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Intermediate phenotypes in psychiatric disorders

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Abstract

The small effect size of most individual risk factors for psychiatric disorders likely reflects biological heterogeneity and diagnostic imprecision, which has encouraged genetic studies of intermediate biologic phenotypes that are closer to the molecular effects of risk genes than are the clinical symptoms. Neuroimaging-based intermediate phenotypes have emerged as particularly promising because they map risk associated gene effects onto physiological processes in brain that are altered in patients and in their healthy relatives. Recent evidence using this approach has elucidated discrete, dissociable biological mechanisms of risk genes at the level of neural circuitries, and their related cognitive functions. This approach may greatly contribute to our understanding of the genetics and pathophysiology of psychiatric disorders.

Risk genes and psychiatric disorders

Most cases of psychiatric disorders are thought to result from complex interactions between multiple genes of mostly small to modest effect and the environment [1]. The identification of these genes and their function has proven to be an extraordinarily challenging endeavor, even using the popular and biologically agnostic *genome-wide association* (GWA) *approach*, which results in the potential identification of genes whose mechanisms of risk association are almost always unknown. This approach, as with earlier linkage and candidate gene approaches, has not produced incontrovertible evidence of association of common genetic variation with clinical diagnosis, though a few promising loci have been found. As psychiatric disorders are syndromal, analogous to most common medical illnesses, it is rational to assume that genetic association is stronger at the level of biological substrates related to syndromal risk. This is analogous to evidence that genes for common medical syndromal disorders show much stronger association to the biological substrates that contribute to risk. Examples include lipid levels and risk for heart disease [2], sodium homeostasis and risk for hypertension [3], and body mass index (BMI) and risk for diabetes [4].

In an attempt to investigate the relevant functions of genes implicated in psychiatric illness, the study of biological substrates has become of increasing interest. In particular, the application of so-called neuroimaging genetics – a technique based on in vivo brain measures - has illustrated how risk genes can modulate specific neural processes, translating gene effects on brain function and structure in a more meaningful way than the clinical association approach alone [5, 6]. Investigators, in general, have favored the study of effects of risk associated genotypes in the brains of healthy individuals [7–11], to circumvent the potential contamination of signal from non-genetic and/or illness-related factors (e.g.

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treatment, symptoms, smoking, general health issues) that make it difficult to interpret results in patients alone. On the other hand, studying “healthy volunteers” exclusively runs the risk of identifying a genetic effect in brain that has little or no relationship to the disorder itself.

Intermediate phenotypes

Genes have pleiotropic biologic effects and can be expected to have diverse effects on the development and function of the brain. The role that genes play in increasing risk for a psychiatric disorder presumably reflects a particular effect of that gene on neural systems that impact on the biology of susceptibility. A crucial point in identifying the mechanisms through which genes confer risk for psychiatric disorders is to define whether the brain phenotype that the risk gene modulates is a biological mechanism implicated in the psychiatric disorder and in risk for the psychiatric disorder. Finding an association between a gene and a brain function does not mean that that association is related to the mechanism of risk for the clinical illness. The analogous conundrum in clinical medicine would be to find that a newly identified risk gene for diabetes also affects BMI, without having evidence that BMI itself is a risk factor for diabetes (which it clearly is).

To link a gene effect in brain to the gene effect on risk for the syndromal diagnosis, it is necessary to show that the brain effect is a biological substrate also linked to illness risk, a so called intermediate phenotype. An intermediate phenotype related to mental illness is a heritable trait that is located in the path of pathogenesis from genetic predisposition to psychopathology [12]. The path goes from relatively simple effects in cells, to more complex effects in neural circuits in the brain, to much more complex effects on the emergent phenomenology of these simpler effects, i.e. behavior and psychiatric syndromes (see Figure 1A).

The search for intermediate phenotypes is best conducted in specific populations that carry risk genes for the disorder without confounding factors related to the state of the disease (e.g. treatment, smoking, etc). Unaffected relatives of patients with psychiatric disorders, ideally healthy cotwins or siblings, fulfill both criteria: they are enriched in risk genes and are healthy. Many aspects of human brain function, behavior and physiology have been studied in such populations with evidence that a variety of such measures are heritable and enriched in relatives [13]. Phenotype studies of relatives of patients with schizophrenia, however, have potentially serious methodological problems which should be appreciated. Relatives may share environmental or behavioral characteristics (e.g. drug or tobacco use, temperament) that can impact on measures of brain function. Moreover, comparisons of relatives across generations are especially problematic because they involve age and life experience factors that are difficult to control.

The literature has many examples of abnormalities in relatives of patients with schizophrenia, from simple tests of processing speed to more complex cognitive operations that mirror those found in patients, implicating a number of potential intermediate phenotypes of interest. Because many of these will turn out to be redundant, it will be important to clarify which are independent, not only for the unnecessary repetition of information that a lack of independency could represent, but most importantly for studies of risk genes. Indeed, it is expected that some risk genes will map onto some intermediate phenotypes and not others; thus, the overlap or autonomy of different intermediate phenotypes is a crucial aspect in dissecting neural mechanisms of genetic risk.

In this article, we review neuroimaging intermediate phenotype findings related to schizophrenia and evidence of genes associated with increased risk for schizophrenia that modulate these intermediate phenotypes (Figure 1B 1–3). We will focus on schizophrenia

and fMRI as an example, but the model can be extended to other psychiatric disorders and other neuroimaging techniques. Based on previous reviews [14–16], studies have been grouped into five different cognitive domains whose circuits have been consistently reported altered in patients with schizophrenia.

Neuroimaging intermediate phenotypes related to working memory and the impact of selected risk genes

Altered fMRI-based activation of prefrontal-parietal circuitry during working memory, the cognitive process to maintain and manipulate information for a short period of time, has been consistently reported in patients with schizophrenia. For the most part, unaffected relatives of patients with schizophrenia show qualitatively similar abnormal engagement of prefrontal cortex (PFC), thalamus, hippocampus-parahippocampus formation (HF) and inferior parietal lobule – especially in the right hemisphere [14, 16, 17–20 ··] (Supplementary Table 1.A). Activation of these regions and of the circuit involving them has been studied using the "imaging genetic" approach for several putative schizophrenia associated genes [8–11, 21–34, 30 ··] (Supplementary Table 2.A). Interestingly, the first GWA positive gene, ZNF804A, has not shown association with this phenotype per se [10] (but see below).

Genetic modulation of PFC coupling between different areas implicated in working memory circuits has also been explored in imaging genetics paradigms with interesting results (e.g. modulation of DLPFC-HF and DLPFC-PFC coupling by ZNF804A [10 ··]; DLPFC or VLPFC coupling with parietal cortex by COMT-GRM3 epistasis [31]). These circuit based associations involve more complex and likely realistic measures of brain function, but PFC coupling with other regions as an intermediate phenotype related to risk for schizophrenia has not been demonstrated yet and requires further exploration. Moreover, in some reports, engagement of the prefrontal cortex may be modulated in different ways by genes based on diagnosis (patients with schizophrenia versus normal controls) [29, 32]. These findings are not easily interpreted, because while they suggest complex gene by disease modulation, they can also be driven by confounders related to disease-state factors, such as gene by medication interactions.

In summary, prefrontal-parietal activation during working memory tasks seems to be a robust intermediate phenotype, consistently reported as abnormal (mostly increased engagement referred to as "inefficiency" or increase noise) in healthy relatives of patients with schizophrenia. Genetic exploration of this circuit suggests a relatively selective modulation of the circuit by some risk genes but not by others (Figure 2.A).

Neuroimaging intermediate phenotypes related to cognitive control/ attention and the impact of selected genes

Cognitive control is an executive function that refers to the ability to direct behavior toward a goal in the presence of conflict and is an integral process of many different cognitive paradigms. The inferior lateral frontal and anterior cingulate (ACC) cortices are key regions implicated in cognitive control [35] and consistently reported altered in patients with schizophrenia [36]. Several studies have explored dysfunction of ACC activation in the context of cognitive control paradigms in unaffected relatives of patients with schizophrenia [14, 16, 37, 38] with somewhat variable results, including decrease [37 ·], increase [38], or no difference [14, 16] in ACC activity (Supplementary Table 1.B). In contrast, altered PFC activation has been consistently demonstrated in healthy relatives during cognitive control tasks, although its independence from other cognitive domains, such as working memory (and *vice versa*) is unclear since this question has not been explicitly explored. Recently,

investigators have started to study the abnormal coupling in cognitive control circuits as a potential intermediate phenotype and preliminary results suggest a decrease of the coupling within prefrontal regions in healthy relatives of patients with schizophrenia [39]. Few studies so far have explored the modulation of risk genes on cognitive control circuits, reporting increase [40], decrease [41] or no effect [42] on ACC activity (Supplementary Table 2.B; Figure 2.B).

Neuroimaging intermediate phenotypes related to episodic memory and the impact of selected genes

The hippocampal formation (hippocampus proper and the parahippocampal cortex) plays a fundamental role in episodic memory - the ability to learn, store and retrieve information [43] - and hippocampus dysfunction has been consistently reported in schizophrenia [44]. Surprisingly, episodic memory studies in healthy relatives of patients with schizophrenia so far have failed to report abnormal hippocampal activation [14], though one study reported abnormal parahippocampal activity [45 ·] (Supplementary Table 1.C). Many studies have explored the role of risk genes in hippocampus modulation during episodic memory tasks in healthy volunteers [8, 9, 11, 28, 30, 46–50] (Supplementary Table 2.C) (Figure 2.C). Since the status of hippocampus function during episodic memory as an intermediate phenotype related to risk for schizophrenia has not been convincingly demonstrated, the link between the neurophysiological effect of these genes on HF and their mechanism for increasing the risk of schizophrenia is unclear.

Neuroimaging intermediate phenotypes related to verbal fluency and the impact of selected genes

Verbal fluency is a classic test of language production that requires the subject to generate words, beginning with a particular letter or within a particular semantic category. Functional MRI studies have reported disturbed patterns of left hemisphere dominance of language processing in verbal fluency in patients with schizophrenia [51], showing increased activity in right hemisphere, with bilateral activation of Broca’s area during word generation tasks. The results in healthy relatives are similar, but few studies have looked at this (Supplementary Table 1.D). Two studies [52, 53 ·], using verb and word generation tasks, reported increased right VLPFC activation in healthy twins discordant for schizophrenia compared to normal control twins, reproducing the same pattern observed in patients. Despite several reports suggesting a modulation of verbal fluency circuits by schizophrenia risk genes [54–62], none of these studies show genetic modulation of right VLPFC activation, which has most consistently been reported as an intermediate phenotype during verbal fluency paradigms (Supplementary Table 2.D; Figure 2.D). An exception was found with NRG1 [55], although it is impossible to exclude a medication effect on gene modulation since the gene effect was only found in patients with schizophrenia.

Using a different verbal fluency fMRI paradigm, a sentence completion task, Whalley et al. found decreased medial frontal and cerebellar activation [63] and aberrant coupling between Broca's area and parietal cortex in subjects at high genetic risk [64]. The effects of three risk genes were explored with this paradigm (G72, NRG1, COMT) (Supplementary Table 2.D) [65–67] and only NRG1 was reported to modulate the medial frontal gyrus [66], although the relationship of this area with verbal fluency processing is not clear.

In conclusion, so far, abnormal activation of the right VLPFC has potential as an intermediate phenotype related to genetic risk for schizophrenia, but risk genes that show effects in other paradigms do not seem to modulate this response.

Neuroimaging intermediate phenotypes related to faces/emotion processing and the impact of genes

Amygdala reactivity to threatening stimuli appears to be abnormal in schizophrenia [68]. Only three studies have examined the genetic liability of faces/emotion processing in subjects at increased genetic risk for schizophrenia [18, 69, 70], with inconsistent results (Supplementary Table 1.E). Indeed, the largest study [18 ·] found no evidence of an abnormality in healthy sibs. This circuit has been extensively explored in imaging genetics, and results seem to suggest that it is vulnerable to modulation by genes increasing the risk for affective disorders [10, 71], while genes associated with risk for schizophrenia seem to be protective (e.g val/val subjects in the COMT functional val/met genotype have reduced amygdala reactivity to threatening stimuli [7, 72] (Supplementary Table 2.E). In conclusion, genes impacting this circuit, if associated with risk for schizophrenia, likely increase the risk for the disorder through a mechanism different from their effect on amygdala reactivity.

Conclusions

The study of brain-based intermediate phenotypes in psychiatry is conceptually appealing and biologically compelling. It is a “no brainer” that genes do not encode for psychiatric symptoms but for simpler molecular processing in cells and information processing in brain. However, the intermediate phenotype approach has to be undertaken with considerable caution and attention to detail. A number of caveats need to be recognized in the literature as it currently exists, including inconsistencies in directionality of findings (hypo- or hyper-activation), biases in criteria for selection of relatives (offspring versus siblings or parents, and the presence of other psychiatric diagnoses in the relatives but not in the comparison groups), small sample sizes not powered to detect small effect sizes, and the relative independency of the different intermediate phenotypes, still not explored for any of them.

These important limitations notwithstanding, the evidence is growing of genetic vulnerability maps of the brain and the way in which they are modulated by risk genes in psychiatry. To date, a consistent observation in unaffected relatives of patients with schizophrenia is abnormal PFC and inferior parietal lobule activation, across different executive cognitive paradigms, and an abnormal lateralization of prefrontal-temporal areas during verbal fluency. These neuroimaging phenotypes have been most frequently studied in relation to schizophrenia-risk genes. Interestingly, there appear to be specific effects of genes on some neuroimaging intermediate phenotypes but not on others, suggesting discrete biological mechanisms of risk in some but not all brain areas/circuits and their related cognitive functions. On the other hand, there are still important gaps between the data on “imaging genetics” and the data on intermediate phenotypes. Many schizophrenia risk genes have been shown to modulate circuits that have not yet been demonstrated to be intermediate phenotypes (e.g. genes modulating prefrontal cortex coupling during working memory tasks or genes modulating hippocampus activity during episodic memory), and not all reported neuroimaging intermediate phenotypes have been systematically investigated with risk genes, despite clear evidence of their being enriched in healthy relatives (e.g., cognitive control). Connecting these two different lines of investigation could greatly contribute to the field's progress in understanding the complex pathophysiology of psychiatric disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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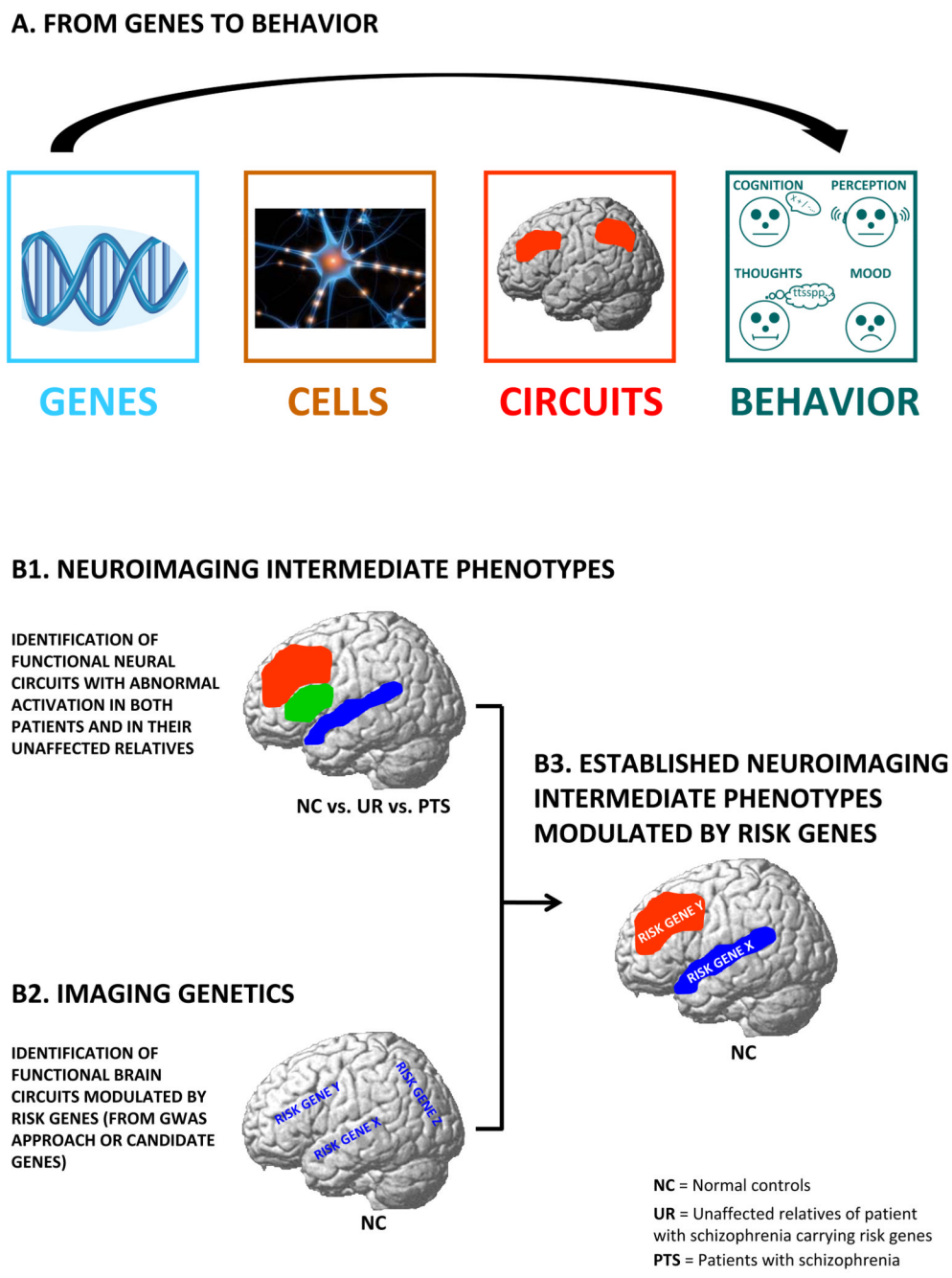

Figure 1.

A. From genes to behavior. Genes encode for molecules, not specific symptoms. The abnormal behaviors observed in psychiatric disorders (such as delusions, hallucinations and cognitive deficits in schizophrenia) are the product of intermediate steps that occur between genes and behavior, such as cell activity and neural circuits. An intermediate phenotype is a heritable trait that is located on the pathogenesis path from genetic predisposition to psychopathology and is likely associated with a more basic and proximal etiological process and therefore more amenable to genetic investigation. **B. Genetic risk on vulnerable brain circuits. B1.** Identification of neuroimaging intermediate phenotypes – which are alterations in neural circuit functions in patients with psychiatric disorders as well as in high genetic

risk subjects (i.e. unaffected relatives). **B2.** Imaging genetics defines neural systems that are modulated by genetic variations, including genetic variations that have been associated with increased risk for psychiatric disorders. **B3.** To increase the probability that the observed biological modulation by the risk genetic variation is the mechanism through which that gene increases the risk for a psychiatric disorder, it is important to demonstrate that the gene modulates a neuroimaging intermediate phenotype.

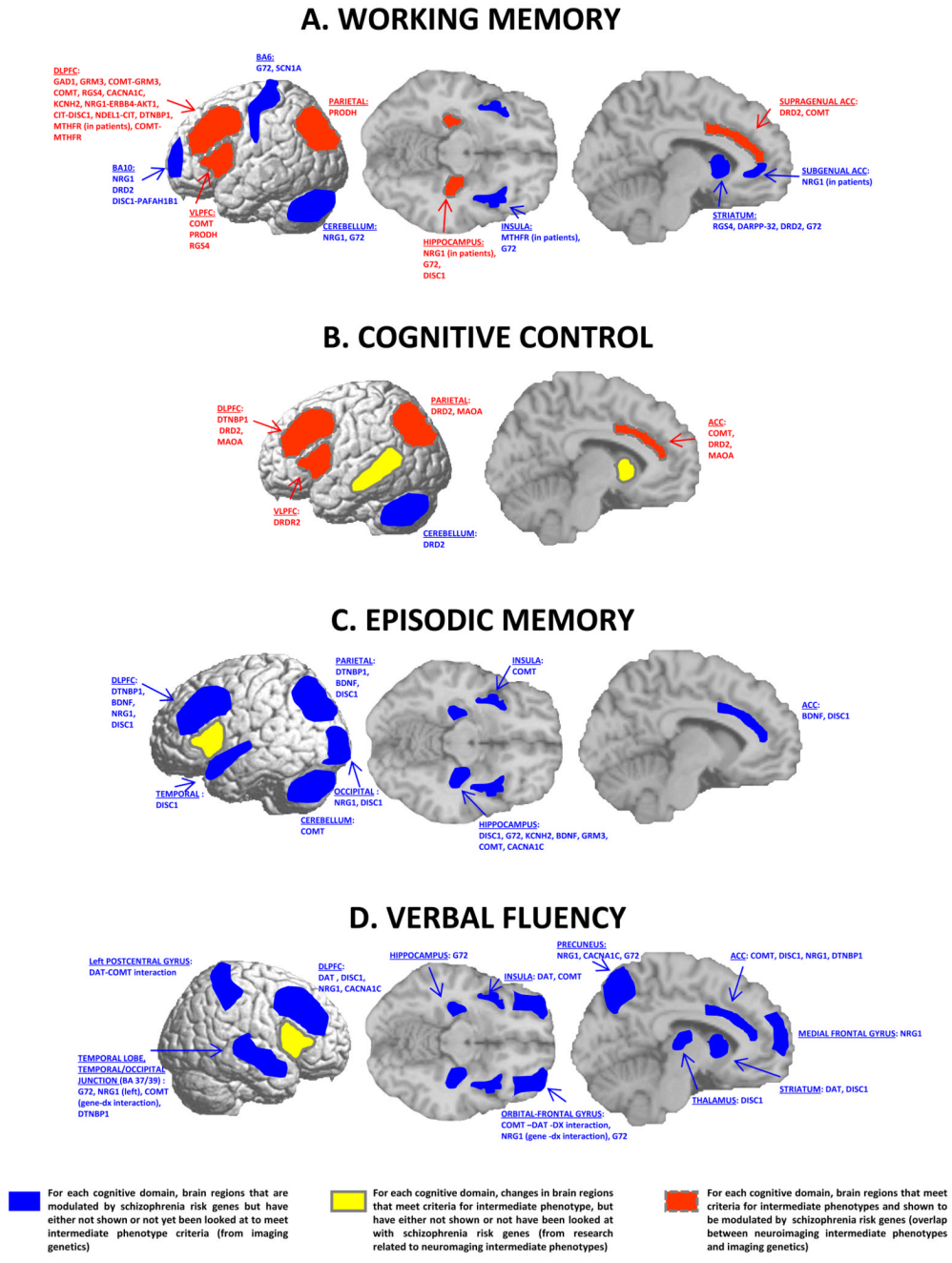

Figure 2. Genetic modulation on vulnerable circuits

A. Working memory. Most brain areas reported altered in patients and their healthy relatives during working memory task are also modulated by a number of risk genes explored with the same paradigm (red fields with square dots) (DLPFC, VLPFC, ACC, parietal cortex, and HF). Many other effects of genes during working memory paradigms have not been show to be intermediate phenotypes (striatum, basal ganglia, subgenual ACC, insula, BA10, BA 4/6, cerebellum) (blue fields). **B. Cognitive control circuit.** Several brain areas within the cognitive control circuit have been reported to be modulated by risk genes during cognitive control processing (PFC, especially ACC, superior temporal gyrus, parietal cortex, and cerebellum). Among these, only PFC (DLPFC, VLPFC and ACC) and parietal

cortex have been consistently reported being altered in patients with schizophrenia and their unaffected relatives with cognitive control paradigms (red fields with square dots). Striatum and middle temporal gyrus (BA 21) have been reported altered in patients with schizophrenia and their healthy relatives during cognitive control, although none of the risk genes studied so far have shown modulation of these regions (yellow fields with solid line).

C. Episodic memory circuit. Studies of potential intermediate phenotypes during episodic memory paradigms are very few and the only area consistently reported altered in patients and in their unaffected relatives is the VLPFC, a region that has not been shown to be modulated by risk genes so far explored with this paradigm (yellow field with solid line). On the other hand, several risk genes have been reported to modulate hippocampal activity during episodic memory, as well as DLPFC, ACC, insula, cerebellum, temporal, parietal, and occipital cortices, all regions whose role as intermediate phenotypes during episodic memory in schizophrenia has not been convincingly demonstrated (blue fields). Thus, there are no brain regions yet that show overlap between the two areas of research (no red fields).

D. Verbal fluency circuit. Right IFG has been reported altered in patients with schizophrenia and their healthy relatives during verbal fluency paradigms. This same area has not been shown to be modulated by risk genes (yellow field with solid line). Many other regions have been reported to be modulated by risk genes during verbal fluency related paradigms, but their role as intermediate phenotypes has not been consistently established (blue fields).

For working memory and cognitive control, only brain areas that were reported consistently altered in at least three studies are presented as potential intermediate phenotypes (from Supplementary Table 1). For episodic memory and verbal fluency, given the paucity of studies, only brain areas with at least one replicated result are reported as intermediate phenotypes (from Supplementary Table 1). For list of genes showing modulation on each circuit, refer to Supplementary Table 2.