OPINION

Intermediate phenotypes and genetic mechanisms of psychiatric disorders

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Abstract | Genes are major contributors to many psychiatric diseases, but their mechanisms of action have long seemed elusive. The intermediate phenotype concept represents a strategy for characterizing the neural systems affected by risk gene variants to elucidate quantitative, mechanistic aspects of brain function implicated in psychiatric disease. Using imaging genetics as an example, we illustrate recent advances, challenges and implications of linking genes to structural and functional variation in brain systems related to cognition and emotion.

Although the genomic revolution transforms all areas of medicine, psychiatry arguably stands to benefit the most. For one, the majority of common psychiatric disorders show high genetic familiality. Heritability (the proportion of total variance in a trait due to genetic variation) has been estimated from twin studies at 81% for schizophrenia[1] and 37% for major depression[2]. As well as being debilitating for sufferers, these diseases impose enormous medical and economic burdens, making understanding the genetic mechanisms of mental disorders crucial. In addition, genetic research is expected to aid in the definition of psychiatric disease entities themselves, which are largely based on clinical phenomenology and lack biological validity. However, understanding the neural mechanisms by which genetic variation increases risk has long been elusive. The genetic architecture of psychiatric risk is complex and is dominated by multiple interacting contributing factors. As genes do not encode for psychopathology, it is reasonable to expect that the association or penetrance of gene effects will be greater at the level of relatively more simple and biologically based phenotypes. Therefore, intermediate phenotypes were initially envisaged to be tools for gene discovery, improving the power of association studies by reducing phenotypic heterogeneity.

However, as we review here, the development of imaging genetics — a strategy for mapping neural structure and activity as a function of genotype in living humans — has encouraged a conceptual transformation by showing that the greater power of intermediate phenotypes lies in using genetic risk variants as tools for the discovery of the mediating neural mechanisms that bridge the gap from DNA sequence to pathological behaviour. Other quantitative parameters from electrophysiology, neurobiochemistry and neuropsychology can also be powerful tools to index intermediate neurobiological processes that are influenced by genetic variation, but these are not in the scope of this article. After briefly recapitulating the development of the intermediate phenotype concept, we review recent advances in the characterization of prefrontal circuits in schizophrenia[3–5] as target neural systems for mechanisms of genetic susceptibility and their epistatic and environmental interactions. Although we use schizophrenia as our example, most of the arguments equally apply to other complex psychiatric disorders such as depression[6] (BOX 1), attention deficit hyperactivity disorder[7], addictions[8] and autism[9]. We conclude with an assessment of the methodological and conceptual challenges of the intermediate phenotype approach.

Intermediate phenotypes in psychiatry

When the tools for gene identification in simple Mendelian disorders — mainly linkage analysis followed by positional cloning — became available, they were applied with enthusiasm to psychiatry[10]. The success of linkage analysis is influenced by several assumptions, including genetic homogeneity, moderate to major gene effects in families and a valid model of inheritance[11]. Given the likelihood that these assumptions do not hold in many complex disorders such as mental illness[11], it is not surprising that the first wave of linkage studies in psychiatry was hindered by weak results and non-replication[10]. Subsequent studies of larger and more carefully characterized samples were more successful and have identified several replicable linkage sites, but these probably reflect regions where multiple genes of small effect are found[12]. Indeed, the weight of the evidence indicates that risk for psychiatric disease is usually conferred by multiple small effect genetic variants interacting with one another and with the environment[13], that is, psychiatric disorders are genetically complex, similar to other common conditions such as hypertension, obesity and diabetes. This implies that no particular constellation of genes or environmental conditions will be characteristic of most ill individuals, and gene–gene and gene–environment interactions, both additive and epistatic, further complicate analysis[14]. The intricate genetic architecture of susceptibility also makes it more difficult to deal with problems such as pleiotropy or variable penetrance found in many, even Mendelian, genetic diseases[15].

The rate-limiting factor in gene identification is often the effect size of a risk allele on phenotypic variance. Many factors contribute to the small effect size of genes in psychiatry. Few variants involve changes in protein structure or function. More often, aspects of gene regulation are implicated, which have relatively subtle biological effects. Importantly, genes do not encode for psychiatric phenomena (for example, hallucinations and panic attacks), and so, almost by definition, the more behavioural the phenotype, the less directly it will be predicted by a genotype (FIG. 1). This leads to the strategy of studying underlying quantitative traits that more directly index biology, analogous to moving from the study of cardiac insufficiency or stroke (complex diseases) to ventricular hypertrophy[16] and cholesterol metabolism. This strategy offers several advantages for behavioural disorders: biological traits are expected to be closer to the genetic substrate, enhancing penetrance; the traits should be observable in genetically at risk but behaviourally unaffected individuals; and, if the traits are sufficiently causally upstream to index a biological process that makes a separable contribution to disease, the genetic architecture should be simplified.

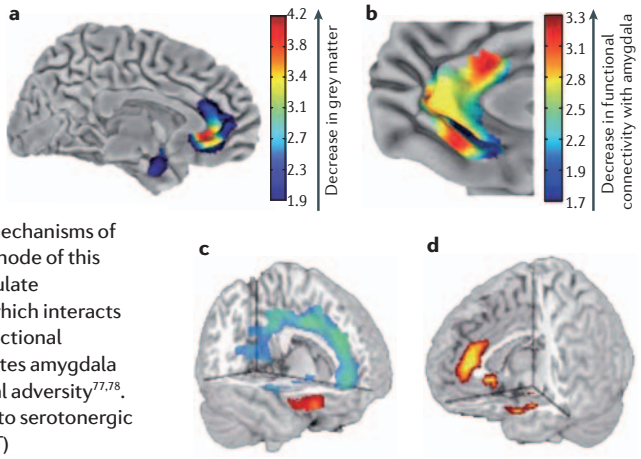
There are two key reasons why the intermediate phenotype concept has resonated strongly with psychiatry[17]. First, the uncertain and phenomenological nature of psychiatric diagnosis makes reference to a biological level of description attractive. Second, the complexity of the human brain

virtually necessitates an effort to parse this problem into tractable biological subprocesses. Almost uniquely in the psychiatric arena, risk factors or intermediate biological phenotypes have come to be known as 'endophenotypes'[17], from the Greek word endos for interior, within. This usage dates back to a seminal paper by Gottesman and Shields[14], who adopted it from a report on evolutionary biology. As used later by Gottesman and Gould[17], and subsequently by the field, it was hoped that endophenotypes would assist both "in the identification of aberrant genes in the hypothesized polygenic systems conferring vulnerabilities to disorders"[17] and in the decomposition of psychiatric diagnosis into biologically valid disease entities (FIG. 2a). Although the original use of the term did not focus on the biological mechanisms of gene effects, but more on psychological processes, it has come to be used in this context as well.

From the outset, it was stressed that using endophenotypes for gene discovery mandates that they be 'sufficiently' heritable[17]. Elaborating on this, several authors[7,18,19] have specified that, as well as being heritable, an endophenotype should: have good psychometric properties; be related to the disorder and its symptoms in the general population; be stable over time; show increased expression in unaffected relatives of probands; cosegregate with the disorder in families; and have common genetic influences with the disorder. It is clear that establishing these criteria for any given measure is an extensive and costly process, and published work suggests that few, if any, endophenotypes actually fulfil them[7]. This issue has become pressing with the application and, arguably, success of neuroimaging in characterizing neural system intermediate phenotypes in psychiatry. After revolutionizing our understanding of the neural underpinnings of normal cognition and altered brain function in disease, neuroimaging has been used to study genetic variation, which has proved surprisingly penetrant at this level[14–21]. For the phenotype discussion, imaging provides, for each participant, an enormous amount of functional–structural data that can potentially characterize gene effects in the brain. However, this unprecedented access to brain biology also carries unique problems: it is unclear, for example, how the issue of multiple testing should be handled in this context, how multimodal datasets can be related to genetic information of growing complexity in the comparatively small populations currently studied, and how reliable and comparable across laboratories and populations these data are. More importantly for some, heritability has not been conclusively demonstrated for many structural and most functional parameters used. The traditional endophenotype model was a strategy for reducing genetic complexity and increasing genetic effect size for facilitating gene discovery. Imaging strategies might prove to be valuable in this respect, but large imaging datasets necessary for gene discovery are difficult to acquire.

Although these problems and possible solutions will be discussed, our primary perspective is that neuroimaging is bringing about a conceptual change in the way in which biological intermediate phenotypes are viewed and pursued in psychiatry and behavioural genetics by enabling a previously inaccessible level of biological characterization and validation of genetic effects (FIG. 2b). Imaging genetics can delineate neural systems that are affected by genetic variation, offering a way of 'functionating' polymorphisms beyond simple clinical statistical association. The assumption of the intermediate phenotype strategy is that gene effects at the level of the brain are a more direct effect of genetic variation than is complex behaviour, and will show association in carriers of risk alleles even if the

Box 1 | **The application of imaging genetics to depression**



The pathophysiology of depression is complex, affecting integrated pathways linking cortical, subcortical and limbic sites and their molecular mediators[6,72,73]. A useful example of the application of imaging genetics to understanding the neural mechanisms of genetic risk concerns a key node of this system, the subgenual cingulate (Brodmann's area 25)[6,74,75], which interacts with the amygdala[76] in a functional feedback circuit that regulates amygdala processing of environmental adversity[77,78]. This circuit is closely linked to serotonergic (5-hydroxytryptamine; 5-HT) neurotransmission[79]. The serotonin transporter gene (*SLC6A4*) contains a variable number of tandem repeats variant in the 5′ promoter region (5-HTTLPR), with reduced transcription of the 5-HTTLPR short (*S*) allele in comparison to the long (*L*) allele. Individuals carrying the *S* allele tend to have increased anxiety-related temperamental traits[80]: this variant predicts risk for depression in conjunction with early environmental adversity[81]. Several studies have found that *S* allele carriers evince an exaggerated response during functional MRI[70,82,83], suggesting that amygdala hyper-reactivity might be a neural substrate of trait anxiety predisposing to psychiatric disease. A reduction in grey matter was found in the subgenual cingulate region of healthy carriers of the *S* allele[6] (**a**). Analyses of functional and structural connectivity confirmed close interactions of this region with the amygdala and suggested an inhibitory feedback circuit[6]. The *S* allele was associated with reduced coupling between the amygdala and the subgenual cingulate cortex (**b**), and the degree of that coupling predicted close to 30% of the variability of trait anxiety in these normal individuals[6], suggesting that the psychiatric risk associated with 5-HTTLPR is mediated by a weakened circuit for the extinction of fear. Two studies have also shown that a frequent regulatory variant (844G>T) of tryptophan hydroxylase 2 biases the reactivity of the amygdala[84,85]. A common variable number of tandem repeats polymorphism[86] in monoamine oxidase A (*MAOA*), which encodes a key enzyme for the catabolism of serotonin and other neurotransmitters during neurodevelopment, has been implicated in violence; the low expression variant also showed a pronounced gene–environment interaction in predicting violent offences in males with adverse early experience[87]. Multimodal imaging results[88] have indicated a similar effect to that of 5-HTTLPR on the structure and function of the amygdala and perigenual cingulate cortex, suggesting a shared serotonergic mechanism of emotional regulation. However, *MAOA* showed more extensive effects in both structure (**c**, reductions in blue) and activation (**d**, reductions in yellow), notably affecting more caudal regions of the cingulate, which are associated with cognitive control — in agreement with previous findings[89] — as well as the orbitofrontal cortex and hippocampus. Panels **a** and **b** reproduced, with permission, from *Nature Neuroscience* REF. 6 © (2005) Macmillan Publishers Ltd. Panels **c** and **d** modified, with permission, from REF. 88 © (2006) National Academy of Sciences.

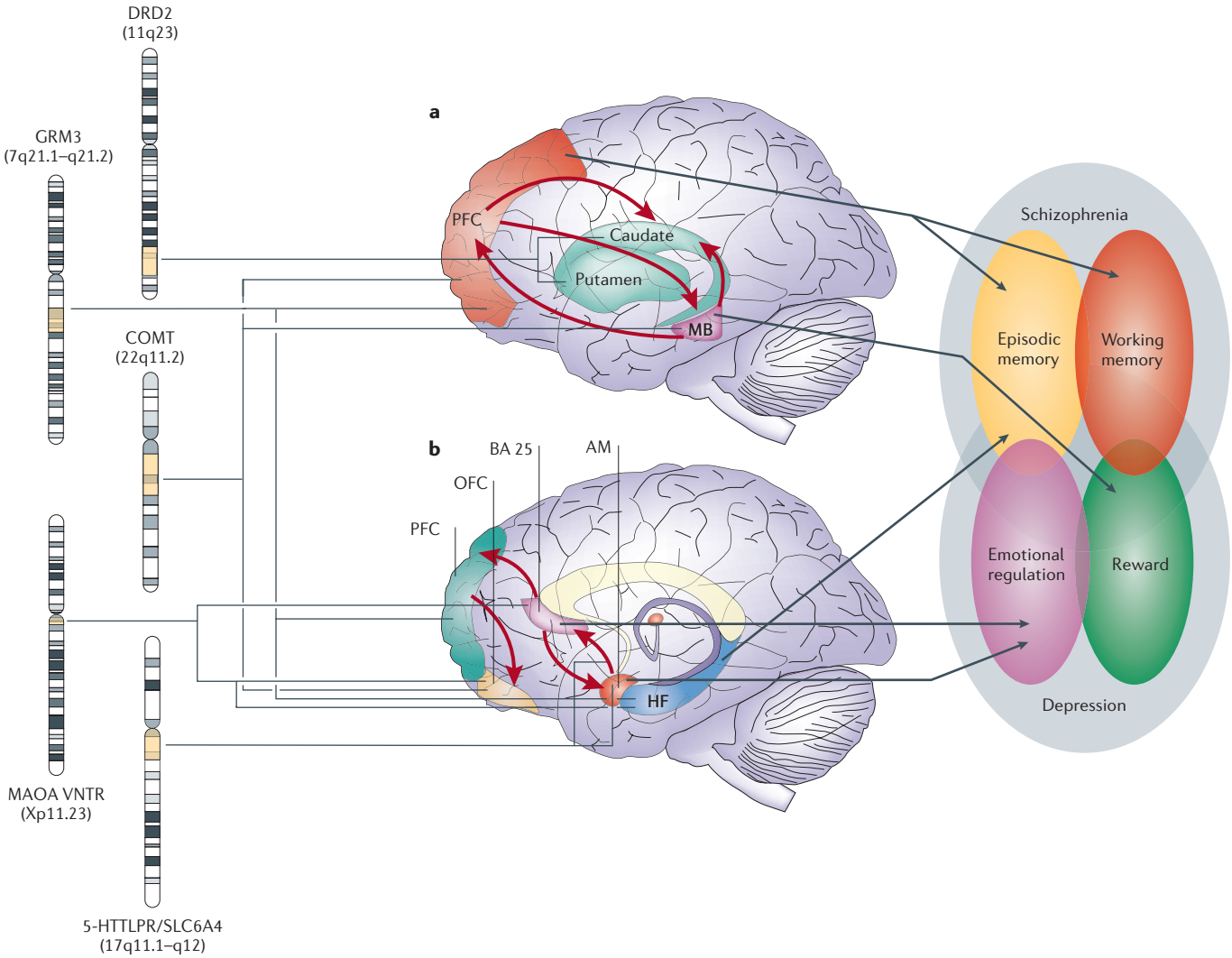


Figure 1 | **The complex path from genes to behavioural and disease phenotype: mediation through brain circuitry.** Multiple genetic risk variants affect, through interaction with each other and the environment, multiple neural systems linked to several neuropsychological and behavioural domains that are impaired, in differing proportions, in psychiatric diseases. No one-to-one mapping exists between genes and neural system mechanisms, or between mechanisms and behaviour. As examples, the following genetic variants are depicted (chromosomal variation in parentheses): GRM3 single nucleotide polymorphism 4 (REF. 57) (7q21.1–q21.2), dopamine receptor D2 (DRD2) Taq 1a[56] (11q23), catechol-*O*-methyltransferase (COMT) Val66Met[4,46] (22q11.2), serotonin transporter length polymorphism (5-HTTLPR/SLC6A4)[6,70] (17q11.1–q12) and monoamine oxidase A variable number tandem repeat (MAOA VNTR)[92] (Xp11.23). These are shown to affect a circuit that links the prefrontal cortex (PFC) with the midbrain (MB) and striatum (caudate and putamen) (**a**), which is relevant for schizophrenia, and a circuit that connects the amygdala (AM) with regulatory cortical and limbic areas (**b**), which is implicated in depression and anxiety (BOX 1). These circuits, in turn, are shown to mediate risk for schizophrenia and depression and various neuropsychological functions. Although illustrative, the connections shown correspond to published work and show that a given gene will influence a variety of neural circuits, which in turn influence several behavioural and clinical parameters. BA 25, Brodmann's area 25; HF, hippocampal formation; OFC, orbitofrontal cortex.

carriers show no clinical diagnostic characteristics. As illustrated below, many risk gene associations to brain-based phenotypes are observed even in healthy individuals. To the considerable degree that susceptibility genes contribute to psychiatric risk, this approach offers a powerful bottom-up strategy to discover biologically valid knowledge about previously unknown mechanisms. Imaging genetics therefore becomes a guide to the discovery of neural circuitry that translates genetic effects into behaviour, and endophenotypes implicate endomechanisms. Although this approach does not depend on the demonstrated heritability of the imaging probe used, to increase the prior probability of observing biologically relevant neural activity, the chosen paradigm must activate brain systems that are plausibly related to the disease under study based on independent evidence. We outline such evidence below for neural systems related to schizophrenia.

We stress that although we use the term endophenotype in deference to an earlier period in the evolution of thinking about this genetic strategy, there is nothing 'hidden' about the biological phenomena in the context of neurobiology and imaging. The term intermediate phenotype is preferred, both because it implies a biological trait that is in a predictable path from gene to behaviour and because the phenotypes and mechanisms are not secondary, but probably primary. This is analogous to its usage in other areas of complex medical genetics. From this perspective, it might be more correct to refer

to the behavioural phenomena as emergent or exophenotypes.

Neural circuits as psychiatric phenotypes. Convergent molecular, cellular, clinical, neurophysiological, neuropsychological and imaging work clearly indicate dorsolateral prefrontal cortex (DLPFC) dysfunction as a key feature of schizophrenia[3]. A defining feature of the DLPFC is its extensive interconnectedness with other brain regions[20,21], making the characterization of these distributed functional networks important[22,23]. We focus on two circuits involving the DLPFC, one interlinking it with the hippocampus and the other with the striatum, as intermediate phenotype targets for genetic association.

In a topographically well-organized manner, the neostriatum receives excitatory glutamatergic projections from the cortex and thalamus, integrates them with monoaminergic inputs and sends them via the globus pallidus and substantia nigra pars reticulata to the thalamus, which projects back to the prefrontal cortex (PFC)[20]. These parallel processing loops are crucial for the integration of sensorimotor, cognitive and emotional information[20]. Lesions to the neostriatal-prefrontal system impair prefrontal-dependent cognitive functions[24] that are characteristic of the cognitive deficits found in schizophrenia[25]. Prefrontal-striatal interactions have been proposed to 'filter' different pieces of information that are competing for cortical processing[26]. This filtering could underlie cognitive symptoms and potential schizophrenia intermediate phenotypes such as the abnormal pre-pulse inhibition of the startle response[26].

The dopaminergic system, originating from the midbrain, is an important modulator of the neostriatal-prefrontal circuit[27] and is the target of a large number of therapeutic and abused drugs. The DLPFC participates in the control of midbrain dopaminergic neurons[28]. These prefrontal–midbrain interactions are crucial for motivated behaviour, working memory[29] and reward-related learning[30]. A large body of work has established an inverted u-shaped relationship between working-memory-related activation of DLPFC neurons and dopaminergic — especially dopamine D1 receptor — stimulation[29], with dopaminergic tone essential for optimizing signal-to-noise ratio, or tuning. In schizophrenia, dopaminergic abnormalities are suggested by the fact that antipsychotic agents block D2 receptors — a cornerstone of the so-called 'dopamine hypothesis'. Although a simple model of

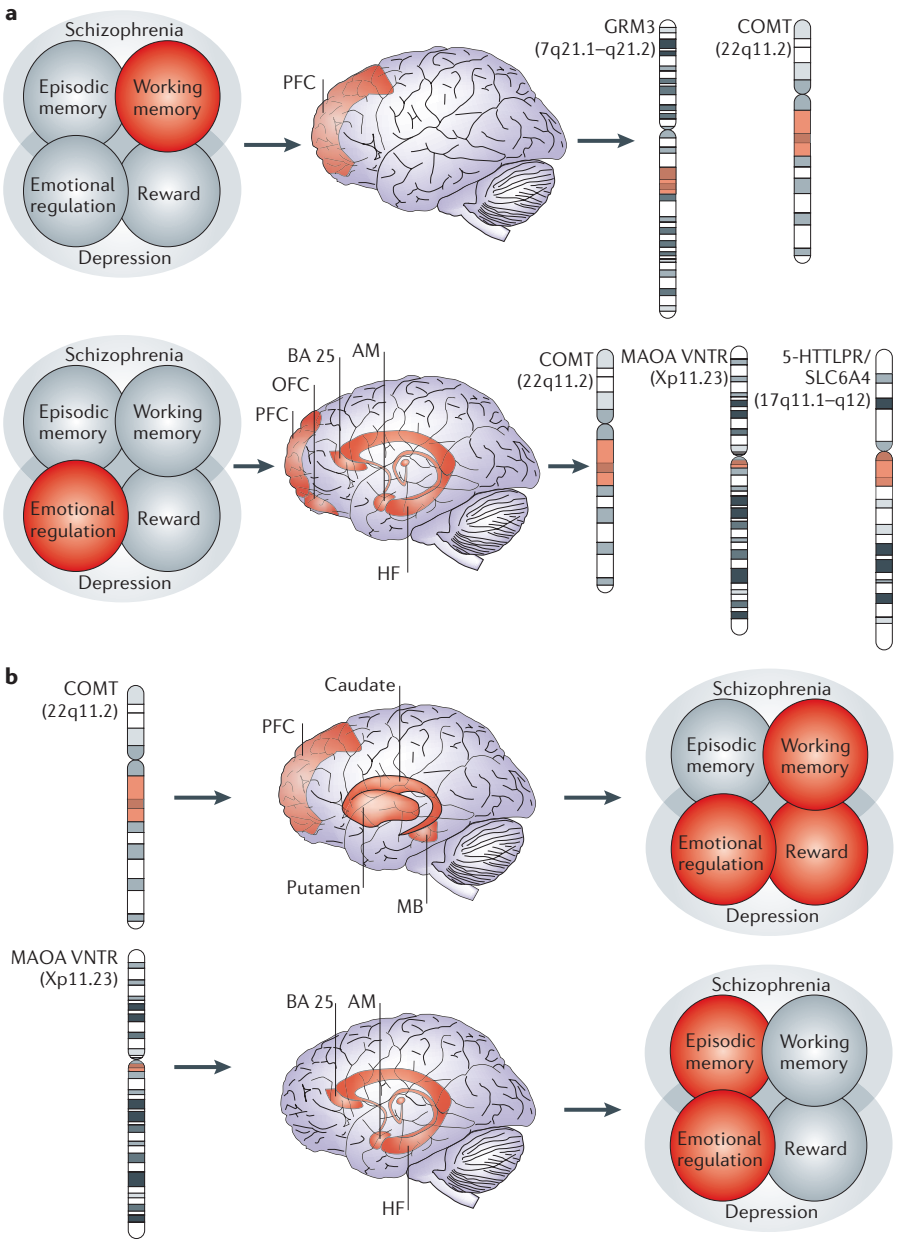


Figure 2 | **Intermediate phenotypes as tools for gene discovery versus neural mechanism characterization.** Examples of two alternative approaches to the identification of genetic variants linked to psychiatric disorders are illustrated, with the relevant genes, neural systems and behavioural phenotypes highlighted in red, and arrows indicating the direction of research inference. **a** | In the gene discovery approach, behavioural or neural systems phenotypes are used to reduce genetic complexity and increase penetrance to identify genes implicated in psychiatric disorders. For example, deficiencies in the electrophysiological response to auditory stimulation were used to identify an association of schizophrenia with the α7 nicotinic cholinergic receptor[93]. In the figure, prefrontal cortex (PFC) dysfunction has been linked to catechol-*O*-methytransferase (COMT) and GRM3 genetic variation[57,65], and emotional regulation has been linked to variation in COMT, monoamine oxidase A (MAOA) and the serotonin transporter length polymorphism (5-HTTLPR/SLC6A4)[6,83,88], and so could have been hypothetically employed as a phenotype to identify these genes. **b** | In the neural mechanism approach, genes known to be associated with psychiatric disorders or behavioural traits are used to discover neural mechanisms mediating their complex emergent phenotypic associations, implicating these mechanisms in the psychiatric disorders to which they have been linked. Examples include the use of the COMT Val158Met polymorphism to characterize prefrontal function and prefrontal–midbrain interactions linked to risk for schizophrenia[4,46], and the delineation of cingulate circuitry regulating amygdala (AM) function mediating risk for anxiety and depression through an investigation of the MAOA variable number tandem repeat (VNTR)[6,70]. BA 25, Brodmann's area 25; HF, hippocampal formation; MB, midbrain; OFC, orbitofrontal cortex.

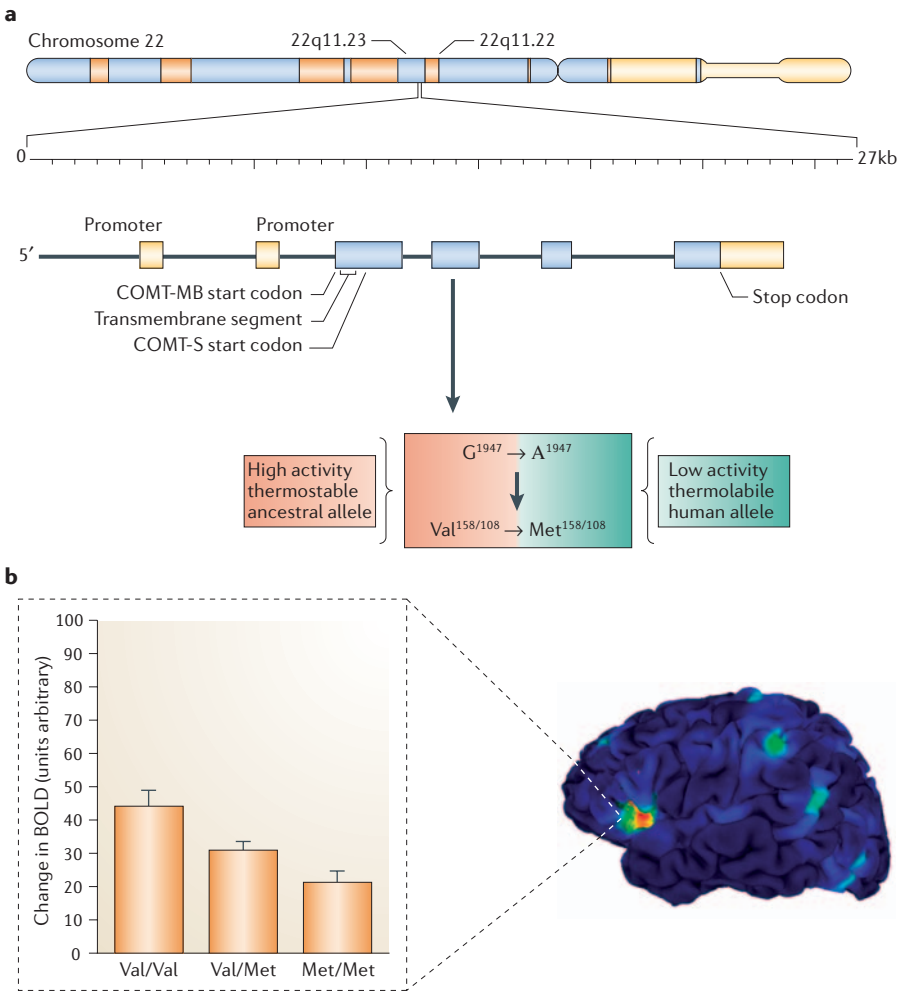


Figure 3 | **COMT Val(108/158)Met polymorphism and its effect on prefrontal function. a** | The catechol-*O*-methyltransferase (*COMT*) gene on chromosome 22q11.23 contains common Val108Met and Val158Met substitutions in exon 4 that affect the thermal stability of the COMT protein (soluble (COMT-S) and membrane bound (COMT-MB) isoforms, respectively). This leads to a conformational change and significant decrease in enzyme activity for Met alleles[45], preferentially increasing prefrontal extrasynaptic dopamine because COMT provides the major clearing step for dopamine released from the synapse in this region[41,42]. As dopamine affects prefrontal cortical neuronal activity[29] (FIG. 4), this leads to changes in prefrontal activation observed during neuroimaging using paradigms that challenge the prefrontal cortex. **b** | Linear effect of the COMT variant on prefrontal cortex activation during a working memory task in 126 healthy controls. BOLD, Blood-oxygen-level-dependent signal that indicates changes in the proportion of oxygenated blood in the brain, which changes in response to neural activity. Panel **b** modified, with permission, from *Mol. Psychiatry* REF. 65 (2006) © Macmillan Publishers Ltd.

increased dopaminergic tone throughout the brain has been disproved, disinhibited neostriatal dopaminergic neurotransmission in schizophrenia is a consistently reported abnormality in actively psychotic patients that has been linked to deficiencies in prefrontal function[5].

The hippocampal formation supports episodic memory and spatial orientation in animals and humans[31], and is strongly implicated in schizophrenia by evidence from neuropathology[32] and from structural[33] and functional[34] neuroimaging. Dense pathways directly and indirectly connect the DLPFC and hippocampal formation[21], and interactions between these regions are implicated in episodic memory[35], as well as in the regulation of emotional–motivational states[36]. Consequently, it has been proposed that DLPFC–hippocampal formation interactions might be particularly disturbed in schizophrenia[37]. This so-called 'disconnection hypothesis'[22] is attractive because neonatal hippocampal formation lesions in animals induce changes in the PFC that manifest postpubertally[38], suggesting an explanation of epidemiological data that links schizophrenia to early neurodevelopmental disturbances[39]. Neuroimaging has indeed provided some evidence of abnormal functional connectivity between these regions in schizophrenia[23]. In monozygotic twins discordant for schizophrenia, abnormal hippocampal morphology predicted prefrontal response[37], suggesting that genetic approaches to interrogate this risk circuit are viable.

Genetic susceptibility mechanisms. On the basis of the intermediate phenotype strategy outlined above, it follows that if the dysfunction of the neostriatal-prefrontal and DLPFC–hippocampal formation circuitry is related to genetic risk for schizophrenia, aspects of prefrontal dysfunction associated with schizophrenia would be found in genetically at risk individuals who are not clinically ill. Results from several studies of healthy relatives of patients with schizophrenia support this proposal. By implication, this suggests that genes related to risk for this disease affect the biology of this neural circuitry and show greater penetrance at this neural systems level than at the level of clinical phenomenology. Probably the best evidence so far for both of these predictions exists for catechol-*O*-methyltransferase (COMT), an important enzyme that degrades cortical dopamine. Dopamine action at the synapse is terminated either by dopamine transporter reuptake, through diffusion out of the synapse, or by COMT catabolism. Because dopamine transporters are scarce in the PFC[40], COMT is a crucial determinant of prefrontal dopamine flux[41,42]. The *COMT* gene is located at 22q11.2, a region implicated in schizophrenia by linkage[43] and by its involvement in the 22q11.2 syndrome, which is the result of a hemideletion that is associated with a strongly increased risk of schizophrenia-like illness[44] (FIG. 3a). A common substitution of valine by methionine (at amino acid 158 of the membrane-bound form of the protein found in the brain) affects the stability of COMT, leading to conformational changes and a subsequent significant decrease in enzyme activity in the brain and in lymphocytes[45].

Neuroimaging studies using a reliable activator for the PFC — the *n*-back working memory task — have shown that this coding variant affects PFC activation[46] (FIG. 3b). In agreement with this discovery, variation in COMT also modulates PFC-dependent neuropsychological performance[47] and the cortical response to amphetamine, which increases synaptic dopamine[48]. The latter finding suggests that the *COMT* genotype places individuals at predictable points along

the putative inverted u-shaped curve that links prefrontal dopamine stimulation and neuronal activities, with homozygotes for the Val-encoding allele — which presumably possesses less synaptic dopamine due to maximal COMT activity — positioned to the left of Met allele carriers, which seem to be located near the optimum of that curve (FIG. 4). Additional evidence for this comes from a positron emission tomography (PET) study[4] showing that the *COMT* genotype has an impact on the prefrontal regulation of midbrain dopamine synthesis in a genotype-dependent directionality consistent with the inverted u-shaped model[49,50] (FIG. 5a). This indicates that risk for schizophrenia associated with this common variant is due to reduced signal-to-noise in the PFC, an idea supported by the finding that working-memory-related and working-memory-unrelated activity in the PFC are inversely coupled to midbrain dopamine synthesis and directionally dependent on *COMT* genotype[4]. This concept also provides a mechanistic explanation of the seemingly counterintuitive finding that, in the 22q11.2 hemideletion syndrome, Met hemizygotes are at a higher risk for psychosis and structural brain change[51] (FIG. 5b). Because of the gene dosage effect, COMT activity is considerably reduced in this syndrome, meaning that Met-allele carriers will now be functionally suboptimal because they are positioned to the right of the optimum peak of the inverted u-shaped curve, whereas Val allele carriers are now closer to optimal: the null chromosome rescues COMT Val hemizygotes, but hurts Met individuals.

Despite striking and consistent data showing an effect of *COMT* genotype on cortical function[52] and dopamine regulation, the evidence for association with schizophrenia *per se* is inconsistent and weak at best[53,54]. This might be viewed as confirmation that genetic associations that are weak on the level of the disease phenotype can be highly penetrant on the biological intermediate level. There are two potentially more lasting contributions of the imaging genetics approach to COMT. First, there is the independent validation and extension of the concept of cortical inefficiency as a key endomechanism that contributes to risk for schizophrenia. Second, there is the delineation of circuit properties impacting on prefrontal function (such as prefrontal–midbrain and prefrontal-hippocampal interaction) that can now be functionally dissected by using genetic information to predict variance in components of this circuit that were previously not under experimental control in humans[4].

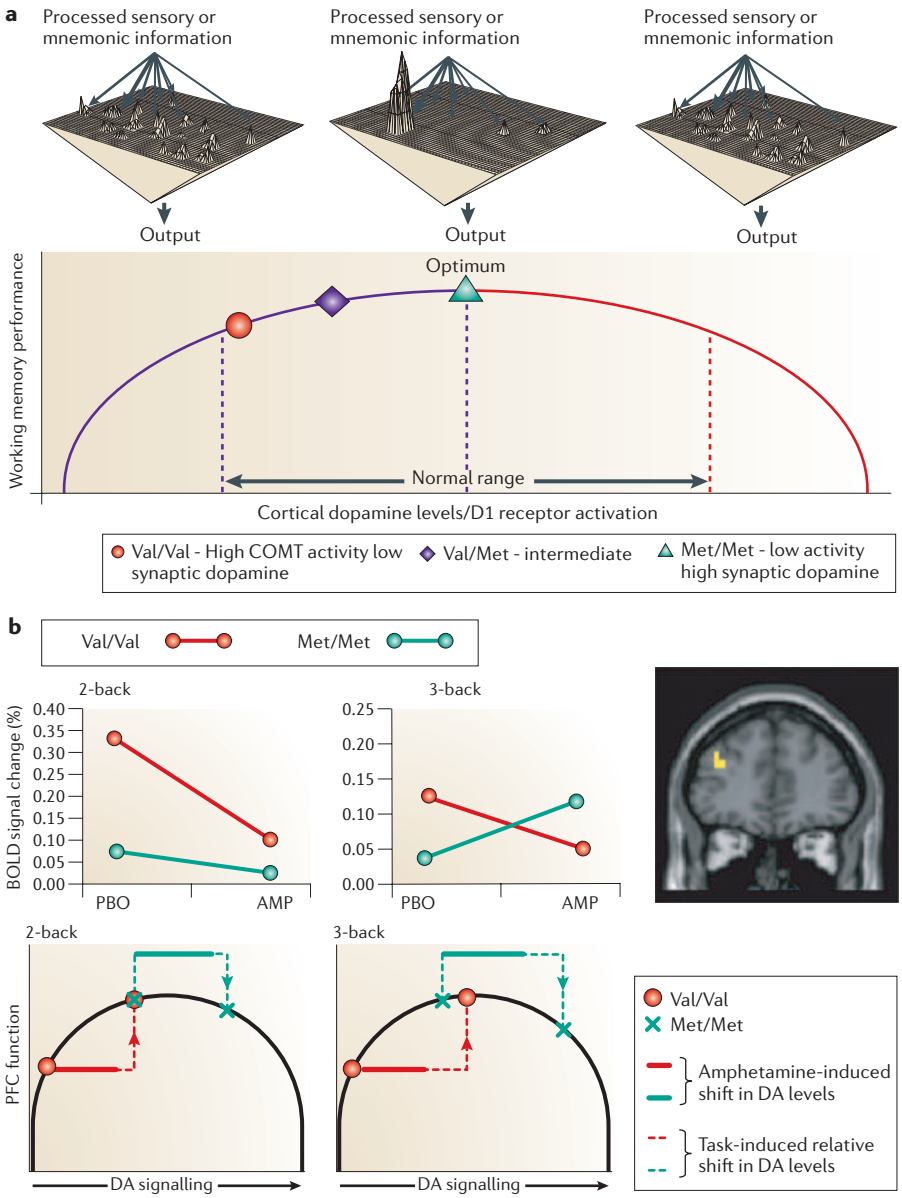


Figure 4 | **Effects of COMT Val(108/158)Met on prefrontal cortex activity linked to extracellular dopamine.** Convergent evidence has established an inverted u-shaped relationship between working-memory-related activation of dorsolateral prefrontal cortex (PFC) neurons and dopaminergic tone (especially D1 receptor stimulation)[29], which has been shown to be essential for optimizing signal-to-noise ratio, or tuning of the neuronal response. This figure demonstrates the use of genetic variation in catechol-*O*-methyltransferase (COMT) to mechanistically characterize neural circuits relevant for schizophrenia that are dependent on this relationship. **a** | An inverted u-shaped curve[94] links extracellular dopamine to prefrontal signal-to-noise ratio (top) and working memory performance (bottom): homozygotes for the Val-encoding allele are positioned at the left (suboptimally low dopamine due to high COMT activity) and Met homozygotes are near the optimum (higher dopamine due to reduced COMT activity). Heterozygotes are intermediate. **b** | Increasing synaptic dopamine (DA) by the administration of amphetamine (AMP) dissociates the functional states of Val and Met homozygotes[48]. At medium working memory load level (2-back task , where subjects need to remember a stimulus presented 2 trials back), Val homozygotes, on the left of the optimum on the inverted u curve, profit from increased dopamine, whereas Met-homozygotes, near the optimum, show little change (left). At high load level (3-back task), dopamine increase by drug intervention now pushes Met homozygotes into the suboptimally high range of dopamine stimulation, leading to reduced prefrontal efficiency (right; localization of activity in the prefrontal cortex is shown on the far right). BOLD, Blood-oxygen-level-dependent signal that indicates changes in the proportion of oxygenated blood in the brain, which changes in response to neural activity; PBO, placebo. Panel **a** (top) data from REF. 95. Panel **a** (bottom) modified, with permission, from REF. 95 © (2000) Society for Neuroscience. Panel **b** reproduced, with permission, from REF. 48 © (2003) National Academy of Sciences.

Because dopamine transporters are abundant in subcortical structures such as the striatum and amygdala, *COMT* variation is not expected to have an impact on the functional activation of the striatum and midbrain. Conversely, dopamine transporter variation should preferentially affect subcortical function. In agreement with these predictions, a recent functional MRI (fMRI) study found an effect of a common variable number of tandem repeat polymorphisms in the dopamine transporter (*DAT1*) gene on the midbrain, but not on prefrontal activation during episodic memory, whereas the *COMT* genotype predicted prefrontal but not midbrain activity[55]. Similarly, D2 receptor function is known to be a principal determinant of striatal neuron function, and a recent imaging study reported an effect on reward-related activation in the striatum of a potentially functional allele (*Taq1A*) in the gene that encodes the D2 receptor[56]. Other genes affecting this circuit are being studied (BOX 2).

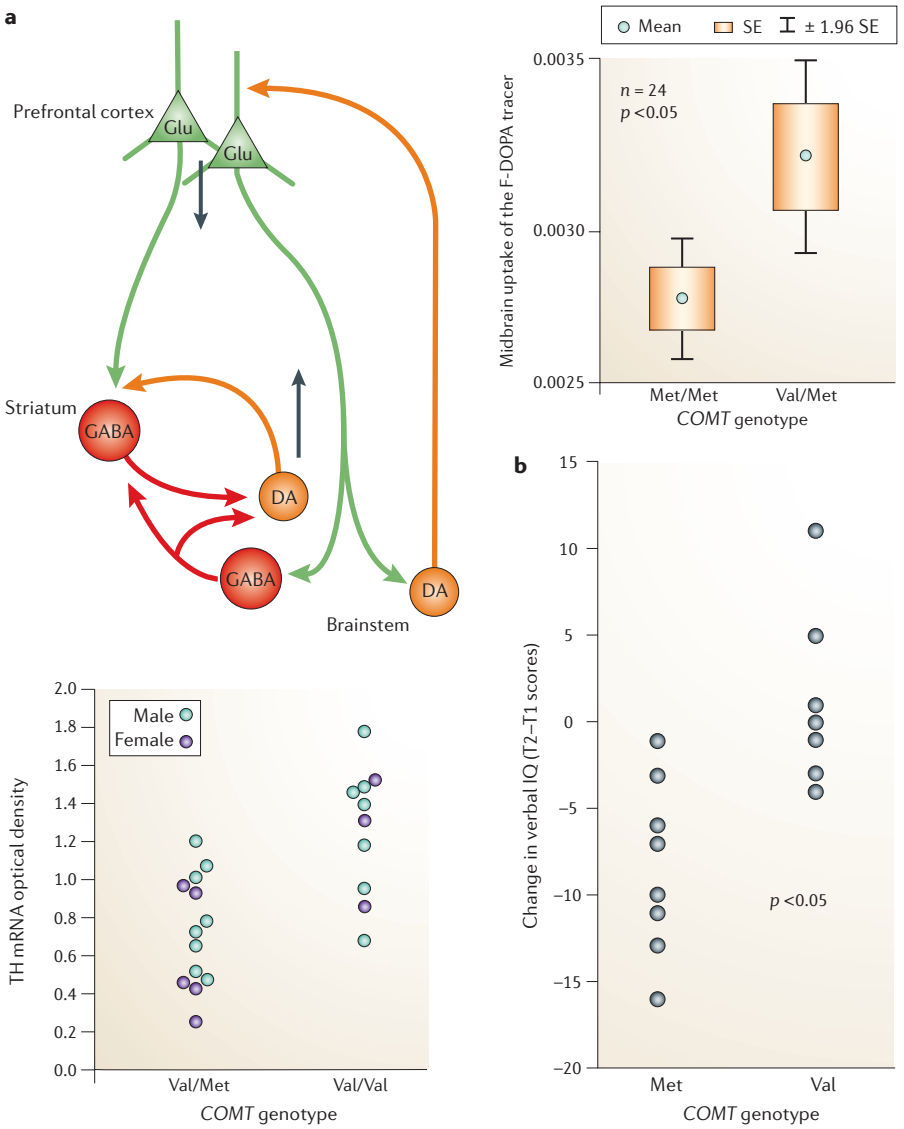


Figure 5 | **Effects of COMT Val(108/158)Met on midbrain–prefrontal interactions and 22q11.2 syndrome. a** | Schematic diagram of prefrontal interactions with the midbrain and striatum, showing that efferent glutamatergic projections of the prefrontal cortex (PFC) interact in a topographically precise manner with dopamine-synthesizing neurons in the midbrain projecting back to the cortex (top left). Convergent evidence from PET[4] (right) and post-mortem brain studies[49] (bottom) of dopamine synthesis indicates increased dopamine synthesis associated with the catechol-*O*-methyltransferase (COMT) Val allele, presumably because of relatively increased catabolism of dopamine in the PFC. **b** | In 22q11.2 syndrome, Met allele carriers are at increased risk for intellectual decay (right), measured as a drop in IQ score in a longitudinal study, and for schizophrenia[51]. The latter results from a gene dosage effect, which implies that that Met allele carriers have suboptimally high extracellular dopamine (because only one of the two genes encoding COMT is present in this syndrome, resulting in reduced gene dosage), whereas Val carriers are now closer to optimal. SE, standard error; T2–T1 scores, from first (T1) to second (T2) measurement timepoint; TH, tyrosine hydroxylase. Panel **a** (top left, bottom) modified, with permission, from REF. 49 © (2003) Society for Neuroscience. Panel **a** (right) modified, with permission, from *Nature Neuroscience* REF. 4 © (2005) Macmillan Publishers Ltd. Panel **b** modified, with permission, from *Nature Neuroscience* REF. 51 © (2005) Macmillan Publishers Ltd.

As predicted by the intermediate phenotype strategy, genes that are potentially related to schizophrenia might seem to have greater penetrance at the level of hippocampal circuitry than at the level of the clinical phenotype. For example, a variation in *GRM3* associated with increased risk for schizophrenia predicted decreased hippocampal activation during encoding assayed by fMRI[57], even in healthy participants. A recent study[58] also found variation in *GRM3* as part of a cluster of variants jointly predicting episodic memory and hippocampal activation, again in normal controls. Another important schizophrenia risk gene is *DISC1* (REF. 50), which is expressed most abundantly within the hippocampus and has been implicated in hippocampal formation development. A common non-conservative single nucleotide polymorphism (Ser704Cys; rs821616) in this gene, although inconsistently associated with clinical diagnosis, strongly predicted reduced hippocampal grey matter volume and abnormal activation of the hippocampus during working memory in normal participants, mirroring findings in patients with schizophrenia[23] and their siblings[59] that might be indicative of abnormal functional interactions with the PFC. Hippocampal volume, together with DLPFC grey matter and memory performance, was also found to be affected by a *DISC1* haplotype overtransmitted to patients with schizophrenia in a twin cohort[60]. The first direct indication of genetic variation affecting circuit properties related to prefronto–hippocampal interactions was reported in a study of the COMT Val158Met polymorphism[61], showing abnormally persistent coupling between DLPFC and hippocampal formation during working memory for Val allele carriers among normal volunteers, which is again similar to observations in schizophrenia[23].

Challenges for the intermediate phenotype approach. The preceding examples illustrate the power of using an imaging genetics approach to delineate neural mechanisms

for genetic risk in the context of single gene effects. However, dealing with genetically complex disorders requires going beyond this stage by dealing with multiple interacting genetic variants in a gene, between separate genes and gene–environment interactions, and with questions of polygenicity versus genetic specificity.

Interacting genetic variants and epistasis. Interacting variants cause specific problems with regard to necessary sample size and mode of inference. An illustrative example is again provided by *COMT*, in which the evidence supports the existence of multiple variants in the gene. A haplotype combining the Val/Met polymorphism (rs4680) with two common single nucleotide polymorphisms at other loci, one upstream in intron 1 (rs737865) and the other in a 3′ untranslated region (rs165599), was highly associated with schizophrenia in a large sample of Israelis of Ashkenazi descent[62]. This haplotype differentially affected the expression of rs4680 alleles in human brain tissue[63], suggesting the presence of a *cis*-acting functional locus in *COMT* that interacts with Val/Met. A population study found that this three marker haplotype is markedly heterogeneous in populations worldwide[64], despite the relatively constant prevalence of schizophrenia, and suggested the relevance of another possible *cis*-acting functional variant (rs2097603) linked upstream in the P2 promoter driving transcription of the predominant form of *COMT* in the brain (membrane-bound *COMT*). This variant also affects COMT activity in lymphocytes and post-mortem brain tissue[45]. So, *COMT* could contain at least three functional polymorphisms that differentially affect its biological actions and confound its clinical associations. The combinatorial possibilities of diplotypes based on varying alleles at these three sites are difficult to model in preclinical systems, but imaging offers a unique potential to identify the functional effects of these combinations. Recent work in our laboratory using a method adapted from haplotype regression showed the interacting effects of these functional variants on prefrontal function[65]. The combined effects of these loci are not linear, which is consistent with predictions based on the inverted u-shaped function described above. Confirmatory convergent evidence comes from a study of executive cognition that found similar non-linear effects of these haplotypes on working memory performance[66]. So, imaging genetics approaches offer strategies for functionating complex genetic interactions at the level of brain function that might not be approachable with non-human models.

Box 2 | **Characterization of additional risk genes in schizophrenia**

Glutamate is the most important excitatory neurotransmitter in the cortex, and the excitability of glutamate neurons is regulated in part by dopamine[35]. *GRM3*, which encodes a metabotropic glutamate receptor responsible for modulating synaptic glutamate, is a candidate gene linked to schizophrenia[57]. A single nucleotide polymorphism in *GRM3* was found to predict prefrontal activation analogous to catechol-*O*-methyltransferase (*COMT*)[57]. This functional convergence of a glutamate and a dopamine gene on a common cortical phenotype illustrates the impact that imaging genetics can have on illuminating core mechanisms behind clinical associations. Recent work in our laboratory has focused on DARPP32, which is expressed in regions receiving dopaminergic innervation, especially the neostriatum (caudate and putamen)[90]. This protein, a phosphatase encoded by *PPP1R1B*, acts as a central molecular switch in dopaminoceptive neurons[90], integrating dopamine and glutamate signals. DARPP32 is a key node in a final common pathway of psychotomimetics in both the frontal cortex and striatum, making it an attractive candidate gene for schizophrenia[90]. We recently identified, through the resequencing of the gene in 298 chromosomes, a frequent haplotype in *PPP1R1B* associated with schizophrenia in a family-based sample[91]. Imaging genetics showed a pronounced and convergent effect on the structure and activation of the neostriatum, as well as on prefrontal–striatal interactions, indicating that *PPP1R1B* contributes to risk for schizophrenia by causing disturbed gating[26] subsequent to impaired fronto-striatal function.

Methodological issues in the characterization of genetic neural mechanisms. As whole-genome scans of hundreds of thousands of genetic variants have become feasible, the selection of variants to study has become a pressing, and so far unsolved, problem. Few variants are well-defined functional polymorphisms. Indeed, many variants that are statistically associated with psychiatric disease are intronic and of no known functional consequence. Although many are likely to be characterized by advances in predicting novel exon, splice, transcription factor or microRNA binding sites, a translational approach will be essential in functionating them by demonstrating, for example, an effect of genetic variation on mRNA expression, protein levels or cellular physiology. It is our opinion that, given the absence of reliable information on the heritability and reliability of most imaging phenotypes currently in use, a statistically significant result in neuroimaging is by itself not sufficient to establish that a given polymorphism is functional, and the complex nature of psychiatric disease predicts that the isolated genetics evidence for association will usually not be unequivocal for a given variant. This leads to a new kind of multiple comparison problem, this time over the number of studied genetic variants, that will need to be addressed by future research. Similarly, it is important to select neuroimaging tasks that tap into neural systems plausibly related to the disease under study, such as working memory tasks that activate DLPFC-striatal systems in schizophrenia.

Given the small contribution of each individual genetic variant to complex phenotypes and the importance of gene–gene interactions, it is crucial to control for occult stratification effects that might confound the analysis of a target variant. This applies to demographic variables such as age, gender, IQ or socioeconomic status, and also to an assessment of genetic stratification that is necessary to investigate whether the studied groups, defined by one genotype, systematically differ in the distribution of other genetic variants. Genomic control or ancestral marker panels should be carried out[67] to investigate this important source of potential confounders.

Further research is also necessary to determine which imaging designs are the most conducive to genetic research. It is clear that the assessment of genetic effects across participants crucially depends on a reliable and robust brain response. Researchers need to balance this requirement against the wish to isolate cognitive subcomponents by subtle manipulation in the context of the limited time and budget available for each participant in these high-volume, long-term experiments. It is our view that a two-step approach is often useful and practical. The first line of research establishes genetic effects using well-tested tasks that reliably elicit strong activation in known functional networks, and more specific neural processes are then elucidated in a second step using specifically tailored experiments. Ideally, behavioural confounders in these tasks should be avoided by design (simplicity), analysis (for example, by using mixed event-related designs[68] allowing for the inclusion of only correct responses) or matching. For the field as a whole, reaching consensus on questions of standardization

of tasks and comparability of data across centres[69] will also allow more rapid progress towards acquisition of the large sample sizes necessary to investigate genetic variation of small penetrance, and gene–gene and gene–environment interactions.

Even for studies of a single established genetic variant, sample sizes must be considered critically. Most studies published so far report significant effects in groups of around 20–40 participants. However, many of these results have not yet been replicated, and a publication bias against negative results in this newly developing field is likely. Moreover, even for the same genetic mechanism the effect size can vary widely depending on which imaging target measure (structural variation, functional activation or functional connections) is examined[6,70]. Further research, ideally contrasting genes of no known or likely function with known functional variants, is necessary to achieve a principled assessment of expected false positive rates that can guide recommendations for statistical inference in this field.

Another area of interest is how to combine independent streams of information, such as structural and functional neuroimaging together with clinical and neuropsychological assessment, into a common analytical framework that can formalize the intuition that convergent information of this kind provides a stronger argument for the functional relevance of the studied genetic variations. It is possible that Bayesian approaches might be a fruitful[71], although as yet unexplored in this domain, way to pursue this goal, as in principle they allow the quantitative consideration of sources of uncertainty relevant to the field, such as the prior probability of a given genetic variation or imaging phenotype being causally related to the disorder under study.

Conclusion

Using schizophrenia as an example, we have discussed recent insights gained from a translational approach to investigate and define neural mechanisms of psychiatric illness based on genetic risk. Although methodological problems in this fast-moving area of research exist and must be tackled in the interest of reliable and replicable results, we believe that this methodology of using genetic variation as a tool for the discovery of brain mechanisms will become a widely applied and fruitful research field in psychiatry and related disciplines. As the characterization of single genetic variants is rapidly proceeding, we predict that research will increasingly turn to dissecting gene–gene and gene–environment interactions, leading to the next crucial step in the field — the identification of converging molecular pathways and their neuronal and systems-level targets, which would then constitute the genetically discovered, biologically valid core pathophysiology of the disorders under study. As these difficult questions posed by interacting genetic variation in complex disease are tackled in the future, we expect that the results mentioned here will then be seen as 'low-hanging fruit' harvested at the beginning of an effort that is ultimately expected to not only reform our view of the taxonomy and pathophysiology of psychiatric disease, but also to point the way to new treatment targets and more principled clinical management.

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