Intermediate or brainless phenotypes for psychiatric research?

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For highly heritable brain disorders, such as schizophrenia and autism, investigating genetic effects on the level of neural systems seems an obvious approach. Nevertheless, the usefulness of the intermediate phenotypes ('endo' phenotypes) continues to be debated energetically. We argue that, while not all intermediate phenotypes are created equal, the hypothesis-driven investigation of the translational cascades linking genetic variation to disturbed behavior is a viable and important strategy that should not be supplanted by an exclusive focus on brainless, clinical/categorical phenotypes investigated in very large numbers of participants.

Introduction

It has been established for decades that for many severe psychiatric illnesses, such as schizophrenia, bipolar disorders and autism, the majority of disease risk is attributable to genetic factors (81% for schizophrenia; Sullivan *et al.* 2003). For most others, such as depression, attention deficit hyperactivity disorder, personality disorders and anxiety disorders, the genetic risk component is still substantial (37% for major depression; Sullivan *et al.* 2000). Put another way, one key to unlocking the as-yet largely unknown pathophysiology of these common and disabling conditions is in the genes. As these are brain disorders, few would doubt that, directly or indirectly, many such genetic variations will be found to act on the human brain. It is also clear that genetic risk variants cannot act directly on the level of psychiatric disease categories, since these are clinical-behavioral entities far removed from the level of molecular biology. Instead, genetic variation will be phenotypically manifest on a variety of levels of description from gene to cell to neural systems to the clinical and social, making understanding phenotypic variation on each of these levels a valid question for research (Gottesman & Gould, 2003). Beyond being a valid line of inquiry, it would be imprudent if research would not try to mine this established circumstance for the discovery of mechanisms mediating genetic risk, because this is one of the very few avenues available to arrive at

mechanistically novel treatments, an area where psychiatry has fallen conspicuously behind other medical disciplines and where the field's track record in reducing mortality and morbidity is disappointing (Insel & Scolnick, 2006).

These considerations notwithstanding, intermediate phenotypes in psychiatry have been subjected to energetic debate (Meyer-Lindenberg & Weinberger, 2006; Munafo *et al.* 2008), including in this journal (Flint & Munafo, 2007). Several of these disagreements revolve around matters of content. However, part of the debate concerns conceptual differences of how intermediate phenotypes are viewed and pursued in psychiatry. It is some of these latter points that I would like to discuss here. While I take my examples mainly from schizophrenia, I believe that the points they illustrate pertain to the application of intermediate phenotypes in all areas of psychiatry.

Distinction between forward and reverse genetics

Most of the psychiatric genetics literature assumes phenotypes to be fixed entities for which genes are then mapped (genes 'for' schizophrenia, personality, brain volume, etc.). This is comforting since it allows the pursuit of these genes in complete ignorance of how these phenotypes arise, as long as they can be reliably measured. In this context, the original conceptualization of an 'endophenotype' by Gottesman & Shields (1967) proposed that they would be useful for 'the identification of genes in the hypothesized polygenic systems conferring vulnerabilities to disorders'. Instead, intermediate phenotypes are usually applied

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in reverse genetics mode: we wish to understand how a given genetic variant acts on the level of cell, brain or cognition (Meyer-Lindenberg & Weinberger, 2006). This was also envisioned by Gottesman & Shields (1967), who suggested that endophenotypes could help in the decomposition of psychiatric diagnosis into biologically valid disease entities. These two applications are by no means mutually exclusive. On the contrary, intermediate phenotypes with high effect sizes and power should be very useful for finding novel genes and the first report of genome-wide scans performed on neuroimaging phenotypes have now appeared (Potkin *et al.* 2009). But the main attraction of intermediate phenotypes is in the hope of understanding the underlying biology, using genes as entry points into the process, an aspect that is sometimes missed entirely in this debate. A related misconception is that intermediate phenotypes have to be genetically 'simpler' than disease phenotypes to be useful. For some intermediate phenotypes, such as neuroticism, there is evidence that this is not the case (Shifman *et al.* 2008). Again however, a simpler genetic architecture, while certainly an advantage for forward genetics, is not a necessity for the understanding of mechanisms in reverse genetics mode instead. The rate limiting factor in gene identification is often the effect size of a risk allele on phenotypic variance and, as detailed below, there is evidence that these can be considerably higher the closer one gets to the biological substrate.

Intermediate phenotypes are biological readouts causally linked across levels of description

One of the main aspects of a translational genetic approach that has advanced our understanding of psychiatric disorders is that knowledge can be transported from one level of biological description to the other. Much is known about how molecular interactions influence neuronal growth and firing rates. The regional distribution of many transcripts, neurotransmitter systems and neuronal cell types has been mapped. A particularly powerful strategy of this type is provided by the functional segregation of the human brain (i.e. an anatomy where the function of an activated area can be inferred to some degree from its location), which provides a canvas for interfacing genetic information with a large body of preclinical and cognitive neuroscience evidence. Using intermediate phenotypes, this massive knowledge can be made useful for psychiatric genetics. For example, mapping genetic effects of a common variant in the serotonin system, impacting on risk for impulsive violence, in human brain using imaging genetics (Meyer-Lindenberg *et al.* 2006*a*; Buckholtz *et al.* 2008)

identifies neural systems that not only correspond to the topography of serotonergic innervation in the brain but are also known to be involved by previous evidence in neuropsychological mechanisms, such as extinction. This provides a systems-level mechanism for previously observed genotype–environment interaction of this gene in behaviour in males (Caspi *et al.* 2002). In schizophrenia, genetically distinct, but biologically linked variants in genes PPP1R1B, encoding DARPP-32 (Meyer-Lindenberg *et al.* 2007) and AKT1 (Tan *et al.* 2008), tagging crucial regulatory proteins in the canonical and non-canonical signal transduction pathways for dopamine, respectively, have been shown to converge on a circuit linking striatum to dorsolateral prefrontal cortex. This clarifies the neural system through which dopaminergic genetic variation plays out to increase risk for psychosis, a system that has been implicated before in schizophrenia in basic neuropsychological functions such as gating (Swerdlow *et al.* 2001). Also in this example as in several others, neural changes found in healthy carriers of risk alleles recapitulate features found, usually in more pronounced form, in patients with schizophrenia and their relatives (Rasetti *et al.* 2009). Characterizing genetic effects across these levels of description, if successful, thus provides not only novel information about the biology of mental illness, but also convergent evidence that these variants are functional – again, a point sometimes missed by traditional geneticists who regard these biological data as fixed phenotypes, ignore the biology linking them and then in the extreme case may demand multiple comparison corrections for them, the exact opposite of what these data mean.

Not all intermediate phenotypes are created equal

Another misunderstanding that stems from the same source is the assumption that any quantitative trait located closer to the level of the biological antecedent is by virtue of that fact a useful intermediate phenotype, or the converse assumption that if one has provided evidence that one such intermediate phenotype is not clearly useful, one has provided arguments against that whole approach. The evidence is, in fact, in favour of the conclusion that some, for example, cognitive, intermediate phenotypes for some diseases and genetic variants may not exhibit higher genetic penetrance or a simpler architecture (Flint & Munafo, 2007). While that argues against a simplistic assumption that cognitive disturbances 'underlie' mental illnesses such as schizophrenia, in the sense that they are obligatory causal antecedents of these disorders, it does not provide an argument against an intermediate phenotypes approach as such.

Imaging genetics: sensitive and specific

In particular, there is now clear and convergent meta-analytic evidence that imaging genetics, one of the most widely used intermediate phenotype approaches, provides data delineating neural systems for genetic risk with high sensitivity and specificity. Regarding specificity, we have shown that, if statistical procedures that are standard in the imaging field are used, results from imaging genetics are conservative both for permuted data and for actual genotypes drawn from a panel of non-conservative single-nucleotide polymorphisms (SNPs) with low prior probability of being associated with cognitive dysfunction or risk for schizophrenia (Meyer-Lindenberg *et al.* 2008). Regarding sensitivity, two meta-analyses of the two most commonly studied genetic variants, 5-HTTLPR with regard to its effect of amygdala function (Munafo *et al.* 2008), and rs4680 COMT Val158Met with regard to its effect on prefrontal cortex function (Mier *et al.* 2009), have provided very similar estimates of effect sizes of 0.7–1.0, corresponding to sample sizes to detect genetic effects at 80% power of around 80 participants. This is at least an order of magnitude better than what is achievable with behavioural diagnoses. This is nowhere illustrated better than with these two genetic variants themselves, which show no consistent association with the psychiatric phenotype for which they were originally nominated in the latest available pooled analyses (Fan *et al.* 2005; Munafo *et al.* 2005; Risch *et al.* 2009).

Should we all just work in genome-wide significant variants?

These latter data highlight a potential difficulty in the field – if compelling data of the impact of a genetic variant on an intermediate phenotype for a mental disorder are demonstrated, does that support the candidacy of this genetic variant even if the evidence for association with the categorical diagnosis is inconclusive, as is the case with practically all variants known today? Clearly, arbitrary genetic variants studied with just any intermediate phenotype would not accomplish this, but would simply be a case of circular reasoning. However, I am not aware that anyone pursues such a strategy. Of course, it is easy to require compelling evidence on both levels before proceeding, but I do not believe this is an issue that can be addressed while being ignorant of or ignoring the underlying biology. Given that mental disorders, as currently diagnosed, must be biologically heterogeneous and genetically complex, inconclusive association data across populations are expected and observed (Harrison & Weinberger,

2005). Genome-Wide Association Study (GWAS) data of categorical diagnoses, while certainly a productive strategy to identify risk variants outside currently studied candidate biological systems, are not a panacea in this regard. Because the relative risks for disease associated with frequent variants are so low, ever-increasing sample sizes to reach the required *p* values are necessary, requiring the combination of samples that can become highly heterogeneous, genetically and etiologically. In fact, recent evidence shows that the number of common polymorphisms associated with disease risk found in such samples may number in the thousands. A recent publication from the International Schizophrenia Consortium (2009) concluded that their data strongly support a polygenic basis to schizophrenia that involves common SNPs and explains at least one-third of the total variation in liability in a score SNP dataset (selected from SNPs that showed a nominally significant *p* value in a discovery sample and was then applied to a target sample for classification of patients and non-patients) including more than 35 000 SNPs, and showed that classification got better as SNPs with more lenient thresholds were included (International Schizophrenia Consortium, 2009).

While it would be splendid, and comfortably safe, if gene discovery solely based on brainless psychiatric phenotypes sufficed, the evidence is therefore that this is not so. In this setting, intermediate phenotypes offer a way forward, not if they are viewed as yet another yes or no binary measure against which to test genetic variants, but rather by answering the question, what does that specific variant do in the brain, and does this fit our data on the pathophysiology of the illness? If it does, and if it fits, I do believe that data like these strengthen the candidacy of this genetic variant and even if one chooses to be sceptical with regard to disease association, it is hard to deny that such data still provide a potential therapeutic entry point into that neural system via the product of the gene, enabling a translational strategy towards mental illness. The progress in this field is therefore not circular, but dialectical. The study of genetic variants refines our understanding of (and therapeutic options with regard to) the neural systems they influence as related to the disorder, and that refined understanding on the neural systems level provides a viable strategy to achieve biologically less heterogeneous groups that will then be useful for forward genetic searches.

Of course, variants that do survive the GWAS procedure are certainly useful to study and intermediate phenotypes, especially neuroimaging, should be used to understand neural mechanisms mediating risk linked to variation in or near the handful of compelling novel genome-wide significant or borderline

significant common variants in ZNF804A, Neuro-granin, TCF4 and genes located in the major histo-compatibility complex (International Schizophrenia Consortium, 2009; Shi *et al.* 2009; Stefansson *et al.* 2009). Recent work on ZNF804A (Esslinger *et al.* 2009), in fact, confirms long-standing assumptions about the pathophysiology of the illness; in this case, the importance of disturbed connectivity in neural circuits linked to the dorsolateral prefrontal cortex in the disorder (Stephan *et al.* 2006).

Moving forward

An intermediate phenotype approach will easily be able to accommodate additional sources of reliable data about genetic variation. In particular, in addition to confirming that a large proportion of heritable risk for schizophrenia is likely to be polygenic (International Schizophrenia Consortium, 2009), not attributable to rare variants, GWAS studies have also discovered rare structural variations in the genome associated with a high risk for schizophrenia that may be subject to negative selection (International Schizophrenia Consortium, 2008; Walsh *et al.* 2008). Since intermediate phenotypes have been quite successful in dissecting neural mechanisms contributing to neurocognitive and behavioural phenotypes in the 22q11 microdeletion (Gothelf *et al.* 2005) and in Williams Syndrome (Meyer-Lindenberg *et al.* 2006*b*), imaging genetics should be used to study brain mechanisms underlying these more common copy number variants. Further, if evidence for multiple rare variants should surface, for example, through a deep sequencing approach, an intermediate phenotypes approach could still be applicable if these rare variants can be appropriately grouped, for example, by gene or region.

But biological heterogeneity is not the only important issue for understanding and treating mental illness that is accessible to an intermediate phenotypes strategy. Interactions with the environment are potentially major factors in the risk architecture for illnesses such as schizophrenia (van Os & Sham, 2003). The delineation of neural mechanisms underlying gene–environment interactions in risk for psychiatric disorders using intermediate phenotypes is in fact a topic of consortia, such as the IMAGEN (Wong & Schumann, 2008) and EU-GEI (van Os *et al.* 2008) initiatives in Europe.

Finally, as mentioned at the beginning, intermediate phenotypes, through an account of the biological cascades through which genetic risk becomes manifest, hold considerable promise for drug discovery. In the available literature, intermediate phenotypes strategies towards personalized treatment strategies based on genetics (Apud *et al.* 2007), promising novel drug targets (Huffaker *et al.* 2009) and imaging genetics data (Tan *et al.* 2007), underpinning mechanistically new drugs in later phase development (Patil *et al.* 2007) can already be found. Again, consortia are forming that hope to mine intermediate phenotypes for much-needed advances in the treatment of severe psychiatric illness such as NEWMEDS (Hughes, 2009).

For all of these approaches, even a complete list of definitive risk variants for psychiatric disorders would just mark the beginning of the final chapter. Only through translational strategies that incorporate intermediate phenotypes across levels of biological description can this genetic information be turned into an engine of discovery for pathophysiology, diagnosis and, perhaps most importantly, treatment.

Declaration of Interest

None.

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