

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

David L. Braff<sup>1,2</sup>, Robert Freedman<sup>3</sup>, Nicholas J. Schork<sup>2,4</sup>, and Irving I. Gottesman<sup>5</sup>

<sup>2</sup>Department of Psychiatry, University of California San Diego, 9500 Gilman Drive, Mail Code 0804, La Jolla, CA 92093-0804;

<sup>3</sup>University of Colorado Health Sciences Center, Campus Box C249-32, PO Box 173364, Colorado Psychiatric Hospital, Room 1F15, Denver, CO 80217-3364; <sup>4</sup>Department of Family and Preventive Medicine and The Center for Human Genetics and Genomics, 9500 Gilman Drive, Mail Code 0603, La Jolla, CA 92093-0603; <sup>5</sup>Department of Psychiatry, University of Minnesota Medical School, 2450 Riverside Avenue, Minneapolis, MN 55454

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 genetics<sup>1</sup> 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.<sup>2,3</sup> Neuropsychiatric disorders, such as schizophrenia, whose genetic component is inferred from twin and family studies,<sup>4</sup> 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

<sup>1</sup>To whom correspondence should be addressed; tel: (619) 543-5570, fax: (619) 543-2493, e-mail: dbraff@ucsd.edu.

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.*<sup>5</sup> 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 Shields<sup>6</sup> and its subsequent reawakening (eg,<sup>7-9</sup>). 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.<sup>8</sup> 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.<sup>10</sup> 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.<sup>11</sup> DNA polymorphisms have been found in candidate genes, such as the NURR1 receptor for retinoic acid<sup>8</sup> and DISC 1,<sup>12</sup> 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.<sup>13</sup>

### 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.<sup>14</sup> 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.<sup>15</sup>

Clearly, important articles such as “Endophenotypes for Psychiatric Disorders: Ready for Prime Time?” by Bearden and Freimer<sup>16</sup> and “Time for a Shift in Focus in Schizophrenia: From Narrow Phenotypes to Broad Endophenotypes” by Weiser et al<sup>17</sup> echo Gottesman’s original and continuing emphasis on the importance of the use of quantitative endophenotypes. Braff and Freedman<sup>8</sup> 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,<sup>18,19</sup> 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,<sup>7</sup>). Because some of these endophenotypes have now led to the clarification of a specific molecular abnormality in schizophrenia patients (eg,<sup>20</sup>), 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 humans<sup>21</sup>) 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,<sup>5</sup>).

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

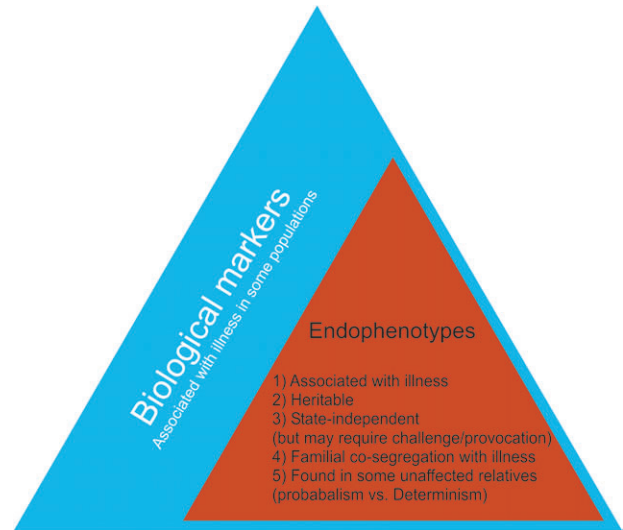
modest magnitude of most log of the odds (LOD) scores reported to date and the uncertainty about the estimated parameters of the genetic models,<sup>22</sup> genetic linkage studies in schizophrenia have often been unable to narrow the regions of interest to less than 5 to 10 cm.

### Criteria for Viable Endophenotypes

Following Gottesman and Gould's rationale (eg,<sup>7,23</sup>), 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*.<sup>24,25</sup> 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 dysbindin<sup>12</sup> have been identified via linkage disequilibrium fine mapping as of considerable neurobiological interest (eg,<sup>31</sup>). DISC-1 translocations, identified in a large schizophrenia-rich Scottish pedigree, have a specific translocation<sup>32</sup> and have probable neurodevelopmental implications. Neuregulin, identified in Scottish<sup>33</sup> and Icelandic<sup>34</sup> 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,<sup>35</sup> 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 <sup>7,8,26-30</sup>). 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.<sup>36,37</sup> 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.<sup>38</sup> 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

Year	Event
1865	Gregor Mendel reports his laws for heritability of traits ("Experiments in Plant Hybridization")
1902	William Bateson coins the term "genetics"
1909	Danish botanist Wilhelm Johannsen uses term "gene" and identifies multiple phenotypes from common genotypes
1910	Nilson Ehle notes seed color (wheat and oats) patterns reflect gene-environment interactions
1941	The term "polygene" used by K. Mather
1953	Watson and Crick note that DNA base pairs are found to constitute the genetic building blocks of life
1950s to present	Modern genetic era
1965	Falconers multifactorial model for non-Mendelian diseases introduced
1972	Gottesman and Shields introduce the term endophenotypes into psychiatric genetics
1983	Huntington's disease gene is identified
1989	Nobel Prize awarded for identification of "colon cancer" endophenotype oncogenes (Bishop and Varmus)
2003	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.<sup>23,39</sup> To date, animal models have been exploited for prepulse inhibition (PPI) of the startle response,<sup>40,41</sup> and the animal model for P50 suppression equivalent (called N40 suppression)<sup>42</sup> that serves as one model for the endophenotype approach because (1) P50 suppression deficits were found in schizophrenia patients and then in their unaffected relatives<sup>43</sup> and the findings were widely replicated, (eg,<sup>44-49</sup>) (2) 11 family and association studies identified a chromosomal region of interest (cf,<sup>20</sup>), (3) the region yielded an association of P50 suppression deficits in schizophrenia via the  $\alpha$ -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  $\alpha$ -7 agonists are feasible.<sup>50</sup>

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  $\alpha$ -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 ( $Z = 5.30$ ).<sup>20</sup> The LOD score was replicated in a large sample of affected sib pairs,<sup>51</sup> 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.<sup>52</sup> 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,<sup>5</sup>). 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.,<sup>25,53</sup>). 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),<sup>53</sup> this pattern of group deficits but divergence of the individual deficit levels is striking.<sup>54,55</sup> 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,<sup>2,3,8,56</sup>). 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<sup>57–61</sup> or other epigenetic factors.<sup>62,63</sup> 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.

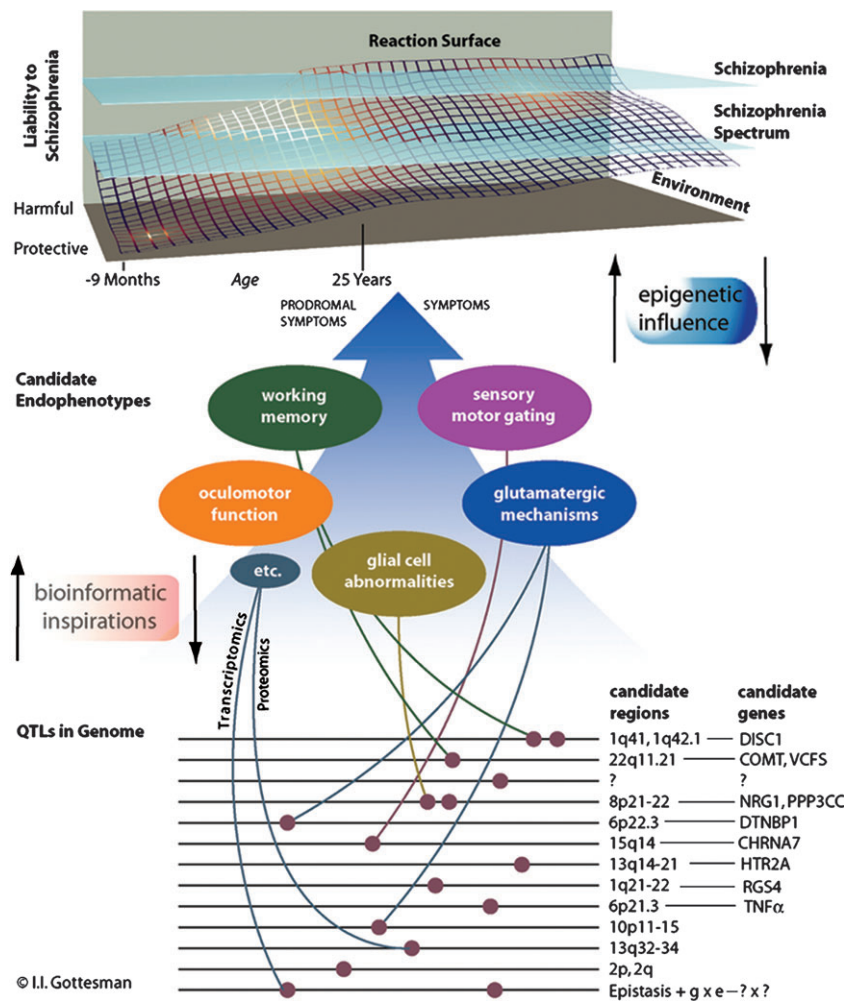
Each of the neurocognitive and neurophysiological functional endophenotypes that are discussed in this issue of the *Schizophrenia Bulletin* have accessible estimates of the effect sizes of their deficits in schizophrenia patients compared with normal comparison subjects (cf.,<sup>64</sup>). Identifying 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.,<sup>5,24,25</sup>). 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.*<sup>65</sup>

### 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.

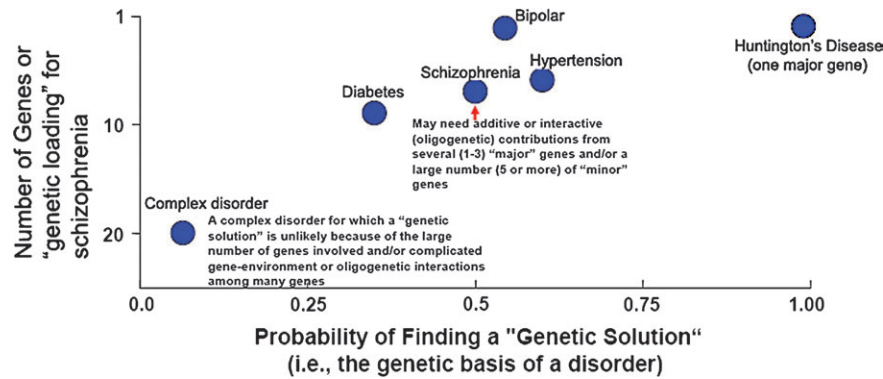
Sampling designs that make use of affected sibling pairs and densely affected multiplex families in allele-sharing genetic linkage analysis studies produce efficient



**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,<sup>1</sup>). 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,<sup>66–75</sup>). 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 unaffec-

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 al*<sup>5</sup> 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.*<sup>65</sup>

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.<sup>65</sup>

### 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 al.*<sup>76</sup> and Schork *et al.*<sup>77,78</sup> 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,<sup>79</sup> elaborated specifically for variance component models of arbitrary complexity by Blangero et al<sup>80,81</sup> and Mitchell et al,<sup>82</sup> and implemented specifically for behavioral traits by Cloninger et al.<sup>83</sup> In addition, endophenotype studies can exploit the novel “whole genome” and “genome partitioning” analysis methods developed by Schork.<sup>84</sup> 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,<sup>85–87</sup>) 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<sup>88–91</sup> and PPI<sup>92</sup> 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 genes<sup>93</sup> and also share partially overlapping neural substrate dysfunction<sup>94</sup> and clinical features.<sup>95</sup> 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  $\alpha$ -7 subunit deficits). A possible example, discussed above and by Turetsky et al,<sup>25</sup> is the link between familial P50 suppression deficits and  $\alpha$ -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  $\alpha$ -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.

#### References

1. Lander ES, Schork NJ. Genetic dissection of complex traits. *Science*. 1994;265:2037–2048.
2. Hyman SE. The NIMH perspective: next steps in schizophrenia research. *Biol Psychiatry*. 2000;47:1–7.
3. Weinberger DR. Schizophrenia: new genes and phenes. *Biol Psychiatry*. 1999a;46:3–7.
4. Gottesman II, Shields J. A polygenic theory of schizophrenia. *Proc Natl Acad Sci U S A*. 1967;58:199–205.
5. Calkins ME, Dobie DJ, Cadenhead KS, et al. The consortium on the genetics of endophenotypes in schizophrenia (COGS): model recruitment, assessment, and endophenotyping methods for a multi-site collaboration. *Schizophr Bull*. October 11, 2006; doi: 10.1093/schbul/sbl044.
6. Gottesman II, Shields J. *Schizophrenia and Genetics: A Twin Study Vantage Point*. New York, NY: Academic Press Inc; 1972.
7. Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry*. 2003;160:636–645.
8. Braff DL, Freedman R. Endophenotypes in studies of the genetics of schizophrenia. In: Davis KL, Charney DS, Coyle JT, Nemeroff C, eds. *Neuropsychopharmacology: The Fifth Generation of Progress*. Philadelphia, PA: Lippincott Williams & Wilkins; 2002:703–716.
9. Cannon TD. The inheritance of intermediate phenotypes for schizophrenia. *Curr Opin Psychiatry*. 2005b;18:135–140.
10. Brzustowicz LM, Hodgkinson KA, Chow EW, et al. Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. *Science*. 2000;288:678–682.
11. Pulver AE. Search for schizophrenia susceptibility genes. *Biol Psychiatry*. 2000;47:221–230.
12. Cannon TD, Hennen W, van Erp TG, et al. Association of DISC1/TRAX haplotypes with schizophrenia, reduced prefrontal gray matter, and impaired short- and long-term memory. *Arch Gen Psychiatry*. 2005a;62:1205–1213.
13. Buervenich S, Carmine A, Arvidsson M, et al. NURR1 mutations in cases of schizophrenia and manic-depressive disorder. *Am J Med Genet*. 2000;96:808–813.
14. Leppert M, Burt R, Hughes JP, et al. Genetic analysis of an inherited predisposition to colon cancer in a family with a variable number of adenomatous polyps. *N Engl J Med*. 1990;322:904–908.
15. Lalouel JM, Le Mignon L, Simon M, et al. Genetic analysis of idiopathic hemochromatosis using both qualitative (disease status) and quantitative (serum iron) information. *Am J Hum Genet*. 1985;37:700–718.
16. Bearden CE, Freimer NB. Endophenotypes for psychiatric disorders: ready for primetime? *Trends Genet*. 2006;22:306–313.
17. Weiser M, van Os J, Davidson M. Time for a shift in focus in schizophrenia: from narrow phenotypes to broad endophenotypes. *Br J Psychiatry*. 2005;187:203–205.
18. Faraone SV, Kremen WS, Lyons MJ, et al. Diagnostic accuracy and linkage analysis: how useful are schizophrenia spectrum phenotypes? *Am J Psychiatry*. 1995a;152:1286–1290.

19. Faraone SV, Seidman LJ, Kremen WS, et al. Neuropsychological functioning among the nonpsychotic relatives of schizophrenic patients: a diagnostic efficiency analysis. *J Abnorm Psychol*. 1995b;104:286–304.
20. Leonard S, Freedman R. Genetics of chromosome 15q13-q14 in schizophrenia. *Biol Psychiatry*. 2006;60:115–122.
21. Insel TR, Collins FS. Psychiatry in the genomics era. *Am J Psychiatry*. 2003;160:616–620.
22. Roberts SB, MacLean CJ, Neale MC, et al. Replication of linkage studies of complex traits: an examination of variation in location estimates. *Am J Hum Genet*. 1999;65:876–884.
23. Gould TD, Gottesman II. Psychiatric endophenotypes and the development of valid animal models. *Genes Brain Behav*. 2006;5:113–119.
24. Gur RE, Calkins ME, Gur RC, et al. The Consortium on the Genetics of Schizophrenia (COGS): neurocognitive endophenotypes. *Schizophr Bull*. In press.
25. Turetsky BI, Calkins ME, Light GA, et al. Neurophysiological endophenotypes of schizophrenia: the viability of selected candidate measures. *Schizophr Bull*. In press.
26. Hasler G, Drevets WC, Gould TD, et al. Toward constructing an endophenotype strategy for bipolar disorders. *Biol Psych*. 2006;60:93–105.
27. Leboyer M, Bellivier F, Nosten-Bertrand M, et al. Psychiatric genetics: search for phenotypes. *Trends Neurosci*. 1998;21:102–105.
28. Lenox RH, Gould TD, Manji HK. Endophenotypes in bipolar disorder. *Am J Med Genet*. 2002;114:391–406.
29. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.
30. Shields J, Gottesman II. Genetic studies of schizophrenia as signposts to biochemistry. In: Iversen LL, Rose SPR, eds. *Biochemistry and Mental Illness*. London: Biochemical Society; 1973:165–174.
31. Miyamoto S, LaMantia AS, Duncan GE, et al. Recent advances in the neurobiology of schizophrenia. *Mol Interv*. 2003;3:27–39.
32. Millar JK, Wilson-Annan JC, Anderson S, et al. Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum Mol Genet*. 2000;9:1415–1423.
33. Stefansson H, Sarginson J, Kong A, et al. Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. *Am J Hum Genet*. 2003a;72:83–87.
34. Stefansson H, Thorgeirsson TE, Gulcher JR, et al. Neuregulin 1 in schizophrenia: out of Iceland. *Mol Psychiatry*. 2003b;8:639–640.
35. Stefansson H, Sigurdsson E, Steinthorsdottir V, et al. Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet*. 2002;71:877–892.
36. Egan MF, Goldberg TE, Kolachana BS, et al. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A*. 2001;98:6917–6922.
37. Glatt SJ, Faraone SV, Tsuang MT. Association between a functional catechol O-methyltransferase gene polymorphism and schizophrenia: meta-analysis of case-control and family-based studies. *Am J Psychiatry*. 2003;160:469–476.
38. Williams HJ, Glaser B, Williams NM, et al. No association between schizophrenia and polymorphisms in COMT in two large samples. *Am J Psychiatry*. 2005;162:1736–1738.
39. Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)*. 2001;156:234–258.
40. Swerdlow NR, Platten A, Shoemaker J, et al. Effects of pergolide on sensorimotor gating of the startle reflex in rats. *Psychopharmacology (Berl)*. 2001;158:230–240.
41. Geyer MA, McIlwain KL, Paylor R. Mouse genetic models for prepulse inhibition: an early review. *Mol Psychiatry*. 2002;7:1039–1053.
42. Stevens KE, Kem WR, Mahnir VM, et al. Selective alpha7-nicotinic agonists normalize inhibition of auditory response in DBA mice. *Psychopharmacology (Berl)*. 1998;136:320–327.
43. Siegel C, Waldo M, Mizner G, et al. Deficits in sensory gating in schizophrenic patients and their relatives. Evidence obtained with auditory evoked responses. *Arch Gen Psychiatry*. 1984;41:617–612.
44. Clementz BA, Geyer MA, Braff DL. Poor P50 suppression among schizophrenia patients and their first-degree biological relatives. *Am J Psychiatry*. 1998a;155:1691–1694.
45. Clementz BA, Geyer MA, Braff DL. P50 suppression among schizophrenia and normal comparison subjects: a methodological analysis. *Biol Psychiatry*. 1997;41:1035–1044.
46. Clementz BA, Geyer MA, Braff DL. Multiple site evaluation of P50 suppression among schizophrenia and normal comparison subjects. *Schizophr Res*. 1998b;30:71–80.
47. Judd LL, McAdams L, Budnick B, et al. Sensory gating deficits in schizophrenia: new results. *Am J Psychiatry*. 1992;149:488–493.
48. Myles-Worsley M. P50 sensory gating in multiplex schizophrenia families from a Pacific island isolate. *Am J Psychiatry*. 2002;159:2007–2012.
49. Waldo MC, Adler LE, Freedman R. Defects in auditory sensory gating and their apparent compensation in relatives of schizophrenics. *Schizophr Res*. 1988;1:19–24.
50. Olincy A, Harris JG, Johnson LL, et al. Proof-of-concept trial of an alpha7 nicotinic agonist in schizophrenia. *Arch Gen Psychiatry*. 2006;63:630–638.
51. Leonard S, Gault J, Moore T, et al. Further investigation of a chromosome 15 locus in schizophrenia: analysis of affected sibpairs from the NIMH Genetics Initiative. *Am J Med Genet*. 1998;81:308–312.
52. Logel JG, Vianson R, et al. Mutation screen of the promoter region of the human a7 neuronal nicotinic receptor subunit in normal and schizophrenic individuals. *Soc Neurosci Abstracts*. 2000;26:373.
53. Braff DL, Light GA, Swerdlow NR. Prepulse inhibition and P50 suppression are both deficient but are not correlated in schizophrenia patients. *Biol Psychiatry*. In press.
54. Kumari V, Soni W, Sharma T. Prepulse inhibition of the startle response in risperidone-treated patients: comparison with typical antipsychotics. *Schizophr Res*. 2002a;55:139–146.
55. de Bruin NM, Ellenbroek BA, van Schaijk WJ, et al. Sensory gating of auditory evoked potentials in rats: effects of repetitive stimulation and the interstimulus interval. *Biol Psychol*. 2001;55:195–213.
56. Freedman R, Coon H, Myles-Worsley M, et al. Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. *Proc Natl Acad Sci U S A*. 1997;94:587–592.
57. Kinney DK, Levy DL, Yurgelun-Todd DA, et al. Inverse relationship of perinatal complications and eye tracking dysfunction in relatives of patients with schizophrenia: evidence for a two-factor model. *Am J Psychiatry*. 1998;155:976–978.
58. Lipska BK, Chrapusta SJ, Egan MF, et al. Neonatal excitotoxic ventral hippocampal damage alters dopamine response

- to mild repeated stress and to chronic haloperidol. *Synapse*. 1995;20:125–130.
59. McNeil TF, Cantor-Graae E, Weinberger DR. Relationship of obstetric complications and differences in size of brain structures in monozygotic twin pairs discordant for schizophrenia. *Am J Psychiatry*. 2000;157:203–212.
  60. Weinberger DR. Hippocampal injury and chronic schizophrenia. *Biol Psychiatry*. 1991;29:509–5011.
  61. Weinberger DR. Cell biology of the hippocampal formation in schizophrenia. *Biol Psychiatry*. 1999b;45:395–402.
  62. Petronis A. Epigenetics and twins: three variations on the theme. *Trends Genet*. 2006;22:347–350.
  63. Wong JJ, Hawkins NJ, Ward RL. Colorectal cancer—a model for epigenetic tumorigenesis. *Gut*. 2006;doi: 10.1136/gut.2005.088799.
  64. Snitz BE, Macdonald AWIII, Carter CS. Cognitive deficits in unaffected first-degree relatives of schizophrenia patients: a meta-analytic review of putative endophenotypes. *Schizophr Bull*. 2006;32:179–194.
  65. Schork NJ, Greenwood T, Braff DL. Statistical genetics in schizophrenia and related neuropsychiatric research. *Schizophr Bull*. In press.
  66. Abecasis GR, Cookson WO, Cardon LR. The power to detect linkage disequilibrium with quantitative traits in selected samples. *Am J Hum Genet*. 2001;68:1463–1474.
  67. Carey G, Williamson J. Linkage analysis of quantitative traits: increased power by using selected samples. *Am J Hum Genet*. 1991;49:786–796.
  68. de Andrade M, Amos CI. Ascertainment issues in variance components models. *Genet Epidemiol*. 2000;19:333–344.
  69. Gu C, Todorov A, Rao DC. Combining extremely concordant sibpairs with extremely discordant sibpairs provides a cost effective way to linkage analysis of quantitative trait loci. *Genet Epidemiol*. 1996;13:513–533.
  70. Iyengar S, Calafell F, Kidd KK. Detection of major genes underlying several quantitative traits associated with a common disease using different ascertainment schemes. *Genet Epidemiol*. 1997;14:809–814.
  71. Risch N, Zhang H. Extreme discordant sib pairs for mapping quantitative trait loci in humans. *Science*. 1995;268:1584–1589.
  72. Risch NJ, Zhang H. Mapping quantitative trait loci with extreme discordant sib pairs: sampling considerations. *Am J Hum Genet*. 1996;58:836–843.
  73. Sham PC, Zhao JH, Cherny SS, et al. Variance-components QTL linkage analysis of selected and non-normal samples: conditioning on trait values. *Genet Epidemiol*. 2000;19(suppl 1):S22–S28.
  74. Todorov AA, Province MA, Borecki IB, et al. Trade-off between sibship size and sampling scheme for detecting quantitative trait loci. *Hum Hered*. 1997;47:1–5.
  75. Ziegler A. Sampling strategies for model free linkage analyses of quantitative traits: implications for sib pair studies of reading and spelling disabilities to minimize the total study cost. *Eur Child Adolesc Psychiatry*. 1999;8(suppl 3):35–39.
  76. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics*. 2001;155:945–959.
  77. Schork NJ, Fallin D, Tiwari HK, Schork MA. Pharmacogenetics. In: Balding DJ, et al, eds. *Handbook of Statistical Genetics*. New York, NY: John Wiley & Sons; 2001b:741–764.
  78. Schork NJ, Fallin D, Thiel B, et al. The future of genetic case/control studies. In: Rao, DC, Province, MA, eds. *Advances In Human Genetics*. New York, NY: Academic Press; 2001: 191–212.
  79. Khoury MJ, Beaty TH, Cohen BH. *Fundamentals of Genetic Epidemiology*. New York, NY: Oxford University Press; 1993.
  80. Blangero J, Williams JT, Almasy L. Quantitative trait locus mapping using human pedigrees. *Hum Biol*. 2000;72:35–62.
  81. Blangero J, Williams JT, Almasy L. Variance component methods for detecting complex trait loci. *Adv Genet*. 2001; 42:151–181.
  82. Mitchell BD, Ghosh S, Schneider JL, et al. Power of variance component linkage analysis to detect epistasis. *Genet Epidemiol*. 1997;14:1017–1022.
  83. Cloninger CR, Van Eerdewegh P, Goate A, et al. Anxiety proneness linked to epistatic loci in genome scan of human personality traits. *Am J Med Genet*. 1998;81:313–317.
  84. Schork NJ. Genome partitioning and whole-genome analysis. In: Rao DC, Province MA, eds. *Advances in Genetics*. New York, NY: Academic Press; 2001a:299–322.
  85. Greenland S. Basic methods for sensitivity analysis of biases. *Int J Epidemiol*. 1996;25:1107–1116.
  86. Greenland S. Useful methods for sensitivity analysis of observational studies. *Biometrics*. 1999;55:990–991.
  87. Rotnitzky A, Scharfstein D, Su TL, et al. Methods for conducting sensitivity analysis of trials with potentially non-ignorable competing causes of censoring. *Biometrics*. 2001;57: 103–113.
  88. Adler LE, Cawthra EM, Donovan KA, et al. Improved p50 auditory gating with ondansetron in medicated schizophrenia patients. *Am J Psychiatry*. 2005;162:386–388.
  89. Light GA, Geyer MA, Clementz BA, et al. Normal P50 suppression in schizophrenia patients treated with atypical antipsychotic medications. *Am J Psychiatry*. 2000;157:767–771.
  90. Nagamoto HT, Adler LE, Hea RA, et al. Gating of auditory P50 in schizophrenics: unique effects of clozapine. *Biol Psychiatry*. 1996;40:181–188.
  91. Nagamoto HT, Adler LE, McRae KA, et al. Auditory P50 in schizophrenics on clozapine: improved gating parallels clinical improvement and changes in plasma 3-methoxy-4-hydroxyphenylglycol. *Neuropsychobiol*. 1999;39:10–17.
  92. Kumari V, Sharma T. Effects of typical and atypical antipsychotics on prepulse inhibition in schizophrenia: a critical evaluation of current evidence and directions for future research. *Psychopharmacology (Berl)*. 2002b;162:97–101.
  93. Kelsoe JR, Spence MA, Loetscher E, et al. A genome survey indicates a possible susceptibility locus for bipolar disorder on chromosome 22. *Proc Natl Acad Sci U S A*. 2001;98: 585–590.
  94. Swerdlow NR, Koob GF. Dopamine, schizophrenia, mania, and depression: toward a unified hypothesis of corticostriato-pallido-thalamic function. *Behav Brain Sci*. 1987;10: 197–245.
  95. Carlson GA, Goodwin FK. The stages of mania. A longitudinal analysis of the manic episode. *Arch Gen Psychiatry*. 1973;28:221–228.