# *Intermediate phenotypes in schizophrenia: a selective review*

*Gilbert A. Preston, MD; Daniel R. Weinberger, MD*

Studies aiming to identify genes of susceptibility for schizophrenia and other complex psychiatric disorders are faced with the confounds of subjective clinical criteria, commonly occurring phenocopies, significant between-subject variability of candidate traits, and the likelihood of allelic and locus heterogeneity that defines the genetics of other complex human brain and somatic disorders.[1-10] A single genotype, for example, may be represented by an array of psychiatric phenotypes; conversely, phenotype per se most likely represents a variable number of interactions between genotypes, epigenetic factors, and the environment. Additionally, research aimed at identification of the molecular origins of schizophrenia must also deal with the complex nature of the human brain. Unlike organs with a few common cellular phenotypes, transcriptomes, and proteomes, individual neurons are often distinct from one another in all of these respects, and in aspects of local and regional micro- and macrocircuitry; hence, human brain

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**Author affiliations:** Genes, Cognition and Psychosis Program, Clinical Brain Disorders Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, Md, USA

**Address for correspondence:** Gilbert A. Preston, MD, Genes, Cognition and Psychosis Program, Clinical Brain Disorders Branch, National Institute of Mental Health, National Institutes of Health, 10 Center Drive, Room 4s235, MSC 1379, 9000 Rockville Pike, Bethesda, MD 20892-1379, USA
(e-mail: prestong@mail.nih.gov)

## Selected abbreviations and acronyms

| | |
|---|---|
| ***DLPFC*** | *dorsolateral prefrontal cortex* |
| ***DZ*** | *dizygotic (twin)* |
| ***ERP*** | *event-related potential* |
| ***MZ*** | *monozygotic (twin)* |
| ***NAA*** | *N-acetylaspartate* |
| ***SNP*** | *single nucleotide polymorphism* |

function reflects dynamic relationships between multiple factors that modulate behavior and phenotype.

Genes only encode for molecules, not specific manifestations of psychopathology. The path from genetic variation to variation in cell biology to manifest behavior must pass through intermediate steps involving neural circuits and systems. A brief summary of the complexity of genetic causation of psychiatric disorders will be detailed in this review. We also discuss the idea that studies of genetic susceptibility in complex polygenic disorders such as schizophrenia might be enhanced by the identification of intermediate phenotypes,[11-13] and we present evidence derived from more than a century-worth of clinical, epidemiological, molecular genetic, and clinical neuroscience investigations in support of the view of cognitive impairment as a core clinical feature of schizophrenia. Most importantly, evidence of heritability of specific impairments discussed here may serve to further empower the search for genes of risk. Necessarily, we shall not discuss all the potential intermediate phenotypes, such as eye-tracking for example, which have been recently reviewed in depth.[14]

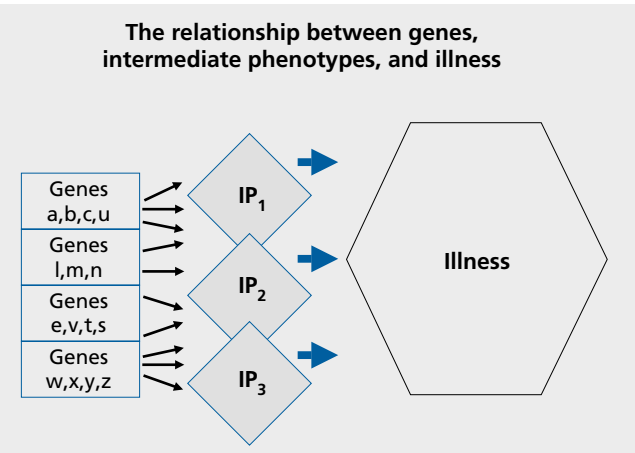


**Figure 1.** A schematic illustration of the assumption that individual traits are controlled by fewer risk alleles than the disorder taken as a whole. This scheme is the principal experimental design incorporated by the majority of studies discussed in this review. It strongly supports trait identification as the means of identifying risk alleles.

## Definitions

An *intermediate phenotype* (often referred to as an endophenotype) is a quantitative biological trait that is reliable and reasonably heritable, ie, shows greater prevalence in unaffected relatives of patients than in the general population. A *complex disorder* arises from a polygenic matrix whose individual components each confer only a small portion of total risk, in contrast to a *monogenic* or *mendelian disorder*. If a candidate intermediate phenotype is to provide meaningful information about a disorder, it should be associated with variant alleles that distinguish patients and their unaffected siblings from healthy controls on quantitative measures. The most useful intermediate phenotype candidates will also be functionally associated with aspects of the core clinical deficits of the disorder. The intensive search for such candidates is based in part on a reasonable, but incompletely substantiated assumption that intermediate phenotypes in schizophrenia are more likely to be modeled by a less complex genetic architecture than the disorder as a whole. *Figure 1* displays a simplified scheme of this concept.

The above statement "less complex genetic architecture than the disorder as a whole," does not imply "simple"; an intermediate phenotype could conceivably be more genetically complex than its parent disorder. However, in the context of this discussion, we will refer to intermediate phenotypes as having a less complex relationship to susceptibility genes than the diagnostic phenotype. The proof of this assumption rests on the demonstration that genetic association is statistically stronger for the intermediate phenotype than for the clinical phenotype. Importantly, the precise means by which a concentration of intermediate phenotypes might explain the clinical presentation of schizophrenia is yet to be determined. Hence, it is also conceivable that schizophrenia is more than a simple sum of distinct traits. In this view, phenotypic expression of the disorder requires the complex interaction of genetic and epigenetic matrices and, to some extent, the influence of environmental factors, acting perhaps through a final common pathway.[10,14] We will briefly consider evidence to support the concept of perturbed synaptic architecture as a putative final common pathway in schizophrenia, and present recent evidence in support of abnormal cortical information processing as the principle clinical feature of the disorder conceivably arising as an emergent property of the synaptic changes. *Figure 2* is a schematic representation of this idea.

## Attractive candidates and alluring alleles: caveats

Finding evidence of an association between variant alleles and a plausible intermediate phenotype depends on determining whether the allele is statistically associated with variance in behavioral or biological measures, eg, scores on cognitive measures. Ideally, a functional variant with well-described effects at the micro- or macroscale should be tested against a well-characterized intermediate phenotype of significant heritability. Absence of evidence to support a variant's impact on brain function should engender caution in the acceptance of purely statistical evidence of clinical association at the level of diagnosis. Even if allelic association with a candidate intermediate phenotype is found, it does not mean that the allele is necessarily the causative genetic factor.[15,16] There are other possibilities. For example, an unrecognized allele may be in "linkage disequilibrium" with the tested allele, thus serving as a "proxy" for the causative factor; or the association may be an artifact, due to experimental, statistical, or population stratification errors. Population stratification signifies genetic population (ethnic group) differences in allelic frequency caused by differences in their origins and geographic separation. For example, if members of two genetically diverse groups score differently on a cognitive measure because of social or cultural factors, the cognitive differences can be misinterpreted as being due to genetic factors.

## Defining relative risk: testing the heritability of an intermediate phenotype

Phenotype reflects the expression of genetic variation. Association implies that a specific genetic variant is related to the phenotype and, thus, the phenotype (here intermediate phenotype) is reasonably heritable, ie, shows greater prevalence in unaffected relatives of patients than in the general population. This concept is a measure of the proportion of healthy siblings displaying the phenotype relative to the proportion of healthy controls with evidence of the phenotype, and is referred to as *relative risk* (RR or lambda). Relative risk computations based on degree of impairment, ie, one or two standard deviations (SDs) below control mean, provide evidence that impairment per se is familial and most likely heritable, and permit the determination of the upper limits of heritability, but are unable to determine the role of

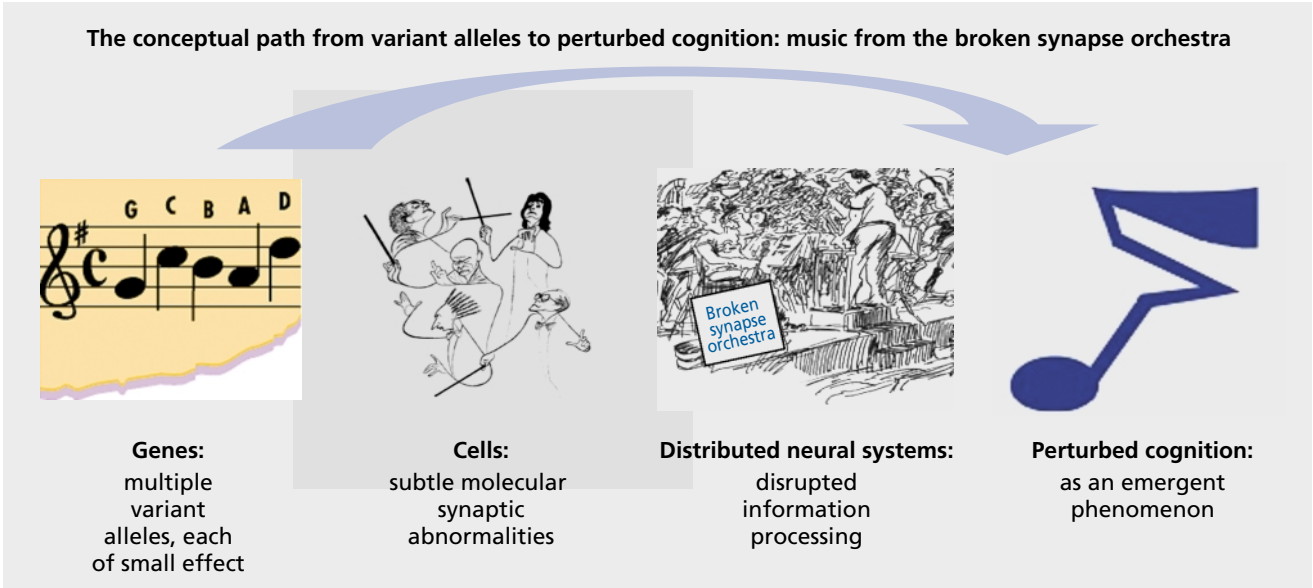


**Figure 2.** Cognitive information processing in the brain, and the illness denoted as "schizophrenia" is cartooned as an emergent property of the holistic interactions of gene matrices > synaptic functions > cognition. The normally occurring products of the putative susceptibility genes discussed in the text are shown to be critically important to aspects of synaptic integrity, plasticity, signal integration, and transmission, the emergent phenomenon of which, in sum, is cognition and, in this case, psychiatric illness. In one sense, this hypothetical construct is analogous to a view of a musical symphony as an emergent property of the dynamic interactions of the musical score, the musicians, instruments, and symphonic conductor.

shared environmental factors. Even so, studies of family aggregations of schizophrenia provide evidence that shared environmental factors do not play a major role in schizophrenia; in turn, this implies that environmental susceptibility factors are specifically experienced only by the proband.[16,17]

Hence, an indispensable first step in the determination of heritability is a careful match of unaffected siblings with healthy controls for age, education, gender, IQ, the absence of diagnosable psychiatric illness, substance abuse, and medical conditions that impair cognitive performance, such as cardiovascular, endocrine, neuropsychiatric, or metabolic disorders. Fulfillment of these requirements permits a more accurate computation of relative risk on other measures of brain function. It is well recognized that the unaffected sibling of a discordant monozygotic (MZ) twin pair is at the highest possible risk for schizophrenia in comparison to any other class of sibling; thus, for example, comparison of cognition in such individuals with healthy MZ twins should conceivably yield the highest probability of identifying putative cognitive intermediate phenotypes in schizophrenia.[18] In particular, if the measured performance of the unaffected twin falls between affected siblings and healthy controls, the measure may be a plausible intermediate phenotype.

## Intermediate phenotypes in somatic disorders: hereditary hematochromatosis

The study of intermediate phenotypes has been especially productive in a number of common medical conditions, such as coronary heart disease,[19] hypertension,[20] and type 2 diabetes mellitus,[21,22] which are similar to psychiatric disorders in the sense that they arise from a polygenic matrix each of which conveys a relatively small increment of risk. In diabetes, for example,[21,22] insulin-receptor resistance has been viewed as intermediate between several susceptibility genes and clinical diabetes; colon polyps are intermediate phenotypes related to colon cancer. Studies of genetic susceptibility in hereditary hemochromatosis (HH), a clinical disorder of iron metabolism, are good examples of the *intermediate phenotype* and the *gene of susceptibility* concepts.[23-26] A simple, robust, and quantitative measure of serum transferrin saturation was first identified as an intermediate phenotype in HH.[23,24] This led to the recognition of the high prevalence of hemachromatosis gene (*HFE*) mutation carriers.[25] The protein encoded by this gene is a membrane protein that associates with $\beta_2$-microglobulin and most likely functions to regulate iron absorption by regulating the interaction of the transferrin receptor with transferrin. As one would expect in a complex genetic disorder, subsequent investigations demonstrated a low prevalence of clinical pathophysiology in carriers of variant alleles of the *HFE* gene, despite high transferrin saturation levels.[25,26] The *HFE* gene is thus recognized as a susceptibility gene in HH, possibly necessary, but insufficient, for expression of clinical hemochromatosis. This illustrates the principle that there are more unaffected carriers of *susceptibility gene* variant alleles than persons with the disorder. It is reasonable to expect insights gained from the study of somatic disorders will draw attention to promising molecular targets for effective therapeutic intervention in schizophrenia.

## Cognitive impairment: a core feature of schizophrenia

Schizophrenia has been recognized clinically as a disorder of cognition since at least 1893, when Emil Kraepelin referred to this illness as "dementia praecox," a chronic, disabling disorder whose core feature was "a disorder of the will," or an impairment of executive functions and working memory in current terminology. Recent clinical studies confirm the role of cognition in the disability of this disorder. For example, McGurk and colleagues[27-29] studied the relationship of neuropsychological measures of executive function and psychiatric symptoms to work outcomes in 30 patients enrolled in a supported employment program. Surprisingly, over a 2-year period, patients' work outcomes were more strongly predicted by levels of function in three key domains of cognitive function than by the severity of their psychotic symptoms; the greater the perturbation of working memory, attentional capacity, and processing speed, the less likely a patient was to remain employed. Similarly, Bryson and Bell[30] studied the influence of cognitive function on work performance in 96 outpatients and found that measures of attention and verbal working memory were the strongest predictors of work performance for the first 13 weeks, and measures of verbal working memory were the strongest predictors of continued employment over the second 13 weeks. Goldberg and colleagues[18] identified specific cognitive measures of genetic risk in schizophrenia, describing large differences in cognitive performance

as a whole between unaffected and affected members of discordant MZ twin pairs, and lesser differences in comparisons between the unaffected discordant twins and controls. Weickert and colleagues[31] studied the pattern of cognitive decline in a large sample of patients with schizophrenia and found that 51% experienced a 10-point or greater decline in IQ from premorbid status, while another 35% had cognitive deficits from early in life. In concert with these findings, Cannon et al[32] studied heritability of several domains of cognitive impairment in a sample of MZ and dizygotic (DZ) twins discordant for schizophrenia, each compared with healthy control twins. Using a 17-item battery of neuropsychological tests, they identified four independently inheritable domains of cognition and demonstrated that abnormalities of working memory were genetically related to risk for schizophrenia. Such studies have attracted increasing attention to the critical nexus of perturbed cognition, variant genotypes, and inherited susceptibility to schizophrenia.

## Candidate intermediate phenotypes in schizophrenia: cognition

Goldberg and colleagues[33] studied cognitive phenotypes in MZ twins discordant for schizophrenia in comparison with MZ twins, both of which were healthy. They found significant differences between the group of unaffected twins of patients and the healthy twin pairs on tests of attention, vigilance, and psychomotor speed. The difference remained even when 10 unaffected twins of a proband were omitted from analysis because they were diagnosed with an Axis I or II disorder. As predicted, the performance of the unaffected twin fell between the affected and control subjects, but failed to match the severity found for the affected twin control comparison. The authors concluded that a lack of equivalent differences in the comparison of cognitive measures between the discordant twins and the healthy controls indicated that the affected discordant twin sustained an environmental insult that additionally impaired cognitive performance. Cannon et al[32] studied heritability of impaired cognitive performance by determining whether such deficits covary with the degree of genetic relationship by comparing scores on a comprehensive neuropsychological test battery of twin pairs discordant for schizophrenia with a well-matched sample of control twin pairs. They found tests of spatial working memory (ie, remembering a sequence of spatial locations over a brief delay), divided attention (ie, simultaneous performance of a counting and visual-search task), intrusions during recall of a word list (ie, falsely "remembering" nonlist items), and choice reaction time to visual targets contributed uniquely to distinguishing the degree of genetic loading for schizophrenia. When combined, scores were more highly correlated within MZ pairs than within DZ pairs, in both discordant and control twins. The authors suggested that their findings supported the assumption of multiple independently inherited dimensions of cognitive deficit in schizophrenia. Interestingly, patients were more impaired than their MZ cotwin on tests of verbal and visual episodic memory, suggesting a preferential impact of nongenetic influences on long-term memory systems. Egan and colleagues[34,35] estimated the relative risk of impaired attention as a candidate intermediate phenotype in schizophrenia, and used the continuous performance test (CPT), 1-9 version, with and without a distraction condition, to study 147 patients with schizophrenia, 193 of their siblings, and 47 normal comparison subjects. Relative risk was estimated by using cutoff scores that were 1, 2, and 3 SDs below the mean of the normal comparison group in CPT both with and without distraction. Fifty percent of the patients, 24% of their siblings, and 18% of the normal comparison subjects scored 1 SD below the mean score of the comparison group for the more difficult distraction version of the CPT. The patients with CPT scores 1 SD below the mean score of the comparison group had a total of 97 siblings. Compared with the comparison group, this subgroup of siblings had significantly lower CPT scores. Relative risk was also significantly higher for the siblings of patients whose scores were 1 SD (RR=2. 1) and 2 SDs (RR=3.3) below the mean of comparison subjects. The authors concluded that poor performance on the CPT is familial, possibly genetic, and could increase the power of genetic studies of schizophrenia.

Although perturbations of executive functions seem to be the most consistently observed candidate intermediate phenotypes in schizophrenia, given the diversity of the human cognitive behavioral *repertoire*, and the apparent complexity of the molecular genetics subserving this complex behavioral array, it is unreasonable to expect any family-based study to mirror a single set of cognitive or physiological deficits.[35-45] Nonetheless, taken as a whole, the weight of neuropsychological studies robustly support assumptions that independent domains of cognitive function are heritable and are differentially linked to risk for schizophrenia.

## Neuroimaging

### Structural imaging

Structural imaging protocols have long attracted attention in schizophrenia research, inasmuch as they are noninvasive and are a relatively easy means by which to collect large numbers of volunteers. The 1976 study of Johnstone and colleagues[46] established a relationship between cerebral ventricular volume and cognitive impairment in schizophrenia. Using x-ray computerized tomography, Weinberger et al[47] subsequently found increased ventricular volume in 12 healthy siblings of patients with schizophrenia compared with well-matched healthy controls, and found that the affected siblings had larger ventricles than their unaffected sibs or healthy controls; a trend towards larger ventricles was also found in the unaffected siblings, suggesting a relationship to state and trait aspects. Reveley and colleagues[48] studied ventricular size in healthy MZ and DZ twins compared with MZ twins discordant for schizophrenia. They reported that ventricular size was highly heritable in healthy MZ twins ($h^2$=0.98), moderately heritable in DZ twins ($h^2$=0.45), and highly heritable in MZ twins discordant for schizophrenia ($h^2$=0.87). The results of this study suggested a strong relationship between family history of psychosis and lateral ventriculomegaly, but the effect of shared environmental factors could not be excluded.
Investigators in this field have recently looked at a differential effect on gray and white matter volumes in the brains of discordant MZ and DZ twins. Hulshoff Pol and colleagues[49] studied whole brain tissue volumes in schizophrenia, intending to determine whether genetic and environmental risk factors are differentially reflected in changes of gray or white matter volume. They used magnetic resonance imaging (MRI) scans to compare 11 MZ and 11 same-gender DZ twin pairs discordant for schizophrenia with 11 MZ and 11 same-gender DZ healthy control twin pairs. Repeated-measures analysis of covariance revealed decreased whole brain volume in the patients with schizophrenia as compared with their cotwins and with healthy twin pairs. Decreased white matter volume was found in probands and unaffected twin siblings compared with healthy twin control pairs, particularly in the MZ twin pairs. A decrease in gray matter was found in the patients compared with their discordant cotwins and compared with healthy twins. The authors suggested that their results indicate that decreases in white matter volume reflect an increased genetic risk to develop schizophrenia, whereas the decreases in gray matter volume are related to environmental risk factors. This initial study provides intriguing data suggesting that there are differential genetic effects on gray and white matter volumes. Further investigations will need to include nontwin siblings, as it is unclear whether twinship per se is associated with discrepant headsizes.[50,51]

### Magnetic resonance spectroscopic imaging

Congruent with structural MRI studies, magnetic resonance spectroscopic imaging (MRSI) studies have that found levels of cerebral metabolites reflect the activity of identical brain regions. For instance, Bertolino and colleagues[52,53] used single-voxel proton magnetic resonance spectroscopy ($^1$H-MRS) to study 10 patients with schizophrenia and 10 controls for evidence of reduced concentrations of *N*-acetylaspartate (NAA), choline-containing compounds (CHO), and creatine/phosphocreatine (CRE), which are metabolites considered to be in vivo signals of neuronal activity, in several brain regions, including dorsolateral prefrontal cortex (DLPFC) and hippocampus. They found that in comparison to controls, patients exhibited significantly diminished levels of NAA/CRE and NAA/CHO in the hippocampus and DLPFC. In a study aimed to determine whether metabolic measures were a plausible intermediate phenotype, Callicott et al[54] studied levels of NAA in the hippocampus and DLPFC of 47 patients with schizophrenia, 60 unaffected siblings, and 66 healthy control subjects, measuring NAA, CRE, and CHO. They found that patients and their unaffected siblings had significant reductions in NAA/CRE hippocampal area compared with controls. Qualitatively defined "low hippocampal NAA/CRE phenotypes" yielded relative risk estimates of between 3.8 and 8.8, suggesting that this characteristic is heritable. These findings, together with the structural imaging and cognitive studies discussed previously, lend support to the concept of a strong association between perturbations of structure, metabolism, and cognitive function in schizophrenia. They also suggest that MRSI-derived measures of neuronal metabolism represent a novel biological phenotype for genetic studies of schizophrenia.

### Functional magnetic resonance imaging

Functional magnetic resonance imaging (fMRI) is a versatile tool in the search for functional deficits in schizophrenia, and will most likely be seen as a paradigm shift in

psychiatric neuroimaging methods, inasmuch as it permits collection of individual, rather than group-averaged, functional neuroimaging data. Even so, a consensus regarding the interpretation of reported findings remains an unfulfilled goal, in spite of the increasing sophistication of this technology.[55,56] Callicott[56] and colleagues studied working memory–related cortical physiology in nonschizophrenic, cognitively intact siblings of patients with schizophrenia with fMRI during performance of the N-back working memory task. They compared 23 unaffected siblings of schizophrenic patients to 18 matched comparison subjects. As a planned replication, they studied another 25 unaffected siblings and 15 comparison subjects. In both cohorts, there were no group differences in working memory performance. Nevertheless, both groups of siblings showed an exaggerated physiological response in the right DLPFC, which was qualitatively similar to results of fMRI studies of patients with schizophrenia. They concluded that inefficient memory processing in the DLPFC, similar to findings in patients with schizophrenia, was associated with genetic risk for schizophrenia. However, similar studies have found that patients' performance is reflected in lesser activation of right-sided DLPFC,[57,58] while others[59] have found agreement with the results of Callicott et al.[56]

A plausible explanation for this seeming contradiction has been offered[15] in a study of working memory in which the authors used the N-back task and 3-tesla fMRI to examine a group of 14 patients with schizophrenia and a matched comparison group of 14 healthy subjects. While all patients' performance was significantly worse on the 2-back working memory task than that of healthy subjects, there were patients with hypoactivated and hyperactivated areas of DLPFC. Subdivision of the patients into high- and low-performing groups exposed areas of greater and lesser prefrontal activation in the high-performing patients, but only underactivated areas in the low-performing patients. These findings suggest that patients with schizophrenia whose performance is similar to that of healthy subjects use greater prefrontal resources, but achieve lower accuracy (ie, inefficiency), while those who fail to sustain a prefrontal information signal-processing network achieve even lower accuracy. In the light of current understanding of complex disorders, it is not surprising to encounter what, at first glance, seems to be an example of endophenotypic heterogeneity, but in regards to identifying genes of risk in schizophrenia, there is strong agreement that information-processing mediated by the DLPFC is significantly compromised.

## Event-related potentials

### *P50 auditory ERPs*

The majority of evidence in support of electrophysiological candidate intermediate phenotypes in schizophrenia derives from studies of event-related potentials (ERPs), particularly components of P50 auditory ERPs.[60-72] Most studies of ERPs in schizophrenia are designed to determine aspects of waveform amplitude or latency that distinguish healthy controls from patients and their unaffected siblings. P50 studies, however, are typically composed of a series of trials in which paired click stimuli (S1, S2) are presented with a 500-ms interstimulus interval and a 8- to 10-s intertrial interval, to an alert subject. The role of the P50 as a candidate intermediate phenotype is principally based on group differences in the ratio S2/S1, or the P50 ratio.[60-65] Similar findings have been reported for the ratio S2/S1 in the N100 ERP.[64,65] Variance between groups in the P50 auditory ERPs has been conceived as reflecting perturbed inhibitory factors, on the basis of the recognition that the amplitude of specific components of a sensory ERP in healthy subjects declines with repeated stimulation, depending upon the interstimulus interval and the refractory period of neural generators. As a result, it has been proposed that dysmodulated sensory signals are permitted access to higher-order cortical processing, an assumption for which direct evidence is lacking.[60-63]

On the basis of evidence that patients with schizophrenia and their unaffected siblings had a larger P50 ratio than healthy comparison groups, previous studies of the P50 found support for this ERP as a plausible candidate intermediate phenotype in schizophrenia. For example, on the Pacific island nation of Palau, Myles-Worsley[63,69] examined P50 sensory gating in 85 schizophrenia patients (56 medicated with typical antipsychotics and 29 unmedicated), 83 of their first-degree relatives (46 parents and 37 siblings), and 29 normal comparison subjects. Abnormal P50 ratios were found in 64.7% of the schizophrenia patients and 51.8% of their first-degree relatives, but only 10.3% of the normal subjects. This proportion of abnormal P50 sensory gating in relatives versus normal subjects resulted in a risk ratio of 5.0. A relative risk of this size is unusual in comparison with relative risk of ±2 in the majority of studies of complex disorders, but evidence suggests the prevalence of schizophrenia in this isolated population may be twice the incidence reported elsewhere. Recently, Leonard and

colleagues[70] found that P50 ratio distinguished schizophrenia patients (P50 ratio >0.5) from healthy controls (P50 ratio <0.5), and predicted the likelihood of a group association with variant single nucleotide polymorphisms (SNPs) in the promoter region of the gene for the $\alpha_7$-nicotinic receptor (*CHNRA7*). In other words, if variant alleles were considered as a class, control subjects were more likely to demonstrate a P50 ratio <0.5, and less likely to have the variant genotype; the converse was the found in patients with schizophrenia. First-degree relatives were not included in this analysis.

Raux[71] and colleagues studied two related $\alpha_7$ genes (*CHRNA7* and *CHRNA7*-like gene) resulting from a partial duplication (from exon 5 to exon 10) in the human genome. They described two types of genetic variation (i) a large deletion resulting in a truncation of the open reading frame; and (ii) a -2 bp deletion in exon 6, which specifically affected the *CHRNA7*-like gene. Genotyping 70 schizophrenic patients and 77 controls who had had P50 ERP recorded, Raux and colleagues found that carrying at least one -2 bp deletion of exon 6 did not constitute a risk factor for schizophrenia; on the contrary, healthy control subjects were more likely to have the -2 bp deletion of exon 6 associated with P50 ratio >0.45. The authors of this study concluded that the -2 bp deletion within the *CHRNA7*-like gene is a risk factor for P50 sensory gating deficit, but not for schizophrenia. In a subsequent study,[72] the same group was unable to replicate the *CHNRA7* core promoter variant findings described by Leonard et al,[70] but found a -194C *CHNRA7* promoter polymorphism that was also associated with a P50 ratio <0.50 as in the Leonard et al study, except that this allele was also more frequent in the control group. The authors suggested that this was a case where the variant allele rendered a protective effect against the P50 ratio deficit, and the -194C polymorphism was in linkage disequilibrium with causal variants of the deficit. Although the evidence to support a functional association of *CHRNA7* promoter variants with susceptibility to schizophrenia is weak, it may still be the case that *CHRNA7* modulates inhibitory factors in the P50 ERP and, in similar fashion perhaps, modulation of clinical symptoms in schizophrenia.

*P300 oddball ERPs*

The P300 oddball ERP is a cognitive electrophysiological paradigm elicited within a 250- to 450-ms poststimulus window when a subject detects a low probability target stimulus. The maximum amplitude of the P300 response is recorded from temporoparietal scalp leads. A number of laboratories[5,73-79] including ours,[5,77] have found the P300 left temporoparietal component amplitude distinguishes patients with schizophrenia from healthy controls. However, whether P300 oddball amplitude is a plausible intermediate phenotype in schizophrenia remains undetermined.

The crux is the question of heritability; the issue is whether siblings share a putative phenotype; and for this the evidence is decidedly mixed. For example, in a series of studies, Turetsky and colleagues[78,79] investigated the P300 oddball paradigm in patients, siblings, and controls, and found that a larger frontal, but not a smaller temporoparietal, component amplitude, was heritable, as only patients had reduced temporoparietal P300 amplitudes relative to controls. They concluded P300 frontal component variance was a candidate intermediate phenotype. In our laboratory, we studied 42 patients with schizophrenia, 62 unaffected siblings, and 34 healthy control subjects with the P300 oddball intending to determine whether two-dimensional topographic scalp-distribution of amplitude and latency distinguished groups.[77] We found patient P300 amplitude was decreased relative to healthy controls over the left parietotemporal area by as much as 54% to 58%; however, there was no difference between unaffected siblings and healthy subjects. P300 latencies were unchanged in both comparisons, calling into doubt the candidacy of the P300 as a plausible intermediate phenotype in this cohort.

In a subsequent study,[5] we revisited the matter of P300 amplitude and latency as intermediate phenotypes in a new, larger cohort of 66 schizophrenia patients, 115 healthy siblings of schizophrenic patients, and 89 unrelated controls. In this study, a principal component analysis was applied on the basis of the notion that P300 abnormalities in siblings of schizophrenic patients may involve a distributed network of relatively weak cortical generators, and because our earlier, smaller study using a topographic analysis of covariance model did not conclude that P300 localized topography predicts risk for schizophrenia. In partial agreement with Turetsky and colleagues,[78,79] we found a smaller temporoparietal, and larger frontal principal component distinguished patients and unaffected siblings from controls. In comparing our two studies, we concluded that principal component analysis was better able to identify signals from multiple weak sources, compared with topographic analysis, and that to some extent, cohort

effects principally explained the differences. A study with a third cohort is in progress in our laboratory.

Blackwood and Muir[75] studied the P300 in members of a single large pedigree in which schizophrenia and depression segregate with a balanced chromosomal translocation in *DISC1* involving the long arm of chromosome 1 and the short arm of chromosome 11. In members of the family with the t(1;11) translocation, P300 amplitude was reduced in carrier relatives compared to relatives with a normal karyotype, regardless of the presence or absence of psychiatric symptoms. The clinical phenotype associated with the t(1;11) translocation included schizophrenia, schizoaffective disorder, recurrent major depression, and bipolar disorder. Hence, there is suggestive evidence that P300 abnormalities are associated with the inheritance of risk for schizophrenia and affective disorders, but the specificity of the association remains unclear. These interesting findings merit efforts to determine whether they are replicable in other population samples.

## Candidate genes

Converging evidence[10-13,80-88] lends strong support to an evolving concept of a core phenotype in schizophrenia that is a reflection of a broad-based perturbation of synaptic functions subserving cortical microcircuit information processing. This is implicit in the cognitive and electrophysiological intermediate phenotype results.[80] The majority of genes thus far identified as likely to confer susceptibility to schizophrenia impact in diverse ways on the development, activity, plasticity, and composition of various synaptic components, eg, axon terminals, cytoplasmic vesicular transport, dendritic structure, and function. For example, the authors of a recent comprehensive review of the molecular origins of schizophrenia[10] identified 12 "good bet" candidate risk genes, while readily acknowledging the subjective and most likely transient nature of their list. Caveats notwithstanding, the authors' stringent criteria included the following:

- Strength of evidence for association with schizophrenia, based on sample size and number of replications in at least three positive independent studies.
- Linkage to a gene locus associated with schizophrenia, based on data from two recent meta-analyses.[87,88]
- Biological plausibility, based on evidence of altered function and expression in vivo or in vitro.[89-91]
- Evidence of altered expression in schizophrenia brain, based on measures of mRNA or protein, or relative expression of isoforms or alleles.[92-102]

The following candidate genes were particularly highlighted: *COMT* (22q11); *DTNBP1* (6p22); *NRG1* (8p12-21); *RGS4* (1q21-22); *GRM3* (7q21-22); *DISC1* (1q42); and *G72* (13q32-34). Importantly, with regards to discussion of intermediate phenotypes in schizophrenia, converging evidence to support the candidacy of the nominated genes derives from morphometric, histopathological, and animal experiment data, implicating hippocampal glutamatergic dysfunction,[91] GABAergic (GABA, γ-aminobutyric acid), and glutamatergic abnormalities in the DLPFC,[92-95] frontocortical dopaminergic inervation,[89,90,96] and signal transmission.[98] From this comes the suggestion that schizophrenia may be viewed as a disorder associated with broad-based disruptions of cortical synaptic functions and perturbed synaptic plasticity on the microcircuit scale[10,81-86,99] and as disruptions of high-order information processing on the neural systems scale.[15,80]

### *COMT*

Egan and colleagues[89] studied abnormalities of prefrontal cortical function in schizophrenia associated with the regulation of prefrontal dopamine, a neurotransmitter that modulates the response of prefrontal neurons during working memory. They examined the relationship of a common functional polymorphism (Val(108/158)Met) in the catechol-*O*-methyltransferase (COMT) gene, which accounts for a fourfold variation in enzyme activity and dopamine catabolism, with both prefrontally mediated cognition and prefrontal cortical physiology. In 175 patients with schizophrenia, 219 unaffected siblings, and 55 controls, *COMT* genotype was related in allele dosage fashion to performance on the Wisconsin Card Sorting Test of executive cognition and explained 4% of variance ($P$=0.001) in frequency of perseverative errors. Consistent with other evidence, these investigators found the load (0, 1, or 2 *met* alleles) of the low-activity *met* allele predicted enhanced cognitive performance. Finally, in a family-based association analysis of 104 trios, they found a significant increase in transmission of the *val* allele to the schizophrenic offspring. These data suggested that the *COMT* *val* allele impairs prefrontal cognition and physiology, and thereby slightly increases risk for schizophrenia.

Goldberg et al[90] used a working memory paradigm to study the effects of genotype on increasing memory load in a large sample of schizophrenia patients, their healthy siblings, and controls. As in the study by Egan et al,[89] par-

ticipants were genotyped for *COMT* at the val158met locus. Goldberg et al found that high-activity *val/val* individuals had the poorest working memory performance, and that *met/met* individuals had the best performance. Siblings and patients with schizophrenia performed significantly worse than controls; the allelic effects on performance were similar in both tasks across groups. These authors concluded that genotype significantly affected working memory, but not subprocesses related to attention, load, or delay. They also proposed that their findings support an additive genetic model in which the effect of allele load is similar in its effects on dorsal prefrontal cortex working memory regardless of the genetic or environmental background in which it is expressed. Taken together, the study of Egan et al, and that of Goldberg et al, together with those of other,[100-102] but not all,[103] investigators support a role for an effect of *COMT* val158met polymorphism on genetic risk, and a critical role in prefrontal cortical function, in families of European descent; it is unclear as to whether *COMT* variants play such a role in other population groups, such as Asians.

*RGS4*

Prasad and colleagues[92] recently reported that genetic polymorphisms in *RGS4*, a gene shown to regulate glutamatergic signaling, were associated with robust volumetric differences across genotypes in the DLPFC of a pooled sample of first-episode, unmedicated schizophrenia patients compared with control subjects. Separately analyzed, the investigators found volumetric differences within the patient group (n=30), but none in control subject (n=27). Notably, considering the critical role of the DLPFC in an array of cognitive domains, the results of this study suggest that *RGS4* polymorphisms contribute to structural alterations in the DLPFC, and may confer risk for schizophrenia via a related mechanism, possibly related to the genetic environment.

*GRM3*

An example of a recent trend in studies aimed at identifying the genetic risk associated with putative intermediate phenotypes is represented by a recent large study of 217 patients with schizophrenia, 311 unaffected siblings, 362 parents, and 136 controls by Egan and colleagues.[93] These investigators employed an array of complementary measures, including the N-back fMRI paradigm, MRI spectroscopy, and postmortem histopathology aimed at testing for an association with schizophrenia and with putative intermediate phenotypes to a previously identified gene candidate, *GRM3*, and exploring potential mechanisms of its effects. *GRM3* encodes the $mGluR_3$ receptor, which modulates synaptic glutamate, dopamine, and GABA. Evidence of aberrant prefrontal cortical function associated with disrupted glutamatergic pathways has been widely reported.[94,96-98,104-108] For instance, investigators have found reduced expression of the excitatory amino acid transporter (EAAT2) mRNA of patients with schizophrenia.[97,104,108] In addition, animal models using $mGluR_{2/3}$ agonists have been shown to block ketamine-induced cognitive deficits and psychosis-like behavioral abnormalities,[109,110] thus suggesting a possible role for *GRM3* in psychotic disorders, such as schizophrenia. *GRM3* is also expressed in astrocytes, where it has been shown to regulate expression of EAAT2, also known as the glial glutamate transporter[104,105] which is critically responsible for reuptake of synaptic glutamate. Together, this evidence supports a potentially important role for this gene in regulation of glutamate neurotransmission.

Previous studies have found that *GRM3* maps to 7q21.1, a region that has shown evidence of linkage with schizophrenia in at least one family study.[111] SNPs in exon 3 and intron 3, 17 kb apart, were previously shown to be associated with schizophrenia[112,113]; in another study, support for *GRM3* association in schizophrenia was strong on an initial sample, but failed to support association on a replication sample.[107] Egan et al[93] found that a common *GRM3* haplotype was strongly associated with schizophrenia ($P$=0.0001); within this haplotype, the *A* allele of an SNP in intron 2 was slightly overtransmitted to probands ($P$=0.02). Subsequently, these investigators studied the effects of this SNP on an array of neurobiological traits related to risk for schizophrenia (ie, putative intermediate phenotypes), and associated with glutamate neurotransmission. For tests of cognition, there was a robust main effect of genotype associated with performance on verbal list learning and verbal fluency, each of which index prefrontal function. The physiological basis of the *GRM3* effect was assessed with the N-back fMRI paradigm; in this, control subjects homozygous for the risk allele showed hyperactivation patterns in both prefrontal and medial temporal (hippocampal) regions, a reflection of inefficiency relative to the other allele carriers matched for the same performance score. This pattern has been observed previously in N-back fMRI studies of DLPFC

in patients with schizophrenia and healthy siblings supporting its plausibility as a cognitive intermediate phenotype related to genetic risk, and illustrating in this study that the risk allele also plays a role in this distinct pattern of prefrontal inefficiency in healthy controls.

Subsequently, the investigators used MRI spectroscopy to measure the effect of SNP 4 on NAA, an indirect measure of prefrontal glutamate neurotransmission, synaptic abundance, and prefrontal extracellular glutamate. NAA has been previously shown to be lower in DLPFC and hippocampus in schizophrenia.[53] In this study right and left, DLPFC NAA was lower in risk allele homozygotes than other genotypes, as would be expected from the cognitive and fMRI results. Group-by-genotype interaction was not significant; even so, there was scant effect of genotype on NAA in the sibling group. Finally, in postmortem human prefrontal cortex, risk allele homozygotes had significantly lower mRNA levels of the glial glutamate transporter EAAT2. The authors suggest these convergent data reflect a specific molecular pathway by which *GRM3* genotype may alter glutamate neurotransmission, and thus prefrontal and hippocampal physiology and cognition, thereby increasing risk for schizophrenia. Nevertheless, the authors cautiously point out the weakness of *GRM3* effects on intermediate phenotypes in this study, particularly due to the multiple statistical testing, which is unavoidable in a study of such complexity. This study is paradigmatic of the opportunities and the impediments encountered in a combined multimodal effort to identify genes underlying the neurobiology of cognitive functions in schizophrenia.

## Conclusions

In many respects, it is eminently reasonable to search for intermediate phenotypes in complex disorders such as schizophrenia, particularly because the matrix of risk alleles shaping the clinical phenotype will likely turn out to be far more complex than might have been expected. Nevertheless, emerging evidence suggests that, in order to more finely parse the genetic infrastructure, it will be necessary to more finely parse and validate the most relevant functional phenotypes. This conjunction of aims may not be easy to achieve. For example, despite a substantial literature on eye-tracking dysfunctions in schizophrenia and unaffected siblings,[1,114,115] and increasingly precise understanding of the physiology of eye movement, it would seem at first glance difficult to imagine how therapeutic normalization would improve the psychosocial outcome for patients with schizophrenia. In essence, that is precisely the reason to identify intermediate phenotypes and the genes modulating their functions; we do not have sufficient evidence to predict which intermediate phenotypes we should ignore. For this reason, it will be important to develop an operational definition of relevance that assigns primacy to putative phenotypes most likely to impact clinical outcome.

The apparently incongruent sensory ERP (P50 and P300) data appears in part to be due to the inherent sensitivity of ERP experiments to methodological diferences.[116,117] In the future, researchers may wish to consider using cognitive ERP, for example,[118] particularly in the context of a combined multimodal approach, and in view of recent advances in quantitative methods of electronencephalographic (EEG) analysis.[119] For example, multimodal experimental design, as in Egan et al,[93] would permit incorporation of tests of cognition from separable domains[120-122] embedded in, for example, EEG or fMRI, in conjunction with corresponding data from molecular genetics. This view is supported by early unimodal studies of the N-back working memory paradigm, which tended to find well-matched siblings and healthy controls performed equivalently; addition of fMRI activation data showed sibling groups use increased neural resources in doing so. It is conceivable that collecting EEG/magnetoencephalogram (MEG) data indexing the temporal evolution of a paradigm such as the N-back would allow an increased understanding of the role of spectral coherence and stimulus-locked phase synchrony in distinguishing group differences in the cognitive process, particularly in light of a developing consensus on a battery of cognitive instruments that may reliably distinguish patients with schizophrenia from comparison groups.[123,124] Finally, the weight of convergent evidence supports initial assumptions that intermediate phenotypes indexing disruptions of cognition would enhance the search for susceptibility genes in schizophrenia. Such developments support the continuing search for intermediate phenotypes, and suggest this will be an increasingly effective strategy empowering the identification of risk alleles in schizophrenia. ❑

### *Fenotipos intermediarios en la esquizofrenia: una revisión selectiva*

*Los estudios orientados a identificar la susceptibilidad genética a la esquizofrenia y otros trastornos psiquiátricos complejos se enfrentan con diferentes factores de confusión tales como los criterios clínicos subjetivos, las fenocopias que se dan comúnmente, la significativa variabilidad entre los sujetos de los rasgos candidatos y la probabilidad de heterogeneidad alélica y de locus que se ha tomado como evidencia para definir la genética de otros complejos trastornos cerebrales y somáticos humanos. Además, la investigación orientada a la identificación de los orígenes moleculares de la esquizofrenia también debe abordar la confusa naturaleza del cerebro humano. A diferencia de órganos con algunos fenotipos celulares, transcriptomas y proteomas comunes, las neuronas individuales a menudo son distintas unas de otras en todos estos aspectos. En esta revisión se presentan trabajos recientes que afirman tentativamente que los estudios de susceptibilidad genética en trastornos poligénicos complejos como la esquizofrenia debieran ser mejorados mediante la identificación de fenotipos intermediarios relacionados con aspectos más fundamentales del desarrollo y función del cerebro. El progreso en la identificación de fenotipos intermediarios significativos para la esquizofrenia ha sido posible gracias al avance de metodologías recientes en neurociencias cognitivas y neuroimágenes, y mediante el empleo de técnicas multimodales combinadas.*

### *Phénotypes intermédiaires dans la schizophrénie : une revue sélective*

*Les études qui ont pour but d'identifier les gènes de susceptibilité à la schizophrénie et aux autres troubles psychiatriques complexes sont confrontées aux facteurs de confusion que sont les critères subjectifs cliniques, les fréquentes phénocopies, la variabilité significative intrasujets des caractères étudiés, et la probabilité de l'hétérogénéité des locus et des allèles dont on sait qu'elle définit la génétique d'autres troubles somatiques et cérébraux complexes chez l'homme. De plus, la recherche consacrée à l'identification des origines moléculaires de la schizophrénie doit aussi composer avec la nature déconcertante du cerveau humain. Contrairement aux organes pourvus de peu de phénotypes cellulaires courants, les transcriptomes et les protéomes, les neurones individuels sont souvent distincts les uns des autres à tous égards. Dans cet article, nous présentons un travail récent qui évalue l'hypothèse selon laquelle les études de susceptibilité génétique dans les troubles complexes polygéniques comme la schizophrénie, pourraient être améliorées par l'identification des phénotypes intermédiaires liés à des aspects plus fondamentaux de la fonction et du développement du cerveau. L'avènement de nouvelles méthodes en neurosciences cognitives et en neuro-imagerie ainsi que l'utilisation de techniques multimodales combinées, ont permis de progresser dans l'identification de phénotypes intermédiaires significatifs dans la schizophrénie.*


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