Functional themes from psychiatric genome-wide screens

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Technological advances and a greater degree of inter-laboratory co-operation mean that genome-wide analyses can now be used to identify genetic variants that are robustly associated with the risk of developing psychiatric and neurological disorders. In contrast to the candidate gene approach, such screens may identify variants within genes which have a hitherto unappreciated role in disorder pathogenesis, and whose brain function is obscure. In this Perspective, I discuss how the behavioral functions of such genes may be investigated using model systems, drawing attention to the potential caveats and limitations with such approaches. The power of focused cross-species studies needs to be effectively exploited to enable useful insights into the molecular pathogenesis of common and disabling disorders, and ultimately to provide better clinical outcomes for patients.

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GENOME-WIDE SCREENS IN PSYCHIATRY

More effective genotyping techniques, greater inter-laboratory collaboration (therefore larger pooled sample sizes), and more precise diagnostic/phenotyping strategies, now permit the identification of robust novel candidate genetic loci for vulnerability to common and disabling psychiatric/neurological disorders using genome-wide screens (GWS; Lee and Lupski, 2006; Cichon et al., 2009). Contemporary psychiatric GWS comprise three types: genome-wide association (GWA), copy number variant (CNV), or linkage. In GWA studies, up to 2.5 million single nucleotide polymorphisms (SNPs) spanning the entire genome may be genotyped in several thousand matched cases and controls. Allelic variants that are more common in cases than in controls are considered to be “associated” with the disorder; these variants may have functional consequences themselves, or alternatively, may be in high linkage disequilibrium (LD) with functional variants (Cichon et al., 2009). Most variants identified by GWAS to date have relatively small effects on risk, although they may be common within the population. In CNV studies, genotyping arrays are used to identify larger chromosomal segments that are either disproportionately frequently duplicated or deleted in cases relative to controls; these mutations tend to be relatively rare, but can exhibit large effects (Lee and Lupski, 2006). Linkage studies, in which selected regions of the genome are genotyped in order to analyze co-segregation with disorder-associated phenotypes, are less fashionable (reflecting the difficulty of obtaining family-based samples), but can be performed as a prelude to identifying a candidate genomic region prior to more comprehensive, directed association analyses (Yang et al., 2011). The lack of success of the linkage-based approach to psychiatric illness to date has suggested that such disorders are not caused by few, highly penetrant transmitted mutations, but rather by many transmitted variants of small effect, or by de novo mutations of large effect.

Genome-wide screens have now been performed for several psychiatric/neurological conditions. Studies on schizophrenia, bipolar disorder, and Alzheimer’s disease for which large, well-annotated sample collections are available have been the most fruitful (Hardy and Williams, 2010; Doherty et al., 2011; Ripke et al., 2011; Sklar et al., 2011), whilst lower-powered studies on disorders such as attention deficit hyperactivity disorder (ADHD) have failed to identify robust candidate gene variants (Neale et al., 2010) but have highlighted genomic regions which when perturbed may influence vulnerability (Williams et al., 2010). Although the biological functions of some significant GWAS gene hits are known a priori, e.g., the vesicular role of the protein encoded by PICALM identified in a recent GWAS in patients with Alzheimer’s disease (Harold et al., 2009), often the precise role of the gene products in the brain is unclear. For genes such as ZNF804A, identified in a GWAS of patients with schizophrenia/schizoaffective disorder (O’Donovan et al., 2008), functional annotation is almost completely absent. Most CNV studies have highlighted the involvement of genomic regions spanning multiple genes, the functions of which may often be unknown, and not all of which may contribute to disorder phenotypes. Once a poorly characterized candidate gene is identified from a GWS (as many more will be in the near future), a key objective should be to examine its normal function. Understanding the behavioral effects of such genes will be critical given that psychiatric disorders are diagnosed according to behavioral criteria and prior to the identification of any overt pathophysiology. Although human studies can provide tantalizing insights into the functions of putatively pathogenic genetic variants (Walters et al., 2010; Esslinger et al., 2011; and genetic neuroimaging, Linden and Thome, 2011), such approaches are necessarily correlative, hampered by ethical/recruitment constraints, and potentially confounded by medication effects. Here,
investigations in model systems, where direct links between genotype and brain function can be explicitly established, will be of benefit. Selecting the most appropriate model system and analytical tools will be crucial to understanding the behavioral pathology of brain disorders.

CHOOSING A MODEL SPECIES

The genomes of organisms such as C. elegans and Drosophila may be experimentally manipulated to investigate developmental effects on primitive nervous systems, and such studies may have relevance for human brain disorders (Kretzschmar, 2005; Wu and Luo, 2005). However, humans and other mammals are more alike both in terms of their genetic complement, and in terms of their basic structural/functional brain homology; as such, this latter group may represent the best type of model for understanding complex psychiatric genetic phenomena. For the past quarter of a century, mice have been the model of choice for investigating the role of genetics in mammalian behavior (Kendler and Greenspan, 2006); they can be produced/maintained in large numbers on consistent genetic backgrounds, and are amenable to neurobehavioral analysis. However, mice are sub-optimal for techniques such as neurosurgery and electrophysiology due to their small size; here, rats are of greater use. As a consequence of their longer history as an experimental model in behavioral neuroscience, the neurobiology of the rat is better defined than that of the mouse, and there are currently more behavioral paradigms available for this species. Moreover, recent breakthroughs in genetic engineering technology mean that the rat is now amenable to genetic manipulation to some extent (Jacob et al., 2010). Whichever rodent model is chosen, there are a number of caveats that should be taken into consideration when modeling human disorders: first, whilst the rodent and human genomes are structurally similar, they do differ significantly in their regulation, expression, and post-transcriptional modification, exemplified by species’ differences in X-inactivation (Okamoto et al., 2011). Also, most experimental rodents are maintained on an inbred background to ensure homozgyosity at every genetic locus (and homogeneity of physiology). In contrast, humans are generally outbred and show substantial allelic variability. Hence, genetic backgrounds could differentially modify the effect of the gene under investigation across species. The use of recombinant inbred strains (including genetic reference panels), which possess a level of genetic, molecular, and cellular complexity matching that of human populations, has been important for identifying genetic loci for complex traits and for testing experimental predictions in a manner more relevant to human disease (Williams, 2009). Second, rodents and humans differ in terms of their brain size, structure, complexity, and associated behavioral functions (Kesner and Churchwell, 2011). Third, in the laboratory, rodents are maintained in small same-sex groups and are often behaviorally tested during the day; in the wild, the sexes are free to interact and are nocturnal. Hence, in terms of social and circadian behaviors, laboratory rodents may not even resemble those living wild, much less humans. Finally, one rodent model cannot recapitulate every key aspect of a multifactorial psychiatric disorder, and nor is it necessarily the case that any one model will exhibit behavioral features specific for one disorder. Despite these caveats, research in rodents has substantially illuminated the neural basis of psychiatric phenotypes in humans, and has led to significant advances in treatment (McArthur and Borsini, 2008).

CHOOSING A SPECIFIC GENETIC MODEL

As genetic manipulation in rats is in its infancy, I have limited my discussion to mouse models; undoubtedly, equivalent rat models will shortly come online. In humans, SNPs may confer risk through a variety of mechanisms: abrogating gene function (nonsense mutation), inducing subtle increases or decreases in gene expression, influencing the fidelity of splicing mechanisms and/or post-transcriptional stability, disrupting protein structure/function (mis-sense mutation), affecting the availability/strength of protein-binding sites or through influencing genomic structure. Deletion CNVs typically act to eradicate the function of multiple genes, whilst duplication CNVs cause over-expression of multiple genes; both types of CNV may also disrupt the function of adjacent genes through altering local chromatin structure. The particular mouse model to be used may be chosen according to the proposed mechanism of action of the “pathogenic” variant in humans. Mutations eliciting subtle effects on gene expression (and therefore behavior) are currently relatively difficult to induce and characterize in rodents. Therefore, models possessing relatively gross changes in gene function, and exhibiting larger effects on brain and behavioral functions, have been, and will probably continue to be, the most practical short-term option. Gene-deletion models may be most appropriate for examining the neurobiological effects of nonsense mutations, expression-reducing variants or deletion CNVs, whilst transgenic mice (which possess a copy of the gene of interest inserted into the genome, ideally at a known site, and thus over-express the gene), may be more relevant to understanding the processes mediated by expression-enhancing variants and duplication CNVs. In order to specify which gene(s) perturbation within a CNV may be pathogenic, a systematic analysis of lines of mice, each with an activating mutation in a CNV gene (in the case of deletion CNVs), or lines of mice, each transgenic for one CNV gene (in the case of duplication CNVs), may be undertaken. Large-scale knockout screens (see below) should soon render the former approach achievable.

The functional consequences of many human SNPs will not be obvious. As a default option, understanding the normal function of a candidate gene may be done in “gene-deletion” models (where wildtype mice possessing two functional alleles of the gene are compared to heterozygotes possessing one functional allele and homozygotes possessing no functional alleles). Traditionally, homologous recombination has been used to create targeted deletions in genes of interest; in conventional “knockout” mice, the function of a critical exon is disrupted from conception, so that homozygous mutants are deficient for the gene product throughout life. Multi-national collaborations [the International Knockout Mouse Consortium (IKMC)1 and the International Mouse Phenotyping Consortium (IMPC)2], have recently been established with the goals of knocking-out and summarily characterizing the vast majority of genes in the mouse genome over the

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1http://www.knockoutmouse.org
2www.mousephenotype.org
next few years. Knockout rodents can also be generated as so-called “conditional forms” whereby the gene of interest is deleted at particular developmental timepoints, or in particular brain regions (Morozov, 2008). Such models permit researchers to identify when and where a given gene product exerts its phenotypic effects and may be particularly informative for understanding the pathogenesis of developmental or degenerative conditions. Other models of value for the field of psychiatric genetics include: mice with defined point mutations in loci of interest originally induced by the chemical mutagen N-ethyl-N-nitrosourea (ENU; Acevedo-Arozena et al., 2008), mice with chromosomal anomalies (Davies et al., 2009; Trent et al., 2011), and inbred mice with spontaneous mutations in genes of interest (Clapcote and Roder, 2006; Ishizuka et al., 2007).

Each genetic mouse model has its own merits and limitations: molecular constructs for generating knockout mice may be extensive and time-consuming, and in some cases, genes may not be amenable to homologous recombination (e.g., those on the Y chromosome). However, knockout mice are generally easy to genotype and might be eradicated by repeatedly breeding to a suitable strain, a costly, labor-intensive process requiring large numbers of mice. Identifying the pathogenic SNP from several in high LD represents a major challenge in human psychiatric genetics. Through using multiple lines of ENU mutants, each with a discrete point mutation within the orthologous LD region, it may ultimately be possible to decipher the causal variant(s). Mice with gross chromosomal mutations may be useful for modeling chromosomal abnormalities with associated neurobehavioral phenotypes (including certain sex chromosome ploidies, Davies, 2011), or the effects of large CNVs, but these mice may have perturbed expression of multiple genes with pleiotropic effects, and therefore cannot provide data on the influence of individual genes. Moreover, generating mice with targeted gross chromosomal manipulations, although possible (O’Doherty et al., 2005), is technically demanding.

Once a particular genetic model has been selected, to limit unnecessary animal usage initial studies should focus on one sex and one age-point at which significant effects are most likely to be identified; positive effects may then be further characterized in the opposite sex, at different age-points, and in different strains. Where a candidate gene/region associated with a “neurodevelopmental” disorder is manipulated, first studies might be limited to male mice, as these disorders tend to be more prevalent and/or more severe in this sex; conversely, for later-onset “affective” disorders, first studies should be performed in female mice, as these disorders tend to be more prevalent and/or more severe in this sex (Rutter et al., 2003). Whether the original association is sex-specific may also inform what sex of model to use initially, e.g., association between SNP rs7597593 within ZNF804A and schizophrenia is apparently driven primarily by association in female patients (Zhang et al., 2011) so examining the specific effects of Zfp804a inactivation/overexpression in female mice may be a useful precursor to studying effects in males. Female rodents are generally avoided in behavioral studies due to the potentially confounding effects of their cyclic hormonal variations. However, there is an increasingly recognized need for pre-clinical behavioral studies to examine females (not least to provide better predictive data for translational pharmacological studies), and provided variations in hormone levels are co-varied for (e.g., by monitoring estrus status), or completely removed (e.g., through ovariectomy), such studies will be worthwhile. In terms of age, mice with mutations of developmental disorder-associated candidate genes might be behaviorally characterized at earlier timepoints than mice with mutations of later-onset “affective” disorder-associated candidate genes; mice with mutations in neurodegenerative disorder-associated candidate genes (e.g., Picalm) should be examined into old age, cost implications notwithstanding.

CHOOSING A BATTERY OF BEHAVIORAL TASKS

A number of fundamental physiological measures should first be obtained in mutant animals; theoretically, risk variants could influence vulnerability via non-specific effects during early pre- and post-natal life. Basic measures might include: indices of gross embryonic/extra-embryonic development, the time of occurrence of developmental milestones in utero and postnatally, mortality/morbidity rates for individual genotypes in utero and postnatally (to resolve whether the gene is having an important developmental role, and to determine the extent to which surviving subjects represent a selected sub-population), interactions with the dam/siblings, bodyweight, and gross motor function. A second level of assays could then be employed; these may provide data of relevance to a particular disorder, and additionally, may help to unconfound the results of any subsequent behavioral analyses. Such assays may include tests of sensory, circadian, and consummatory functions, locomotor activity, assays of reactivity to novelty and exploration, tests of fear and anxiety, and paradigms taxing motivation. Several of the “primary” and “secondary” assays listed above will be performed routinely in the phenotyping screen undertaken by the IMPC.

The behavioral assays to be used in the third tier of analysis should be guided principally by the core symptoms of the particular disorder(s) associated with the candidate variant. Obviously, human-specific constructs such as “theory of mind” cannot be effectively modeled in rodents. However, there are now many behavioral paradigms available for rodents which examine sensorimotor, social, and cognitive behaviors, including those relevant to autism (Silverman et al., 2010). Many rodent cognitive tasks are based on analogous human tasks used in the clinic, and generate data with significant cross-species translatability (Humphrey et al., 1999; Winstanley et al., 2006; Zeeb et al., 2009; Bussey et al., 2011). Ideally, as many as possible of the core symptoms of the associated disorder should be assessed in the genetic model; assays should not be limited to those that are “easiest” to perform, most commonly used or already established in the laboratory, and, where necessary, appropriate collaborations should be initiated. It is surprising that many rodent models with disruptions in putative ADHD-associated genes, are only tested for one (non-specific) core feature of the disorder (hyperactivity) and not for
the other core features of attentional or impulsive dysfunction, despite the fact that assays of attention and impulsivity can now be performed routinely (Humby et al., 1999; Isles et al., 2003; Win-
stanley et al., 2006; Humby and Wilkinson, 2011). Where possible, multiple assays should be used to obtain converging evidence for effects on behavioral functions.

Once a robust behavioral phenotype has been established and specified, its physiological, cellular, and molecular under-
pinnings can be investigated in detail and manipulated phar-
macologically. A number of additional manipulations examining the influence of putative “environmental” protective/risk factors for the disorder of interest on the behavioral phenotype may be performed; such manipulations might include administration of established/experimental therapeutic drugs, acute/chronic stress, altered housing/social conditions, dietary perturbations, environmental enrichment, or in utero/postnatal exposure to toxins.

Studying relatively simple models such as rodents with muta-
tions in single candidate genes will be useful for understanding the role of those specific genes in basic mammalian brain development/function, and for highlighting molecular and neural path-
ways of interest. However, by themselves, such studies will not fully explain the pathogenesis of complex psychiatric disorders which often exhibit symptoms unique to humans. A major challenge in the near future will be to produce more elegant models which more faithfully mimic the constellation of behavioral, neu-
robiological, and molecular pathologies seen in brain disorders (i.e., improved face validity). This could theoretically be done by generating and examining mice with mutations in multiple con-
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referenced candidate genes whose effects mirror those of the putative pathogenic variants in humans (i.e., improved construct valid-
ity); such mice could then be exposed to environmental factors of relevance to the pathogenesis of the disorder. A conceptually sim-
ilar approach has already been taken for some neurodegenerative conditions with promising results (Lewis et al., 2010).

CONCLUSION

Genome-wide screens are highlighting more genetic variants involved in the pathogenesis of psychiatric/neurological disorders. Many of these are within genes of obscure function. Genetically amenable rodent models, despite their limitations, will be of value in understanding the pathways through which risk variants may influence vulnerability to disorders of the brain, and hence in identifying novel therapeutic targets. They will also be of use in the systematic examination of how environmental risk factors interact with genetic predispositions to affect behavioral phenotypes. Studies in rodent models should allow psychiatrists to better predict the behavioral repertoire, clinical course, and response to treatment of patients possessing particular genetic risk variants.

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