

Deconstructing Schizophrenia: An Overview of the Use of Endophenotypes in Order to Understand a Complex Disorder

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The genetics of schizophrenia has been approached utilizing a variety of methods. One emerging strategy is the use of endophenotypes in order to understand and identify the functional importance of genetically transmitted, brain-based deficits across schizophrenia kindreds. The endophenotype strategy is a topic of this issue of *Schizophrenia Bulletin*. Endophenotypes are quantitative, heritable, trait-related deficits typically assessed by laboratory-based methods rather than clinical observation. Endophenotypes are seen as closer to genetic variation than are clinical symptoms of schizophrenia, and are therefore closely linked to heritable risk factors. There has been a broad expansion of opportunities available to psychiatric neuroscientists who use the endophenotype strategy to understand the genetic basis of schizophrenia. In this context, genetic variation such as single nucleotide polymorphisms (SNPs) induces abnormalities in endophenotypic domains such as neurocognition, neurodevelopment, metabolism, and neurophysiology. This article discusses the challenges that abound in genetic research of schizophrenia, including issues in ascertainment, epistasis, ethnic diversity, and the potentially normalizing effects of second-generation antipsychotic medications on neurocognitive and neurophysiological measures. Robust strategies for meeting these challenges are discussed in this review and the subsequent articles in this issue. This article summarizes conceptual advances and progress in the measurement and use of endophenotypes in schizophrenia that form the basis of the multisite National Institute of Mental Health

Consortium on the Genetics of Schizophrenia. The endophenotype strategy offers powerful and exciting opportunities to understand the genetically conferred neurobiological vulnerabilities and possible new strong inference and molecularly based treatments for schizophrenia.

Key words: endophenotype/schizophrenia/cognition/genetics

Introduction

The power and appeal of the molecular biology mantra, “DNA to RNA to protein,” in explicating cell biology comes from its virtually universal appearance in all species from microorganisms to human beings. The genomes of many species are being mapped and sequenced, so that the entire genome and, ultimately, the corresponding biological activity of genes can be identified. Because these genes are identified, it is reasonable to ask how this information can be related to the heritable risk for all diseases with complex genetics¹ including psychiatric disorders (c.f. Table 1). For Huntington’s disease, a singular Mendelian dominant disorder, changes in the genetic coding of the amino acid sequence can be closely associated with functional changes in neurobiological integrity. In contrast, for a complex psychiatric disorder like schizophrenia, as defined by *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria, the relationship is obviously not as simple.^{2,3} Neuropsychiatric disorders, such as schizophrenia, whose genetic component is inferred from twin and family studies,⁴ are conceptualized as multifactorial, and most likely reflect the combined influence and interactions of genetic, epigenetic, stochastic, and nongenetic factors. Furthermore, in contrast to simple Mendelian dominant illnesses, there is no reason to presuppose that only one gene is responsible for a complex psychiatric disorder, such as schizophrenia. Individuals who are ill probably have several genetic differences from the rest of the population, and there are likely different genes or sets of genes associated with schizophrenia disorders in different populations. Thus, how best to use the power of molecular genetics to identify susceptibility genes of

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complex genetic psychiatric disorders, such as schizophrenia, remains a scientific challenge that is only now beginning to be resolved.

This issue focuses on the usefulness of the endophenotype strategy to understanding the genetic architecture of schizophrenia, with an emphasis on neurocognitive and neurophysiological measures such as those used in the National Institute of Mental Health (NIMH) linked R01 Consortium on the Genetics of Schizophrenia (COGS), as described in this issue by Calkins et al.⁵ The endophenotype strategy has reemerged as an important tool in neuropsychiatric research strategies after a long latency since its introduction to studies of psychopathology by Gottesman and Shields⁶ and its subsequent reawakening (eg,⁷⁻⁹). PubMed on July 21, 2006, revealed some 426 uses since 1987, of which over 200 have been published since 2005. This dramatic emergence of endophenotype articles reflects many factors including the limited reproducibility of genetic and neurobiological studies directed toward etiologies of the disorders in the DSM, and an improved appreciation for the complex relationships between genes and behavior. Endophenotypes are not discernible to the unaided eye or ear—they are not the signs or symptoms of psychiatry. The prototype of an endophenotype that Gottesman and Shields had in mind 25 years ago was the role played by an abnormal glucose tolerance test, after glucose challenge to the asymptomatic cotwins and close relatives of type 1 diabetic patients, in identifying those at greatest genetic risk in the context of a multifactorial (polygenic) threshold model of etiology. Disease heterogeneity is often guaranteed, rather than simplified, through our current (DSM) diagnostic system; inherent benefits of endophenotypes include more specific disease concepts and process definitions. Endophenotypes for psychopathology can be neurocognitive, neurophysiological, neurodevelopmental, biochemical, endocrinological, or neuroanatomical. Heritability and stability (independent of state) represent key components of any useful endophenotype. Importantly, they characterize an approach that reduces the complexity of symptoms and multifaceted behaviors by using measurement strategies that use quantitative units of analysis that are amenable to being assessed in the laboratory.

A simple and commonly applied strategy of molecular genetics to psychiatric disorders is to assume that the distribution of illness in a family represents the effect of a single gene with a large effect and to apply genetic analysis techniques to identify genes.⁸ This approach does not necessarily overlook the complexity of psychiatric illness, but it assumes that the effect of a single major gene will still be discernable in a complex genetic background, if samples sizes are large enough or if the population is sufficiently homogeneous.¹⁰ An attractive feature of this approach is that the search for genes is not constrained by preexisting hypotheses about the neurobiology of the

illness, but a problem is that neurobiological information is not used to inform these analyses. A second commonly applied strategy is essentially the opposite approach, ie, to make an assumption about the biology of the illness and then examine candidate genes associated with that biology to determine if they are mutated. Both approaches have been successful to a limited extent for understanding the genetics of schizophrenia. This second approach is now called whole genome association studies and advances in gene chip technology make it feasible. Replicable linkages for schizophrenia have been obtained at several locations. For example, genetic regions have listed 10 major regions implicated in schizophrenia, but, as yet, crucial genetic mutations have generally not been identified at most of these sites.¹¹ DNA polymorphisms have been found in candidate genes, such as the NURR1 receptor for retinoic acid⁸ and DISC 1,¹² pathways critical for neuronal development, but these polymorphisms seem to be found in only a small proportion of schizophrenia patients, perhaps accurately reflecting the etiological heterogeneity of this disorder and the futility of applying a one gene, one disorder model for the group of schizophrenias.¹³

The Endophenotype Approach to Schizophrenia

This issue of *Schizophrenia Bulletin* focuses on a third approach that attempts to make simultaneous use of the power of molecular biology and neurobiology by identifying specific brain dysfunction that might be caused by a family of individual genetic polymorphisms, abnormal in the aggregate. The rationale comes from one basis of molecular biology: if there are discrete genetic abnormalities associated with schizophrenia, then each of them might cause a specific protein change that is reflected in a corresponding discrete functional abnormality. Even if several genes are abnormal (along with environmental contributions), the functional abnormality from each gene should be identifiable. Theoretically, the relationship between functional abnormalities and genes, discovered by genetic linkage or candidate gene analysis, should be stronger than the association to the illness itself, because the illness results from a mixture of multiple genetic as well as multiple nongenetic abnormalities that may vary between different individuals and families. Although this approach has not yet led to the identification of multiple interacting genetic abnormalities that are associated with the onset of schizophrenia, this endophenotype strategy has also been extremely important for gene discovery in other complex medical illnesses. For example, in a form of colon cancer, multiple polyp formation, rather than cancer itself, is the genetically heritable trait.¹⁴ This is a single gene defect that is fully penetrant though age of onset differs, in the genetic disease. In spontaneous forms (95% of colon cancers have an activated protein C mutation), the same gene becomes an

oncogene but the disease and the polyp formation is not inherited. Likewise, in hemochromatosis, high serum iron is the more penetrant heritable trait than the illness itself.¹⁵

Clearly, important articles such as "Endophenotypes for Psychiatric Disorders: Ready for Prime Time?" by Bearden and Freimer¹⁶ and "Time for a Shift in Focus in Schizophrenia: From Narrow Phenotypes to Broad Endophenotypes" by Weiser et al¹⁷ echo Gottesman's original and continuing emphasis on the importance of the use of quantitative endophenotypes. Braff and Freedman⁸ subsequently pointed out, these endophenotypes are likely closer to a specific genetic abnormality and corresponding protein change than are DSM-defined categorical but fundamentally qualitative and subjective diagnostic entities. A fundamental issue in the endophenotype approach is the strategy of identifying and validating potential endophenotypes. Identification usually comes from studies of schizophrenia-linked deficits. A second step is to identify evidence of segregation and heritability in "clinically unaffected" relatives. Genetic analysis depends on genotype-endophenotype correlations within individual pedigree members and is a more challenging test than a determination of schizophrenia-normal subject differences.

Gottesman has pointed out that endophenotype or intermediate phenotype is often used as the descriptive term for these discrete, genetically determined intervening variables that may be part of a complex illness and are not readily discerned by the naked eye, but may need a laboratory-based assessment or even "challenge" (eg, glucose tolerance test) in order to be identified. The search for relevant endophenotypes is challenging and complex because there are no a priori criteria for deciding if a particular element of schizophrenia or any other psychiatric illness reflects the effect of a single gene. Putative endophenotypes have ranged from clinically defined diagnostic dimensions, such as the presence of measured and quantified schizotypy in relatives of schizophrenia patients,^{18,19} to the salient, face valid neurocognitive and neurophysiological measures described in this issue, to structural measures of specific, functionally important regions of the brain to metabolic, neurodevelopmental and even temperament-related genes. Thus, an important distinction should be made between endophenotyping and clinical subtyping (cf,⁷). Because some of these endophenotypes have now led to the clarification of a specific molecular abnormality in schizophrenia patients (eg,²⁰), there is evidence that they are linked to the disorder itself. In this context, even if endophenotypes turn out to be determined by multiple vs single genes, their complex genetic architecture may well be simpler than the clinically useful, but still ambiguous or qualitative diagnostic category of schizophrenia as reflected in our diagnostic manuals.

There are several important issues to consider in the linking of disease susceptibility endophenotypes. (1)

Because endophenotypes are putatively caused by genetic polymorphisms, genes sometimes begin to regulate their neurobiological expression at conception. By the time the endophenotypes are measured in adulthood, their expression may have been modified by lifelong nongenetic factors such as developmental events, aging itself interacting with vulnerable neural substrates, brain injury, medication administration and substance abuse and/or exposure, etc. Thus, not all genes will have direct effect on endophenotypes. Some phenotypes may represent effects during development of adaptive changes in one region that are, in fact, due to the direct action of protein changes in more distant, but functionally related brain regions and circuits where the disease allele is actually expressed. (2) Most of the 16 000 genes expressed in the brain (out of a total of 30 000 genes found in humans²¹) are often expressed differentially, in different brain areas and interact with genetic and nongenetic factors, so that their ultimate function may include much more than the simple endophenotype being measured. The neural substrates of many of these endophenotypes are known: some overlap structurally and functionally. (3) Many genes expressed in the brain are involved in the development of neurons, brain development, cellular migration, and morphology, so perhaps their most important functional effects may have occurred prenatally. (4) Mendel's second law points out that every genetic trait segregates independently in a family, so that, if schizophrenia consists of multifactorial traits, there should be some clinically unaffected siblings who express specific endophenotypes independently of other endophenotypes. These siblings may be better subjects for characterizing the endophenotype than the patients themselves, whose multiple deficits may obscure the unique endophenotype. (5) Because the aim of genetic studies is generally to identify individually affected subjects who have or do not have a particular genetic abnormality, the measurement of the putative endophenotype must clearly separate the most affected and unaffected individuals, regardless of whether a quantitative or categorical (discrete) variable is used. The range of effect sizes for several putative endophenotypes is thus critically important, as discussed below and in the accompanying articles in this issue. (6) Measuring endophenotypes, even at one site, is often a complex endeavor. For this reason, both selection of endophenotypes and quality assurance of endophenotyping is of paramount importance, especially in multisite studies described in this issue (cf,⁵).

A recent review of the progress of genetic research in schizophrenia revealed a number of replicated linkages. Yet, these linkage findings have not led to an avalanche of identification of causative genes in schizophrenia, in part because many of the initial findings and replications have not sufficiently narrowed the regions of interest to allow for cloning of a genetic variant, based on its linkage to nearby markers on the same chromosome. Given the

modest magnitude of most log of the odds (LOD) scores reported to date and the uncertainty about the estimated parameters of the genetic models,²² genetic linkage studies in schizophrenia have often been unable to narrow the regions of interest to less than 5 to 10 cm.

Criteria for Viable Endophenotypes

Following Gottesman and Gould's rationale (eg,^{7,23}), the reasonable criteria for a viable and generative endophenotype are as follows: (1) the deficits in the endophenotype are associated with schizophrenia, (2) these deficits are heritable, (3) the endophenotype deficits are stable (although a trait-relevant challenge may be required to elicit them) and trait-related: their appearance is relatively independent of state-related fluctuations in the individuals' status, although factors such as age may affect the endophenotype, (4) the endophenotype and disorder show cosegregation, and (5) the proband's specific endophenotype deficit is found at higher rates in the probands' relatives than in the general population. Critical reviews of various endophenotypes follow in this issue of *Schizophrenia Bulletin*.^{24,25} Figure 1 introduces the concept of endophenotype to help clarify its distinction within the broader class of biological markers.

Developmental and Metabolic Genes Not Discussed at Length in This Issue

In the realm of developmentally important genetics in schizophrenia, DISC 1 and 2, neuregulin, and dysbindin¹² have been identified via linkage disequilibrium fine mapping as of considerable neurobiological interest (eg,³¹). DISC-1 translocations, identified in a large schizophrenia-rich Scottish pedigree, have a specific translocation³² and have probable neurodevelopmental implications. Neuregulin, identified in Scottish³³ and Icelandic³⁴ pedigrees, is a candidate gene in schizophrenia. Because it is associated with neural regulation and mediation of CNS processes and development as well as with key schizophrenia-related neurotransmitters such as NMDA, GABA, and glutamate,³⁵ it also is an interesting candidate gene in schizophrenia. Likewise, dysbindin, originally identified via the study of densely affected Irish pedigrees, seems to be functionally important in processes such as signal transduction. Thus, although these genes have been identified via large pedigree studies in order to find DNA regions that harbor genes related to schizophrenia, it turns out that upon specific interrogation, more is being discovered about their functions and patterns of heritability. This approach utilizes a type of "strong inference"-based approach, whereby known endophenotypes are investigated because it is known that the endophenotypic deficit is stable, reliable, and heritable in schizophrenia kindreds.

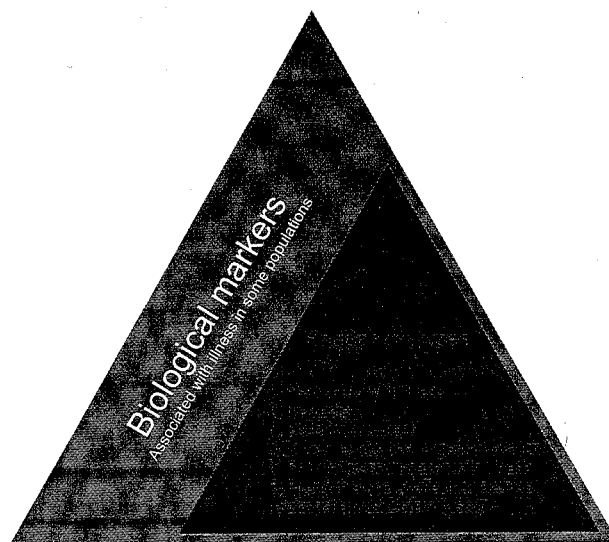


Fig. 1. Biological Markers (Known as Subclinical Traits or Vulnerability Markers) may be Primarily Environmental, Epigenetic, or Multifactorial and Then Origin. For this reason, criteria useful for the identification of endophenotypes, a special subset of such markers for studies in psychiatric genetics have been proposed, adapted, and refined over time (see^{7,8,26-30}). Current criteria for an endophenotype, to be distinguished from biological markers, are designed to direct clinical research in psychiatry toward genetically and biologically meaningful conclusions. © 2005, I.I. Gottesman and used with permission.

Another related realm of endophenotype-related genetic dysfunction is biochemical, such as the catechol-O-methyl-transferase (COMT) findings, where the valine/methionine polymorphism has been found to be associated with schizophrenia.^{36,37} In addition, the COMT story is face valid and compelling. Since the 1950s, trans-methylation dysfunctions and related hypotheses have abounded. Despite this long history, the COMT results have been "mixed," with failures to replicate and executive function associations being reported and not replicated.³⁸ Clearly, the COMT story is just now being clearly explicated.

Unique Advantages of the Endophenotype Approach: From Gene to Treatments

There are several potential crucial advantages to the endophenotype approach: (1) Physiological and more elementary neural-based endophenotypes may more directly reflect the activities of synaptic and other neuronal mechanisms than does the more complex illness itself, and therefore they are more likely to reflect major gene effects. (2) Both schizophrenia patients and their unaffected relatives may show a fairly extensive range of scores on the endophenotypes, making such measures ideal for quantitative trait linkage analysis. Analysis of quantitative measurements related to the clinical phenotype may provide more power to detect linkage, compared with clinically defined schizophrenia. (3) To

Table 1. The Timeline of 150 Years of Genetics/Genomics and Endophenotype Development

1865	1902	1909	1910	1941	1953
Gregor Mendel reports his laws for heritability of traits ("Experiments in Plant Hybridization")	William Bateson coins the term "genetics"	Danish botanist Wilhelm Johanssen uses term "gene" and identifies multiple phenotypes from common genotypes ^{34,6}	Nilson Ehle notes seed color (wheat and oats) patterns reflect gene-environment interactions	The term "polygene" used by K. Mather	Watson and Crick note that DNA base pairs are found to constitute the genetic building blocks of life
1950s to present	1965	1972	1983	1989	2003
• Modern genetic era • Epigenetic factors affect phenotypes via environment, gene-gene interactions, or stochastic mechanisms • Mendelian dominant diseases in humans are few	Falconers multifactorial model for non-Mendelian diseases introduced	Gottesman and Shields introduce the term endophenotypes into psychiatric genetics	Huntington's disease gene is identified	Nobel Prize awarded for identification of "colon cancer" endophenotype oncogenes (Bishop and Varmus)	The Human Genome Project 3 000 000 000 base pairs constitute "only" 30 000 genes, not 100 000 or more as was once thought to be the case; 16 000 expressed in brain
					2007 and Beyond
					A challenging and bright (not easy) future

Partially adapted from Gottesman and Gould *Am J Psych*. 2003;160:636-645.

the extent that the biology of the endophenotype is understood or can be investigated via brain-imaging studies and infrahuman animal model research, candidate genes can be identified more systematically in areas of linkage. (4) Endophenotypes lend themselves directly to animal models.^{23,39} To date, animal models have been exploited for prepulse inhibition (PPI) of the startle response,^{40,41} and the animal model for P50 suppression equivalent (called N40 suppression)⁴² that serves as one model for the endophenotype approach because (1) P50 suppression deficits were found in schizophrenia patients and then in their unaffected relatives⁴³ and the findings were widely replicated, (eg,⁴⁴⁻⁴⁹) (2) 11 family and association studies identified a chromosomal region of interest (cf,²⁰), (3) the region yielded an association of P50 suppression deficits in schizophrenia via the α -7 subunit of the nicotinic receptor, (4) a specific genetic single nucleotide polymorphism (SNP) in the promoter region of this gene was identified, (5) now, molecularly based treatments with α -7 agonists are feasible.⁵⁰

Thus, the P50 suppression story illustrates the unfolding of the use of endophenotypes. Initially, the endophenotype was used for a genome-wide scans, and this identified several promising loci with less than dramatic LOD scores. However, one of these loci was at the site of a candidate gene, the α -7 subunit of the nicotinic acetylcholine receptor. New markers were identified in genomic clones containing the candidate gene, and the LOD score with one of these markers reached genome-wide significance ($Z = 5.30$).²⁰ The LOD score was replicated in a large sample of affected sib pairs,⁵¹ and the identification (via positional cloning) of several functional polymorphisms in the promoter region of the gene has been completed, a groundbreaking advance in the use of the endophenotype strategy.⁵² From this example, it appears that the power of linkage is enhanced, not only by the increased penetrance of the endophenotype but also by the ability to relate the endophenotype to a specific biological function. In related work, the cloning of the amyotrophic lateral sclerosis gene was facilitated by knowledge that one of the genes in the linkage region was expressed in spinal motor neurons.

Some Caveats and Challenges to the Endophenotype Approach

Several potential problems with neurocognitive, neurophysiological, and other endophenotypes must also be addressed. First, the technology needed to reliably acquire neurophysiological and neurocognitive phenotypes is complex and must be carefully adapted to large multisite-population studies (cf,⁵). Secondly, although heritability and segregation patterns, as well as animal models, provide some evidence for whether or not an endophenotype reflects a major gene effect, identifying a causative gene (through association studies) or a LOD

score of sufficient merit (using a gene linkage approach) is the only definitive answer to that question. Some endophenotypes may be too complex to show robust major gene effects, but will still be useful in understanding which aspects of the pathophysiology of the disorder are linked to particular loci or sets of loci. Thus, the endophenotype approach frequently uses the following template that has been refined in the COGS multisite consortium: (1) Determine the segregation and cosegregation of these phenotypes and families. (2) Perform linkage analysis on those phenotypes that appear to show genetic transmission. The results of the segregation studies help identify the genetic aspects of schizophrenia from a physiological and cognitive perspective, and they may also determine which of the various pathophysiological features of schizophrenia have a common neuronal basis. The results of the linkage studies ultimately will be used in subsequent projects to identify candidate genes, supported by both linkage and neurobiological findings, for molecular sequencing. (3) The data from projects such as the COGS can then be made available to other researchers, multiplying the potential benefits of this type of multisite study.

Even within specific domains of function where groups of patients have deficits in measure A and measure B, deficits in individual patients may be divergent as illustrated by P50 suppression and PPI (cf.^{25,53}). Although groups of schizophrenia patients may have P50 suppression and PPI deficits, there is a divergence in which patients have the deficits, reflecting the heterogeneity of the "group of schizophrenias." Different subjects thus have divergent loading for P50 suppression and PPI deficits. Thus, despite the face valid similarities of measures that are labeled as gating deficits (eg, P50 gating, PPI gating),⁵³ this pattern of group deficits but divergence of the individual deficit levels is striking.^{54,55} It does appear that there are independent mechanisms of deficits that may well reflect divergent neurobiological substrate dysfunction and genetic architectures.

A visual synthesis of the chain of ideas expressed so far may be useful, although still sketchy, and has a clear heuristic value for researchers contemplating their next steps. It is provided in figure 2 and further serves as context in which to appreciate the articles to follow.

Candidate Endophenotypes for Genetic Studies

Given the considerations discussed above, while the somewhat ambiguous DSM-IV diagnostic criteria for schizophrenia may be clinically and administratively useful, they are not likely to be optimally useful as meaningful and potentially exploitable phenotypes in genetic studies (eg,^{2,3,8,56}). In accounting for the genetic diathesis or vulnerability to schizophrenia, genetic factors account for 50–80% of the liability to developing the disorder—the remaining variability resides in nongenetic second hits, such as neonatal or in utero neural damage to the devel-

oping hippocampus^{57–61} or other epigenetic factors.^{62,63} The importance of the use of endophenotypes to parse the genetic vulnerability for schizophrenia from the impact of a second, nongenetic hit, is partly illustrated by the fact that clinically unaffected relatives of schizophrenic patients have deficits in one or more endophenotypes, but do not have the disorder of schizophrenia. The value of familial, nondiagnosis-based candidate endophenotypes as vulnerability markers extend beyond schizophrenic research, to many disorders, from diabetes to hypertension to bipolar disorder.

Each of the neurocognitive and neurophysiological functional endophenotypes that are discussed in this issue of the *Schizophrenia Bulletin* have accessible estimates of the effect sizes of their deficits in schizophrenia patients compared with normal comparison subjects (cf.⁶⁴). Identifying the frequency and mode of transmission of these endophenotype abnormalities for genetic studies is a major first step that is the focus of many current investigations including the COGS. Once this first step is accomplished, strategies will be employed to identify the precise genetic architecture underlying these endophenotypes in schizophrenia. Endophenotypes are usually selected because of (1) their existing replication and importance in schizophrenia research, (2) their appearance in unaffected relatives of schizophrenia patients, and, in most cases, (3) their already at least partially identified neurobiological substrates. Most of the extensively studied endophenotypes have clear between-site reliability, heritability, and stability clinical research based on the COGS (cf.^{5,24,25}). For many of the endophenotypes, this information is currently being assessed and will advance our knowledge of heritability patterns as current research within the COGS and other venues progresses. Heritability is discussed in other articles of this issue, using statistical measures most commonly cited in the published literature and reviewed by Schork et al.⁶⁵

Ascertainment Issues

Basically, much research focuses on exploring the genetic architecture of quantitative endophenotypes underlying schizophrenia susceptibility rather than on the genetic basis of schizophrenia itself. This point is important to emphasize for the following 2 reasons:

(1) in order to explore the determinants of a quantitative trait, one must exploit adequate "variation" in that trait as exhibited in appropriate samples and (2) from an epidemiologic perspective, in order to understand how a determinant "contributes" to disease, one must have adequate numbers of both normal (ie, nonaffected) and affected subjects to supply contrast that can be exploited via statistical genetics methods.

Sampling designs that make use of affected sibling pairs and densely affected multiplex families in allele-sharing genetic linkage analysis studies produce efficient

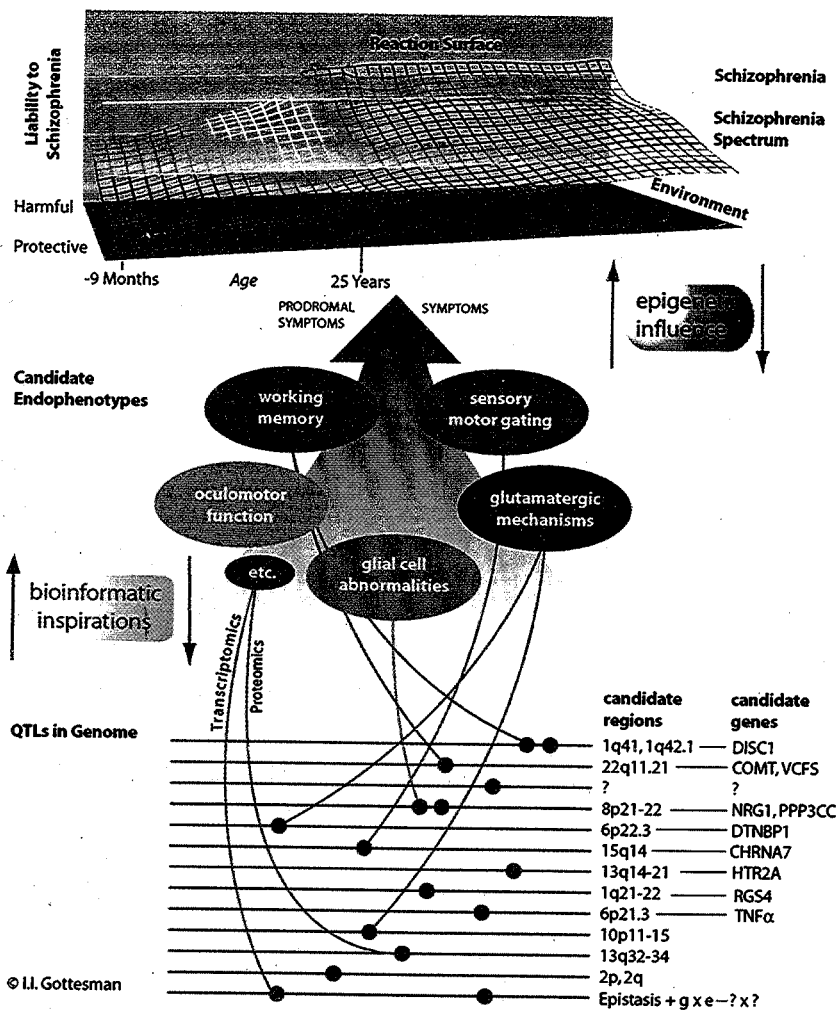


Fig. 2. Gene Regions, Genes, and Candidate Endophenotypes are Implicated in a Biological Systems Approach to Schizophrenia Research. The reaction surface suggests the dynamic developmental interplay among genetic, environmental, stochastic, and epigenetic factors that produce cumulative liability to developing schizophrenia spectrum and schizophrenia disorders above each of the 2 thresholds shown. Endophenotypes are characterized by simpler neurobiological and genetics antecedents than psychiatric disorders. The schizophrenia phenotype, as an example, is associated with a number of candidate genes and chromosomal regions, the influence of which can be observed at the levels of either behavior or endophenotypes. Endophenotypes, located closer to genes in the pathway from genes to behaviors, have fewer genes associated, and thus are more amenable to genetic investigations and studies in model systems. This skeleton (genes to endophenotypes to behaviors), allowing for epigenetic, "environmental," and purely stochastic influences upon clinical observations, and inspired by bioinformatics and the HapMap, can be applied to other diseases with complex genetics using the input of disease-specific candidate genes/regions, single nucleotide polymorphisms, and endophenotypes. None of the sections of this figure can be definitive; many more elements exist and await discovery (represented by "etc." and question marks). © 2005, I.I. Gottesman and used by permission.

and powerful designs for the study of a single qualitative disease (eg, ¹). However, such designs are not optimal for studying quantitative endophenotypic variation, where contrasts between individuals with high and low endophenotype values generate power. This fact—especially as it bears on linkage analysis—has been explored in great detail (eg, ⁶⁶⁻⁷⁵). Thus, sib pair and multiplex families would not be optimal for studying "quantitative" phenotypes underlying or contributing to "qualitative" disease outcomes like schizophrenia, because, if high scores on these phenotypes truly associate with schizophrenia, then by having families with exclusively multiply affected individuals (and the consequent lack of unaffected

teds in those families) one would actually "reduce" the variation exhibited by this trait in the samples and thereby reduce power for studying the determinants of the quantitative phenotypes. The "contrast-based" sampling strategies enrich samples for both high and low endophenotype values, while ensuring that enough unaffected siblings will be ascertained to provide needed "contrasts." The degree to which such variation exists among schizophrenia and normal control subjects is a primary question to be addressed. In addition, because we do not know a priori the degree to which each endophenotype contributes to, or is associated with, schizophrenia, researchers maximize the chances of success of capturing

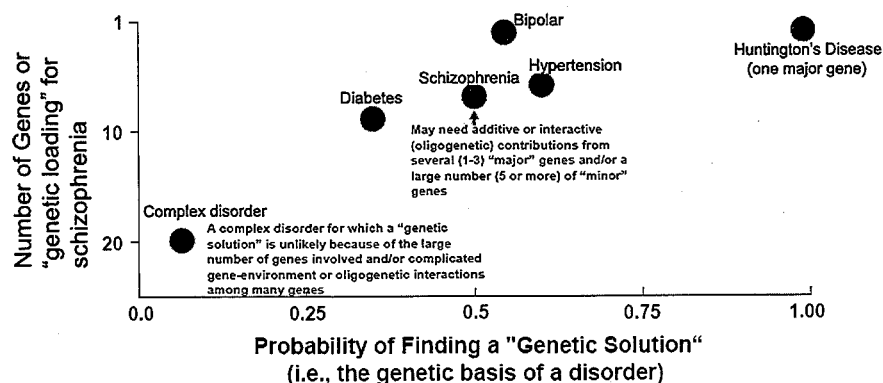


Fig. 3. This Figure Indicates the "Ease" of Defining the Genetic Architecture of Single Gene, Mendelian Dominant Disorders (eg, Huntington's) vs the More Daunting Task of Understanding the Genetic Architecture of Complex Disorders Such as Diabetes, Schizophrenia, Bipolar Disorder, and Hypertension.

enough variation in the "set" of proposed endophenotypes with the COGS sampling scheme described by Calkins et al⁵ in this issue. On the other hand, when multigenerational families are utilized for endophenotypic and genetic information, the design may introduce another ascertainment bias: it is possible that cohorts of families who volunteer for research projects are more intact and less alienated from each other and thus are not truly representative schizophrenia patients. From an endophenotypic standpoint, this possibility is falsifiable by comparing endophenotypic scores and other measures of symptoms and social functioning of probands in these kindreds with singleton schizophrenia patients ascertained in a community sample. Whatever be the state of affairs, we can still study genetic determinants and interrelationships of the endophenotypes, as well as their role in schizophrenia susceptibility and pathology in various populations of interest (e.g., See Figure 3 for an illustration of the study of genetic determinants of simple vs. complex human disorders). Also, even in more extensive studies such as the COGS, ascertained kindreds inevitably have multiple affected individuals, providing appropriate within- and between-family contrasts useful for generating hypotheses on how each endophenotype contributes to unique subtypes of schizophrenia. These types of issues are discussed in detail by Schork et al.⁶⁵

Some, if not all, of the endophenotypes discussed in this issue can be used to assess traditional and novel subtypes of schizophrenia, via unique data analysis such as cluster-analytic methods. However, in order to make claims about the potential role-proposed endophenotypes have in defining and contributing to schizophrenia (globally or by subtypes), we need to compare and contrast these endophenotypes between adequate numbers of affected and unaffected individuals within and across the ascertained families. By sampling only affected sib pairs or dense multiplex families, there are no such contrasts. Note that within-family variability in the endophe-

notypes would not be as great if one merely collected dense multiplex families but these and other strategies also have their own unique advantages.⁶⁵

Ethnic Diversity

Use of racial contrasts such as Caucasian and African American subjects (as utilized in the COGS) is often meant to explore ethnic and genetic background factors that contribute to variation in endophenotypes without producing so much diversity in samples so as to create problems with (potentially) overt genetic heterogeneity. For example, there is known to be appreciable genetic heterogeneity, with unknown functional significance, within urban US ethnic and racial populations, so that adding additional ethnic groups to sampling strategies may wash out potential linkage effects and confound adequate characterization of the genetic architecture of a proposed endophenotype. When the contribution of race, ethnicity, and genetic background to variation is considered as part of the study of endophenotypes, the genetic architecture of these endophenotypes using traditional strategies involving stratification of the sample and the use of racial categories as covariates, as well as the more novel approaches to unearthing cryptic substructure within experimental samples using the strategies outlined by Pritchard et al⁷⁶ and Schork et al^{77,78} are important. These strategies consider the genetic similarity (eg, the microsatellite marker genotype information obtained from the genome scan) of all pairs of individuals in a sample amounting to a "genetic cluster analysis." If evidence for empirically defined clusters of genetically similar individuals are found in the sample, these clusters can be accommodated in subsequent analyses meant to determine their impact on variation in the schizophrenia endophenotypes under scrutiny. Ethnicity can be an even more complex, challenging problem in endophenotypic and genetic research.

Epistasis (Gene by Gene Interactions)

Studies of endophenotypes in families frequently use a strategy for assessing epistasis that is specifically designed for variance components analysis that considers the product of the identity-by-descent allele sharing matrices computed from marker loci within 2 different regions of the genome as a new coefficient matrix in the variance-covariance matrix decomposition of the variance component model. Testing a single parameter associated with this matrix (that is used to quantify the percentage of variation explained by the epistatic interaction of loci with the 2 different regions) allows geneticists to explore the effect of epistasis on proposed quantitative endophenotypes. This strategy was discussed initially by Khoury et al.,⁷⁹ elaborated specifically for variance component models of arbitrary complexity by Blangero et al.^{80,81} and Mitchell et al.,⁸² and implemented specifically for behavioral traits by Cloninger et al.⁸³ In addition, endophenotype studies can exploit the novel "whole genome" and "genome partitioning" analysis methods developed by Schork.⁸⁴ These methods consider and test the contribution of any genomic locus, subregion, chromosome, set of chromosomes, or an entire genome, to variation in a quantitative trait while controlling for, or accommodating, the contribution of other loci or regions. Thus, while these methods do not focus on interaction, they allow for the consideration of the heterogeneous genetic and multifactorial basis of quantitative endophenotypes.

Medication Effects

Notably, the potentially normalizing effects of antipsychotic medications on endophenotypes have neither totally obscured evidence for familial transmission of the quantitative phenotypes nor voided linkage findings. In fact, because medications are a potential source of environmentally induced phenotypic variation and their normalization, their net effect would probably be to reduce evidence for linkage, and they are not likely to produce false-positive results. Of course, this is an issue that only future data-based studies can resolve. Carefully selected endophenotypes where both between- and within-group publications and pilot data indicate that medication confounds are not significant or would not significantly create analytic problems are best suited for study in whole family studies with diagnostic "contrast" where probands are receiving antipsychotic medications. An optimal strategy for dealing with potential minor medication effects can be handled in 3 complementary ways: (1) Based on experience, 10–20% of probands will enter most studies in a nonmedicated state (the ideal population to study but one very difficult to ascertain), allowing experimenters to further assess medication effects both within- and between-subjects. (2) A new statistical strategy for addressing medication effects is being

used in the COGS project. In the absence of data comparing every conceivable minor medication effect with each endophenotype, imputing values for the probands, can be utilized. The selection of endophenotypes that have small medication effects, high short-term correlations between medicated and unmedicated states, and concomitant low variability allows for such imputed medication effects modeling and is thus a viable strategy. (3) A sensitivity analysis (eg,^{85–87}) can be used that will test the robustness of results to varying assumptions about the magnitude of medication effects. Sensitivity analyses can be repeated under 3 sets of assumptions: (a) no medication effect, (b) a medication effect equal to half of the maximal effect suggested by the literature, and (c) a medication effect equal to the maximal effect suggested by the literature. Thus, investigators can consider the (a) analyses as primary analyses. The (b) and (c) strategies can be used in an exploratory fashion to suggest hypotheses that would require cross-validation. For these latter analyses, the maximal medication effects for each phenotype (expressed as standard deviation units) must be ascertained. This 3-pronged strategy can be utilized to successfully complete endophenotyping projects with appropriate rigor. Still, the normalizing role of atypical antipsychotic medications on endophenotypes such as P50 suppression^{88–91} and PPI⁹² combined with the difficulty of ascertaining nonmedicated but ill patients is a major obstacle for the endophenotype strategy and all sorts of other schizophrenia research. Because, it is becoming increasingly clear that many neurocognitive and neurophysiological endophenotypes are at least partially normalized by second-generation antipsychotic medications, there currently exists a state of affairs that will offer challenges to endophenotype-based (and other) gene finding approaches in schizophrenia research.

Diagnostic Specificity

Robust diagnostic specificity, especially across schizophrenia and bipolar disorders, is often lacking for endophenotypes and reflects the fact that "different" disorders (eg, schizophrenia, bipolar disorder) may share genes⁹³ and also share partially overlapping neural substrate dysfunction⁹⁴ and clinical features.⁹⁵ Again, quantitative phenotypes offer some advantages in providing answers to this conundrum. If other disorders share genes as well as some endophenotypic variation with schizophrenia, this can be explored in future studies of "mixed" diagnostic groups (eg, schizophrenia, bipolar, schizoaffective manic type). Endophenotypic variation can be used to characterize schizophrenia and schizophrenia subtypes as a prelude to these other studies.

Future Directions

When all is said and (hopefully) the requisite research is done, what unique advantages accrue from deconstructing

schizophrenia via endophenotyping? First, because schizophrenia is heterogeneous, each of the group of schizophrenias may eventually be characterized endophenotypically. Both bivariate and cluster-analytic methods may yield endophenotypic profiles that can reveal the neurobiology and genetic architecture of different putative groups of schizophrenia patients.

Secondly, from behavioral/laboratory deficits that have known heritabilities and neural substrates, specific abnormal molecular targets may be identified via endophenotyping and subsequent genetic analyses. Just as endophenotypes are thought to more closely reflect a single gene product than the illness itself does, so too is drug therapy directed to a single gene product, such as a neurotransmitter mechanism, rather than to the illness as a whole. Although some drugs like antipsychotics are not directed toward receptors known to be genetically altered in schizophrenia, the identification of genetic mechanisms nonetheless opens possibilities for the development of drug mechanisms that were not heretofore considered. More specific therapies can then be developed based on patients' group membership (eg, frontal cortical dopamine hypoactivity, hippocampal cholinergic α -7 subunit deficits). A possible example, discussed above and by Turetsky et al,²⁵ is the link between familial P50 suppression deficits and α -7 subunit promoter region SNPs in the cholinergic receptor. This result logically leads one to identify poor P50 suppressors among schizophrenia patients and administer (after appropriate safety and clinical trials) an α -7 agonist as adjunctive or monotherapy. Other examples might be based on behavioral and endophenotypic deficit patterns and the genetics of endophenotypes in schizophrenia from neurocognition, to neurophysiology, to neurodevelopmental (eg, neuregulin), to metabolic (eg, COMT) deficits.

Thus, we may be on the cusp of developing endophenotypically and genetically identified compounds in order to treat schizophrenia via strong inference-based biomedical research rather than serendipity. The following articles in this issue cover some of these possibilities, with an emphasis on COGS-related measures (neurocognition and neurophysiology), although the COGS structure can and will also examine the other domains listed above in our search to understand the neurobiology and genetic architecture of schizophrenia and also to develop new molecular biologically derived, more robust and specific treatments for this complex and disabling group of disorders.

Acknowledgments

David L. Braff and his laboratory are supported by the Bowman Family Foundation research partnership with the National Alliance for Research on Schizophrenia and Depression, a grant from the Department of Veteran Affairs (VISN 22 Mental Illness Research,

Education, and Clinical Center), and NIMH grants MH-042228 and MH-065571 (COGS). The authors thank Gregory A. Light, PhD, Joyce Sprock, and Emmeline R. Crowley for their comments and editorial assistance.

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