from the point of neuropsychiatry, although the same is certainly true for many other disciplines of neuroscience, both genetics and imaging have been imbued with very high hopes to provide the breakthroughs needed to finally solve the mystery of mental illness, which leads to a biologically based understanding and taxonomy of these illnesses, and to find new treatments. Corresponding to those very high expectations, both genetics and neuroimaging have also been viewed with a degree of disappointment that these breakthroughs have not (yet) happened in the time frame originally envisioned (Insel, 2010; Insel and Scolnick, 2006).

Given these communalities, it could be assumed that combination of these two approaches to better understand human neurobiology and disease might be natural, especially since the tools to validate
and mechanistically underpin their findings in ever more sophisticated animal and cellular models is rapidly advancing. However, it is probably fair to say that the process has not been smooth. Since I was fortunate enough to be at NIMH when the first results showing an association of common genetic variants in human brain function were obtained (Egan et al., 2001; Hariri et al., 2002; Heinz et al., 2000; Small et al., 2000), I could see that almost from the very beginning, geneticists were skeptical whether single variants could have effects that big, and have continued to suspect that many imaging genetic findings are false positives. That debate at time became controversial enough that it received the attention of the general scientific community including features journals such as nature (Abbott, 2008).

Part of that debate is conceptual and concerns different viewpoints on the common goal of understanding neurobiological causes and modifiers of normal cognitive function and, especially, mental illness. Consider Fig. 1, which was introduced by Daniel Weinberger (this recent example is taken from Rasetti and Weinberger, 2011) and has by now become almost ubiquitous in imaging genetics talks. It depicts a cascade in which genetic variation impacts on neural function, which leads to systems-level dysfunction, which then alters information processing in brain linked to mental illness. The linear arrangement belies the fact that the interactions between the various levels are in fact very complicated, a fact that was originally intended to be shown by the divergent errors between each level, and depicted more directly in other conceptual papers from the Weinberger lab, where the complexities of genotype-phenotype interactions in schizophrenia were discussed in detail (Weinberger et al., 2001). In later visualizations (Meyer-Lindenberg and Weinberger, 2006), we have therefore tried to depict the relationships between genes, the brain and behavior as a network (Fig. 2). A given genetic variant is likely to impact on several neural phenotypes (pleiotropy), genetic variants interact which each other (epistasis) and with the environment (G×E), and there is no one to one mapping between neural systems and the behavioral level, or, indeed, between behavioral components such as cognitive sub-processes and mental illness.

Even if this complexity is properly understood, the motivations of imagers and geneticists to engage in unraveling this network still differ (Meyer-Lindenberg, 2010b). For geneticists, the impetus is often to find new genetic causes and modifiers of a phenotype that they regard as given. In other words, a geneticist will tend to read Fig. 1 from left to right, from gene to brain to behavior. From this angle, neuroimaging phenotypes may be attractive, because they are closer to the biology of genetic function than illness or cognitive phenotypes are. If that is true, the penetrance of genetic variation on that level should be higher and it should be easier to find genes that impact on this phenotype. That, in a nutshell, was the original impetus for the “endophenotype” concept of Gottesman and Shields (1967). It has especially taken hold in psychiatric neuroscience, because the need to find a biological basis is greatest in this discipline. Psychiatric illnesses are currently defined as behavioral and psychopathological syndromes observed by the clinician or the patient, combined with course criteria, and have little biological validity by themselves.

For imagers, the direction of reading of Fig. 1 tends to be the reverse, from right to left. They are interested in discovering how cognitive functions and mental illnesses work on the level of brain and therefore regard genes as a means to that goal. Taking a gene that has been associated with a given mental illness or cognitive function or biological process relevant for brain functions such as neural development, they will use imaging to try and understand what neural mechanisms are associated with that genetic variant. In other words, from the point of view of geneticists, a given neuroimaging phenotype (regarded as fixed) is a tool for finding new genetic variants, from the point of view of the neuroimager, the genetic variant viewed as given is the engine of discovery for discovering the neural function. Of course, these two points of view are not only not contradictory, they should be mutually reinforcing. Nevertheless, a considerable amount of cultural debate and misunderstanding has been sparked by these diverging vistas on a common landscape.

Returning to the initial question of whether imaging genetics is even possible, initial reviewers very reasonably demanded replication studies, for example in the landmark paper on 5-HTTLPR and amygdala function in Science (Hariri et al., 2002). At the same time, in the laboratory where I was working in Danny Weinberger’s program at the time, headed by Karen Berman, we took a different tack to provide a genetic “proof of principle”: We studied an illness in which the genetic “lesion” was unambiguous and there was a clear associated neuropsychiatric phenotype, and we tried to see whether we could link the two using neuroimaging. That condition was Williams Syndrome, a rare hemizygous deletion of about 28 genes on chromosome 7, that has a distinctive uneven profile of peaks and valleys in neuropsychological function and behavior, the most conspicuous of these two being a marked deficit in visual constructive function (the ability to create a whole from its parts, like a puzzle from its pieces) and marked hypersocial behavior (Meyer-Lindenberg et al., 2006b). In series of studies, we and others showed how mechanisms for these behavioral features of Williams Syndrome could be uncovered using functional and structural neuroimaging (Meyer-Lindenberg et al., 2004; Reiss et al., 2004).

### Candidate gene studies

From those early beginnings, candidate gene studies have seen explosive growth in imaging genetics. A candidate gene is a genetic variant

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**Fig. 1.** A figure depicting a cascade from gene to cell to the systems to the behavior level, as introduced by Daniel Weinberger. See Rasetti and Weinberger (2011) for a recent depiction.
that is studied based on an investigator hypothesis, for example because it is involved in a neural feature one wants to study, or because there is prior association evidence of that variant with an interesting neuropsychiatric or cognitive phenotype. Much of that was by necessity, because the hypothesis-free identification of genetic variants associated with neuropsychiatric phenotypes through methods such as genome-wide association was not achieved until 2008. Other methods such as linkage have been used to support studies of genes positioned at that locus, an example would be dysbindin (DTNBP1), but these are probably best thought of as candidate genes as well. Much of the debates surrounding imaging genetics has centered on studying these candidate genes. Specifically, it has been argued that we do not know enough about the biology of mental illness to construct plausible hypothesis, and much of the association evidence for single genetic variants that has been used as a point of departure for imaging geneticists has been criticized by geneticists for being inconsistent or weak. Candidate gene studies have been linked to “packing your own lunch box and then checking what’s inside” (S. Hyman, in (Abbott, 2008)).

This position overlooks two things. First, candidate genes are extraordinarily useful if the scientific goal is to study the biological system in which that candidate gene has its effect. For example, if one is interested in the dopaminergic system, the study of candidate genes in the dopamine system makes eminent sense, and if one is interested in modifiers of the therapeutic effect and side effects of antipsychotic drugs, and knows that these block dopamine D2 receptors, studying the effects of genetic variants of this receptor is informative (Zhang et al., 2007), as is the further genetic dissection of postsynaptic dopaminergic neurotransmission through appropriate candidate genes, such as AKT1 or PPP1R1B (Meyer-Lindenberg et al., 2007; Tan et al., 2008). From this viewpoint, studying a candidate gene is as similar source of controlled variance in a system as is the use of a pharmacological manipulation, for example.

Secondly, it is important to consider that the neural system uncovered by imaging genetics can itself be highly informative. If the brain changes with which a given genetic variant are found associated map onto a system seen abnormal in a given illness, this finding should strengthen the evidence for the association of that variant with that illness. Such a finding should also begin to provide a neural systems-level account of the mechanism through which it acts. Of course, given the multitude of confounds that affect studies in patients, such as medication or hospitalization history or associated behaviors ranging such as smoking, it cannot be simply assumed that a neuroimaging finding obtained in patients with a heritable illness compared to controls necessarily represents an intermediate phenotype. However, these are exactly the kinds of confounds that imaging genetics studies conducted in healthy risk allele carriers can avoid, making converging findings from patients and imaging genetics studies very useful. Even more importantly, the neural systems themselves have been studied extensively at multiple levels of neurobiological description, allowing the imaging genetic researcher to tie in a wealth of additional information into understanding the action of the genetic variant, and through this link this information to the neurobiology of the studied disorder. For example, the discovery that the common genetic risk factor associated with depression in the setting of environmental adversity (Caspi et al., 2003) impairs interactions between the cingulate and amygdala (Pezawas et al., 2005) is informative because firstly, both of these structures are implicated in depression (Murray et al., 2011). Secondly, much is known about functional interactions of cingulate and amygdala in the context of the regulation of negative emotion, fear and extinction (Quirk et al., 2003), therefore providing candidate mechanisms that might underlie gene environment interactions is observed for this variant. While it is clear that this research is only a step towards biological models of illness that will then have to be inferentially and experimentally tested, it is also clear that none of these insights are available on the
level of formal genetics operating on predefined phenotypes. Much of the attraction of imaging genetics lies in that process of mechanistic discovery.

Another point of debate is the question of the neural systems studied themselves. Because brain activation studies, which are commonly employed in imaging genetic's work, by their nature focus on the neural system that is activated by the paradigm (often, a cognitive activation task), another hypothesis enters the study in addition to the genetic variant(s) examined: namely, whether the neural system(s) that the task can access do in fact have a disease-related genetic causal component. One important step in this direction is to produce evidence of disease-related heritability, for example through the study of siblings (Goldman et al., 2009; Goldman et al., 2008; Rasetti et al., 2009). Otherwise, variance in a particular experiment of interest tied to a given genetic variant could arise from other systematic variance such as method, illness effects, or cohort effects. Further criteria that should be satisfied by an intermediate phenotype are laid out in the classical papers by Gottesman and colleagues (Gottesman and Gould, 2003; Gottesman and Shields, 1967) and discussed in the context of imaging genetics by (Meyer-Lindenberg and Weinberger, 2006).

So much for the conceptual issues. What can neuroimaging methodologically contribute to the candidate gene debate? Several papers have addressed the issue of candidate gene imaging genetics findings being false positives (Meyer-Lindenberg et al., 2008; Silver et al., 2011). These papers have concluded that, using currently available methods that are standard in the field in neuroimaging, false positives are well or even conservatively controlled. That also means that neuroimaging phenotypes can be used in conjunction with broader genetic searches, such as genome-wide association, if appropriate statistical control is extended over the range of genetic variants studied (see below). Secondly, an increasing number of genetic variants have been meta-analyzed. These meta-analyses (Mier et al., 2010; Munafò et al., 2008) have concluded that fMRI is indeed able to show effects of the studied genetic variants with a degree of consistency and, even more importantly, with a markedly higher penetrance than that available through studies of these same variants at the behavioral level. In other words, the hope of the original endophenotype concept (Gottesman and Shields, 1967), namely that neuroimaging phenotypes, might exhibit higher penetration for genetic variants because they are biologically closer to the genes, has so far been reflected in these meta-analyses. Interestingly, other analyses strongly suggest that not all methods that go below the level of clinical observation and behavior are created equal in this respect. For example, a meta-analysis of the effects of the common Val158Met variant in the COMT gene showed that neither neurocognitive, nor electrophysiological phenotypes showed higher penetrance than that exhibited on the clinical level (Flint and Munafò, 2007). While the conclusions of this study may have been affected by the inclusion of both patient and control samples and heterogeneity of the measures examined, it could therefore be that fMRI occupies a privileged position in the imaging genetics enterprise (Meyer-Lindenberg, 2010b). If so, it is probably because it combines exquisite sensitivity to functional change with a high spatial resolution in mapping that change onto the brain.

**Genome-wide significant variants**

Largely driven by developments in genetics, the landscape of imaging genetics is now changing. It was an important moment when in 2008, genome-wide association technology, which allows for the hypothesis-free testing of the majority of common genetic variants in the human genome on a chip, identified the first genetic risk variant associated with a neuropsychiatric phenotype, psychosis (O'Donovan et al., 2008). This risk variant in a gene called ZNF804A, is to this day one of the best supported genetic risk variants for psychosis, both in schizophrenia and bipolar disorder (Williams et al., 2011). Using imaging genetics, it was found that this variant impacted specifically on the connectivity between brain areas (Esslinger et al., 2009), mirroring findings found in subjects with overt disease (Meyer-Lindenberg et al., 2005) and suggesting a role of the gene in the development or elaboration of connectivity. Subsequent work confirmed an impact of these genetic variants on brain structures or functional systems relevant for schizophrenia, such as the ‘theory of mind’-system (Walter et al., 2011), and replicated the initial findings with regard to the prefrontal–hippocampal interaction (Rasetti et al., 2011). About two handfuls of genetic risk variants have been identified that show this level of hypotheses-free support (Purcell et al., 2009; Shi et al., 2009; Stefansson et al., 2009) and most of them have been studied in functional neuroimaging. An instructive example is afforded by the gene CACNA1C, first implicated in a meta-analysis of genome-wide association studies of bipolar disorder (Ferreira et al., 2008), but since confirmed to schizophrenia and depression (Green et al., 2010), which was in previous work shown to encode for a calcium channel important in hippocampal function in rodents (Moosmang et al., 2005). Using an imaging genetic approach, an impact on hippocampus function could be confirmed (Erickson et al., 2010) and extended to dorsolateral (Bigos et al., 2010) and medial prefrontal cortex (Erickson et al., 2010). Other neural phenotypes linked to schizophrenia of psychosis were also impacted by this risk variant. Similar work has been performed for variants such as Neurogranin (Pohlack et al., 2011) or HOMER1 (Rietschel et al., 2010). While in general, imaging genetics findings for these genome-wide associated variants have shown considerable convergence, the number of studies is not large enough for meta-analyses yet. Since, in essence, imaging genetics is a form of association study, inconsistencies in allelic or haplotype association effects or directionality must be expected in this approach as well, which could be due to population heterogeneity or differences in the used ascertainment paradigms.

Another important insight from genome-wide association studies that will occupy the future of imaging genetics research is the recognition that a sizeable amount of variation in the human genome is contained in copy number variants (CNV), larger scale, but rare, deletions, duplications or inversions of genetic material. Of a multitude of CNV found in the genome, several have been strongly associated with neuropsychiatric phenotypes, especially schizophrenia, autism and mental retardation (Walsh et al., 2008). It will be an important future goal of imaging genetics to study these rare variants, because they are the highest risk genetic risk factors for these illnesses yet available. Of course, finding and studying these rare conditions, whose frequency is around 1% or less in the normal population, is difficult. Several research programs of this type are currently underway. In evaluating these data, it will be interesting to look for both shared and distinct neural signatures of the various CNVs to identify core system(s) that are related to genetic high risk for mental illness. It will be equally interesting to find distinguishing features of the individual CNVs, which are likely to be associated with complex neuropsychiatric phenotypes of which conventional mental illness categories are only a facet. In doing so, researchers will be able to use the imaging methodology that has been refined on the copy number variants that were known prior to the GWAS area, such as Williams Syndrome, mentioned above, or 22q11 syndrome (Gothelf et al., 2005).

Even unambiguously supported, single common genetic variants and CNVs are extensively studied in the future, this will only provide part of the answer. To provide a fuller picture, imaging genetics methodology must evolve to confront the full complexity of the heritability of neuropsychiatric phenotypes (Fig. 2).

**Confronting complexity: Epistasis, pleiotropy, and G×E**

Recent data from formal genetics have shown that there is a clear need to move far beyond single variants when trying to explain the considerable heritability of psychiatric disorders. In a paper by the international schizophrenia consortium (Purcell et al., 2009), it was suggested that many tens of thousands of common genetic variants
may be necessary to account for the heritability of the schizophrenia, one of the most heritable psychiatric disorders. Such large-scale genotype sets, and their interactions, may in fact be able to explain the heritability of common traits and disorders, and therefore account for the “mystery of the missing heritability” (Yang et al., 2010). If that is so, imaging genetics must develop methods that will allow relating the effects of a large number of genetic variants on equally multidimensional neuroimaging phenotypes. In addition, the question of the interactions of genetic variants needs to be considered. In neuroimaging, methods for quantifying, the effects of haplotypes and other sets of interacting variants that may only be probabilistically known have been developed and applied (Meyer-Lindenberg et al., 2006c).

Another approach has been to search the space of epistatic interactions by first testing for interactions on the level of the clinical association, and then verifying these interactions using neuroimaging methodology (Nicoodemus et al., 2010). Multivariate approaches that tackle this problem, for example using sparse regression (Vounou et al., 2010), independent component analysis (Meda et al., 2010), gene-set association tests (Subramanian et al., 2005), pathway association tests (Linkste et al., 2010), machine learning or factor-oriented association tests (Liu et al., 2009) are appearing in the literature, but have not yet been tested on the amount of variants that would be necessary to be truly explanatory, at least based on the estimates of the formal genomic papers. While gene-set or pathway association tests bring in additional biological knowledge (about genes, gene groups, and their biological interactions) into the process, sparse regression, independent components and machine learning techniques are fundamentally atheoretical (although several admit, for example through a preselection step that filters the group of variants entering the analysis, including such information in preprocessing). Further developing these methods and integrating them with each other and with a quantification of rare and structural genetic variants will continue to be an important research frontier of imaging genetics.

This issue will become even more pressing as the field moves into whole genome sequencing, which will provide, for each subject, the entire genomic sequence and therefore an unprecedented amount of genetic detail. What the strategy exactly should be to relate neuroimaging phenotypes, which will always be smaller than the amount of information available genomically to these new datasets, is presently unclear. There is a need for the field here to discuss the relationship between large volumes of genetic data, sample size and directed hypotheses. A deviation to ‘hypothesis free’ analyses could be as detrimental as a sole reliance on candidate gene studies. Limited, but multiple allele or multiple gene or regional queries (e.g., based on metabolic pathways or guided by chromosomal deletion syndromes such as 22q11 that often increase risk for psychiatric disease) can be practical with very large sequence-based datasets even when the sample size will remain relatively small.

All of these lines of development will also increase the necessity for large-scale collaborative work even further. Such work must be done at several sites and over a longer time period, which will require the field of imaging genetics to pay much closer attention to the details of multi-site collaborations, for example in accounting for scanner variability, day by day variability and quality control, and the reliability of the employed paradigms. Successful examples of such multi-side imaging genetics cooperations have started to appear in the literature (Esslinger et al., 2009; Schumann et al., 2010) and are underway, for example the ENIGMA project (http://enigma.loni.ucla.edu/about/). These studies also provide examples for analytical techniques that can address some of the heterogeneity in these large samples, in the context of the general linear model but also by meta-analyzing samples from different sites.

A further frontier that will have to be tackled by imaging genetics research is the integration of environmental factors. The interactions between genotype, environment, disease or cognitive status and intermediate phenotypes are by no means as simple as a linear reading of Fig. 1 suggests. A recent review (Kendler and Neale, 2010) demonstrates that there are several causal settings in which an association of genotype and phenotype can be observed. One important consideration is the presence of interactions or correlations between genes and environment. Already by themselves, environmental risk factors are highly relevant, even for highly heritable disorders such as schizophrenia or autism, where an environmental contribution is clear from epidemiological studies (van Os et al., 2010). Neuroimaging work has begun to define neural mechanisms that might mediate environmental risk factors such as unstable social status (Zink et al., 2008) or urbanicity (Lederbogen et al., 2011). Interestingly, the results of this work converge with imaging genetics studies that have characterized the impacts of risk variants that by themselves show a degree of gene environment interaction or correlation, such as 5-HTTLPR or MAO-A (Meyer-Lindenberg et al., 2006a). This interesting convergence on a systems-level suggests neural mechanisms by which environmental adversity might be reflected in an inability to process negative emotions (Buckholtz and Meyer-Lindenberg, 2008) that will need to be pursued further in future work. An as yet almost unexplored terrain is the potential impact of epigenetic factors on neural intermediate phenotypes in humans. Epigenetics could account for a considerable amount of variants in disorders that have heritable and familiar components. Unfortunately, brain tissue is only available invasively in humans and research strategies that require cellular materials are therefore strongly limited. It is, however, encouraging, that in a recent pioneering paper, Bertolino and co-workers were able to show correlations between an epigenetic modification of the COMT gene and the imaging genetics phenotype which is generally associated, prefrontal efficiency (Ursini et al., 2011). The question remains, however, whether correlations observed between different tissue compartments that show a little overlap in lifetime effects on the epigenetic programming might not themselves be regulated by trait factors, in this example, by COMT genotype. As the translational enterprise of neuropsychiatry unfolds further, imaging genetics will have to integrate itself with other techniques to quantify mediating biological processes. Similar to epigenetics, at the cellular compartments allows the ascertainment of proteomic, lipidomic and expression profiles that could in principle be related to neural intermediate phenotypes. An exciting new development is the possibility to create neurons in vitro from a given patient through induced pluripotent stem cell technology (Brennand et al., 2011). This could allow trait-like parameters of neural development, measures in these newly generated neurons, to be related to neural intermediate phenotypes ascertained by imaging.

In summary, imaging genetics has had a stimulating role on the field of fMRI research in general, and on the understanding of neuropsychiatric phenotypes in particular, by delineating neural systems that may mediate genetic risk for these disorders. This is an important contribution to creating a neural risk architecture of these illnesses, which will in turn be pivotal for their evidence-based taxonomy and for personalization of treatment. While these goals are still in the future, the position of imaging genetics in the translational enterprise, its possible privileged position in being able to map genetic effects of high penetrance while simultaneously being relatable to the behavioral phenotypes that neuropsychiatrists study, also promise a much-needed impetus to speed up drug development in psychiatry, as I have recently discussed elsewhere (Meyer-Lindenberg, 2010a). Looking back from the initial studies looking at single common genetic variants and small deletions, the field has come a long way. Clearly, it has also, as outlined, a long way to go to truly confront the complexity inherent in its subject matter, but I am optimistic that the imaging genetics approach will continue to grow with its challenges. I look forward to an updated version of this “future review” after the next decade has passed.

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