Neurogenetics of Psychiatric Disorders

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Preface

Neurogenetics forms the bridge between biology and neuroscience on one hand and genetics on the other. Clinical applications of neurogenetics are evolving, and it is anticipated that within a few years they will expand well beyond the current genetic testing for the few simple "one gene" forms of illness (such as is the case for Huntington's disease and some forms of Alzheimer's disease).

There is a growing expectation that clinicians will soon be able to exploit and utilize pharmacogenetics in the care of their patients. It is further anticipated that knowledge of genetic subtypes of neuropsychiatric disorders will result in a rethinking of the disease concept as it is currently known in the Diagnostic and Statistical Manual of Mental Disorders IV structure of American (and worldwide) psychiatry. Finally, the National Institutes of Health has made restructuring of the clinical research engine a priority, and it has a stated focus on translating the research from bench to bedside.

The focus of this publication is the translation of bench-based research to the clinical bedside, with the aim of ushering in the enthusiasm and practicality of these results at the clinical level. For the most part, textbooks on psychiatric disorders lack updated information on the biology of disease genes. We believe that these disease genes will become the essential platform upon which neuroscience will build a productive application of science in the clinical setting. This book offers the most current overview of how basic neuroscience contributes to the clinical practice of psychiatry.

The book begins with an introduction to the current state of genetics in major mental illnesses, including an overview of the relevant history and findings of genetics from the past century. We use Alzheimer's disease as the prototype for the pathways heading to integration of genetics, biology, and clinical studies in the cognitive and behavioral realm and illustrate the points elaborated above. We follow with a discussion of major mental illnesses, such as schizophrenia, as well as the rare single-gene neuropsychiatric diseases. What we have learned from

these disease states will serve as an example for how we can approach the more common psychiatric disorders in both the adult and child.

Next, we focus on the disease models involving candidate genes in model organisms. This section is highly significant since such models will ultimately bridge basic science or genes to their clinical applications. It is the translation of such knowledge that has been absent for the clinician. We begin this section with chapters that organize candidate gene products classified by their functions. In particular, we discuss the gene products either as neurotransmission or neurodevelopment associated in the pathophysiology of schizophrenia. Following these chapters, we provide an overview of genetics-based models, including rodents and lower vertebrates. For the final chapter, we develop and present a unified model that includes concepts of genetic and environmental cross-talks for the disorders. We intend to show that this research will soon become the basis for treatment of these disorders. New drugs will no longer be "me too" drugs developed on the basis of similarities to existing medications, but will be the result of specific inquiry of the biological and genetic mechanisms discovered in the wake of the genome project.

It is imperative for the clinician to appreciate how a gene is causally associated with disease. At the same time, we must pay close attention to the ethics and implications of such work, and a chapter summarizing the current discussions in the field is included.

Finally, we conclude with what is on the horizon for translational research. It is our hope that this publication will serve as a reference source for clinicians, researchers, and other health care professionals seeking answers to the many questions related to the understanding of the neurobiology and the relevant treatment implications of neurogenetics of psychiatric disorders. It is our hope that the clinician, upon reading this book, will be able to "translate" the research and its applicability to their patients.

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Alzheimer's Disease: A Complex Paradigm

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INTRODUCTION

In 1901 a 51-year-old woman suffering from progressive mental deterioration came under the care of Dr. Alois Alzheimer while he worked as an attending physician in Frankfurt. A few years later in 1906 Alois Alzheimer gave a remarkable lecture on his patient at the 37th Meeting of the Southwest German Psychiatrists, in which he described for the first time a form of dementia that, subsequently at the suggestion of Emil Kraepelin, became known as Alzheimer's disease (AD). After the patient's death and upon microscopic examination of her cerebral cortex Alois Alzheimer found tangled bundles of fibers, which he termed neurofibrillary tangles, and abnormal accumulations of material around the nerves, which he termed senile plaques [for an English translation of the 1907 paper see Alzheimer et al. 1995 (1)]. These findings today still represent the hallmark of AD pathology and are required for the postmortem definite diagnosis of the disease. Unlike the original patient, most individuals affected by AD show initial symptoms at a later age. In the beginning of the twentieth century when AD was first described most people died before the age of 60 and the disorder was an unusual and interesting oddity. Today, with the increase in life expectancy and the dramatic increase of the population of elderly especially in the Western countries, AD represents one of the major health problems of our times. It currently has a prevalence that varies from 5% to 50% for different age groups of people older than 65 years (2) and it affects women 1.5 times more often than men (3). In the

year 2000 there were an estimated 4.5 million AD cases in the United States and there will be an expected 13.2 million by 2050 (2). With an average onset in the mid-70s and an average of eight years of gradual deterioration leading to death, AD has a tremendous impact not only on the families of the patients but also on the society and the health care systems. This makes the elucidation of the causes of the disease extremely important and pressing.

Clinically AD is diagnosed by the progressive memory loss with increasing inability to participate in daily activities. As the disease progresses, the patients fail to recognize the members of their family and often forget their own identity, eventually losing contact with the world around them. The National Institute of Neurological Disorders and Stroke (NINDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) have defined widely accepted criteria for the diagnosis of AD. Based on these criteria living patients are diagnosed as possible or probable AD, although a definitive diagnosis is only made pathologically by the brain autopsy findings. Macroscopic autopsy findings in the AD brain include gross cerebral atrophy, mainly in the frontal, temporal, and parietal regions, and ex vacuo ventricular dilation. Microscopically, an increased number of neuritic plaques in the cerebral cortex is pathognomonic. The core of the neuritic plaques is composed of amyloid consisting primarily of a small peptide known as amyloid beta (Aβ), whereas reactive astrocytes and microglia may appear at the periphery. Aβ is derived from the larger amyloid precursor protein (APP) through two steps of proteolytic cleavage involving the enzymatic activities of beta and gamma secretase. Based on the age of onset (AOO) of the symptoms AD is divided into early onset (EOAD) and late onset form (LOAD), with the 65th year of life established as the threshold and adapted by the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 4th edition, published by the American Psychiatric Association). In the past it was only the early onset disease that was referred to as AD, whereas later onset cases were described as senile dementia and often viewed as a normal consequence of aging. The similarity of the phenotypes of the early and late onset and the lack of grounds to consider senile dementia a part of normal aging is now clear and the diagnosis of AD is also used for the elderly. The majority (95%) of AD cases are late onset and advanced age is by far the most important risk factor for developing the disease followed by family history. Of the early onset cases approximately half are inherited as an autosomal dominant trait, usually having an onset in the 40s or 50s. For this reason the early onset cases are also often referred to as familial AD. This does not imply that LOAD does not have an inherited component. On the contrary it has been shown that there is an increased risk for first-degree relatives (4,5) and an estimated heritability of more than 0.7 (6,7). Yet despite the strong genetic effect, there is no pattern suggesting Mendelian inheritance. Thus the main difference in genetic terms between EOAD and LOAD is that the former is often a Mendelian disorder, whereas the latter shows complex genetics.

The multiple modes of transmission together with a history of successful gene detection for both early and late onset disease make AD a paradigm of

complex neuropsychiatric disorders. AD was one of the first neuropsychiatric disorders to be studied intensively through linkage and association studies. Compared to many others it has a number of advantages for genetic research: (*i*) It has a variant that is inherited in a Mendelian fashion (*ii*) it has clear pathological findings that allow accurate (yet postmortem) diagnosis and (*iii*) the autopsy findings point to specific proteins providing a lead for research. Yet, like all other complex disorders, there are also a number of factors making the genetic studies on AD difficult: (*i*) Despite its high heritability, its complex inheritance pattern suggests the presence of multiple genes, each possibly with a moderate or small effect and therefore hard to detect with linkage or association studies. (*ii*) Although well-recognized clinical signs and symptoms have been developed into various diagnostic criteria (NINDS-ADRDA criteria and DSM-IV criteria) and allow accurate diagnosis in a living patient, as in other neuropsychiatric disorders the diagnosis is still not as certain as for such diseases as diabetes, asthma, or hypertension. This is of great importance as even few diagnostic errors can have strong negative effects on linkage results. (*iii*) Another problem very pronounced in AD but also present in other complex disorders is the late AOO. This often leads to lack of parental DNA in the pedigrees reducing them to pairs of siblings and greatly reducing the amount of genetic information that can be extracted. At the same time the phenotype of unaffected siblings is not used for analysis as it is not reliable, which leads to further reduction in the information and the power of the studies to detect linkage.

GENETICS OF EARLY ONSET ALZHEIMER'S DISEASE: FINDING GENES

The existence of an autosomal dominant variant of AD quickly led to the discovery of genes by positional cloning despite the genetic heterogeneity observed among EOAD families. Three genes have been identified that are responsible for the vast majority of autosomal dominant AD: *APP* mutations are present in about 7%, whereas *PSEN1* (presenilin 1) mutations account for the majority of familial EOAD cases with *PSEN2* mutations also having a small share (8).

The Amyloid Precursor Protein Gene

As early as 1969, a connection had been made between AD and chromosome 21 through the observation that trisomy 21 patients develop Alzheimer's-like dementia and pathology (9). It was later shown based on the amino acid sequence of the amyloid peptide that the deposits found in the brain of trisomy 21 and Alzheimer's patients share the same main component (10). The *APP* gene coding for the precursor protein from which the amyloid peptide is cleaved off, was also soon found to be located on chromosome 21 (11,12). This generated a great interest in the possibility that the *APP* gene is involved in the disease. Linkage studies were and still are the golden standard for disease gene identification; however, genotyping multiple polymorphic markers was very cumbersome and

expensive at that time. Having prior evidence for a specific chromosome and even a specific gene was a very important advantage for those interested in the genetics of AD. The initial studies of scattered markers were negative (13) but soon positive results for linkage were reported (14). This was followed by more linkage studies with conflicting results (15,16) due to the underlying genetic heterogeneity and much confusion regarding the role of *APP* in AD. Within a few years, though, in 1991, a missense mutation was discovered in the *APP* gene segregating with the disease. Using a single family that was showing linkage at the *APP* locus, Goate et al. (17) sequenced the *APP* gene and found a mutation causing a substitution of a conserved Val to Ile and cosegregating with the disease. One hundred controls did not show the same variant. After screening more AD families, they detected a second pedigree segregating this mutation, and using another nearby polymorphism they showed that the two families were not related. The same mutation was later found by Naruse et al. (18) in two separate Japanese cases of familial EOAD and in a third Japanese family by Yoshioka et al. (19). Two other mutations changing the same amino acid were also found later by Murrell et al. (20) (Val717Phe) and Chartier-Harlin et al. (21) (Val717Gly). Despite these positive findings, however, many studies were failing to detect *APP* mutations, which led Tanzi et al. (22) to conclude that *APP* gene mutations account for a very small portion of familial AD. The remaining genes were found not long after that. The Human Gene Mutation Database (22a) today reports 16 nucleotide substitutions in *APP* associated with AD, all located toward the carboxy terminus of the amyloid precursor protein.

Although *APP* mutations account for less than 10% of EOAD families and 0.5% of all AD cases, the discovery of this first AD gene was a breakthrough. *APP* and beta amyloid became the focus of AD research leading to major discoveries toward untangling the pathogenesis of AD. Today the "amyloid hypothesis" that suggests beta-amyloid to be the main pathogenic factor in AD, remains in the forefront of the field and treatments based on this hypothesis are currently being tested. Very recently a new mechanism of *APP* involvement in AD was described. Rovelet-Lecrux et al. (23) reported duplication of the APP locus on chromosome 21 in five families with EOAD and cerebral amyloid angiopathy. Out of 12 EOAD cases negative for PSEN1 PSEN2 and APP mutations, five showed APP duplications, suggesting that this mechanism might explain a significant number of cases.

The Presenilins

Despite the small share of *APP* mutations in the LOAD pathogenesis, it was the first gene to be discovered mainly because of its connection to the amyloid plaques and to Down syndrome. At the same time, however, the types of polymorphic markers used, the genotyping methods, and the analytical tools were rapidly allowing for higher throughput and for linkage to be more intensively pursued. In 1992 a number of reports of linkage for EOAD to a locus on chromosome 14 termed AD3 appeared in the literature (24–28). Three years later,

Sherrington et al. (29) identified the second EOAD gene, the one responsible for the linkage, by positional cloning. Using six large pedigrees, each having an logarithm of the odds (LOD) score to the region exceeding 3.0, and defining the segregating haplotypes and recombination in the linked region relative to a contig of yeast artificial chromosomes that they constructed, they were able to narrow down to a minimal region of interest, which they then used for selection of transcripts. They screened the putative cDNAs for conservation and evidence of splicing, identified full-length clones, and aligned them to a partial transcription map of the region. For 19 putative transcripts, they went on to amplify and sequence cDNA from patients and controls. Sixteen of these 19 transcripts were not from known genes and one of those, which they named S182, contained a series of nucleotide changes altering the amino acid sequence and observed only in patients. This gene was subsequently called *PSEN1*. The function of the *PSEN1* product was unknown and it immediately became the subject of intense investigation. Recent work indicates that presenilin together with nicastrin, Aph-1, and Pen-2 are responsible for γ-secretase, the enzymatic activity that cleaves Aβ off of the precursor, and that all four proteins are necessary for full proteolytic activity (30).

A third gene for familial EOAD was discovered soon after *PSEN1*. Levy-Lahad et al. (31) studied a family of Volga German descent affected with AD with an earlier AOO and longer disease duration than the *PSEN1* families. After excluding linkage to the *APP* and *PSEN1* they performed expressed sequence tag (EST) database searches and identified a sequence that showed homology to the *PSEN1* gene. The deduced protein product of this gene on chromosome 1 shared 80.5% sequence identity with *PSEN1* and carried mutations on conserved residues in affected members in their family, which showed linkage to the same locus. Mutations in *PSEN2* were also found in all affected members of an Italian pedigree (32). It is interesting that the Volga German pedigree included two affected individuals who lacked the identified mutation and had a later AOO. This type of intrafamilial heterogeneity is likely behind the difficulties we are still facing in linkage mapping of complex disorders including the later onset cases of AD.

Beta-amyloid, the peptide found in the senile plaques in the Alzheimer's patient's brain is a 40 to 42 amino acid fragment of the *APP* gene, resulting from its normal proteolytic processing. The amyloid precursor undergoes proteolytic cleavage as shown in Figure 1. There are two processing pathways depending on the first proteolytic step. If the first cleavage is performed by α-secretase (Fig. 1A), an activity attributed to metalloproteases *ADAM9*, *ADAM10*, and *ADAM17* (33), it precludes the formation of the amyloidogenic Aβ peptide as it is performed within the 40 to 42 amino acids of Aβ. If the first cleavage is performed by β-secretase (Fig. 1B), an activity attributed to the product of the *BACE* gene on chromosome 11, then the second cleavage, common in both pathways, produces Aβ. The normal functions of the resulting peptides from the two pathways are not entirely clear and they are still under investigation. The cleavage site of γ-secretase shows some variation, which results in variable size of Aβ fragments, mostly 40 and 42 amino acids

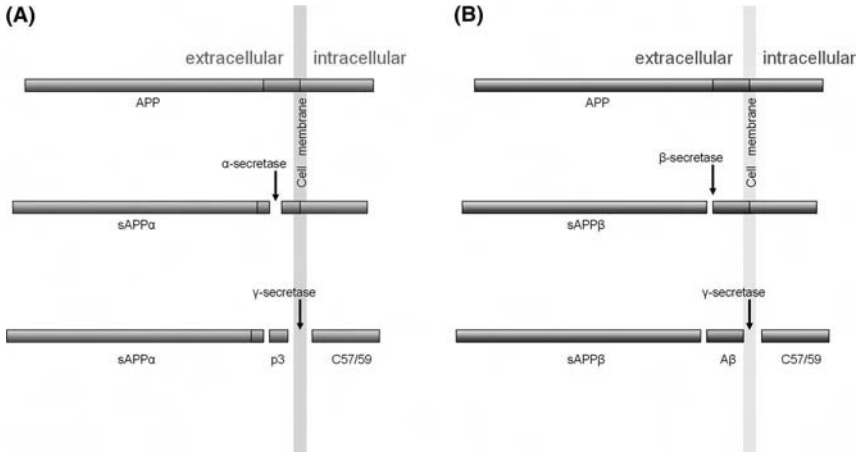

Figure 1 Proteolytic processing of the amyloid precursor protein. (**A**) Cleavage by α-secretase prevents the formation of Aβ. (**B**) Cleavage by β-secretase followed by γ-secretase leads to the amyloidogenic peptide Aβ.

long (Aβ-40 and Aβ-42). The longer peptide aggregates more readily and forms plaques. It has been shown that mutations in *APP* as well as in *PSEN1* relate to the relative abundance of the Aβ42 peptide, thus tipping the balance toward the variant with more amyloidogenic potential, and it is argued that this imbalance leads to the formation of plaques, neuronal death, and progression to dementia. A lot of questions still remain regarding the function of the presenilins and the cleaved *APP* in the healthy brain. It is clear that there is still a lot to learn about the disease, as none of the discovered genes appear to have a strong effect in the late onset form of the disease despite the common neuropathological findings of the two variants. Nevertheless, γ-secretase is now a major candidate target for pharmacological intervention. The targets of this enzymatic activity, unique for its property to cleave inside the cell membrane, extend beyond APP to include the NOTCH receptor protein. The widespread importance of NOTCH signaling creates a potential problem for attempting to inhibit γ-secretase activity as a treatment for AD. Further knowledge on the details of the complex of proteins forming γ-secretase and other possible treatment targets might provide solutions for this problem.

The discovery of *APP* and the presenilins have provided grounds for further research and better understanding of AD. Thanks to the discovery of these genes and through the manipulation of the corresponding genes in the mouse, there are now a number of successful AD mouse models that express mutations in *APP*, *PSEN1*, and *PSEN2*, as well as animals expressing more than one of these mutations (34). Such animals show a phenotype strongly resembling AD with age-related accumulations of Aβ-containing neuritic plaques in the hippocampus and cerebral cortex, activation of astrocytes and microglial cells in regions containing plaques, and degeneration of cholinergic nerve terminals. These animal models

are very useful for developing and testing treatments that target different candidate pathogenic mechanisms such as glial activation or Aβ levels.

GENETICS OF LATE ONSET ALZHEIMER'S DISEASE: THE COMPLEX DISEASE CHALLENGE

Other than the AOO no other differences in the clinical presentation between LOAD and EOAD have been described. From the genetic standpoint one major difference is the mode of inheritance which in LOAD is not compatible with Mendelian but rather with complex inheritance most likely including multiple genes and contributions from environmental exposures. Twin studies have shown monozygotic twins to have a concordance rate of 59% compared with around 30% for dizygotic twins (35). The estimated cumulative risk to first-degree relatives of AD-affected probands approaches 50% by the age of 90 years compared to 10% to 15% in the general population (5). Heritability has been estimated at around 0.7 (7). In that respect LOAD is very much similar to other major neuropsychiatric disorders including schizophrenia and bipolar affective disorder and, as we will describe, the search for genes has provided results that very much resemble those disorders with one major exception; a single gene, *APOE*, has been identified whose involvement in LOAD has been very well established with numerous and consistent replications of the initially observed association. This has been very encouraging for the field of neuropsychiatric genetics and it has provided insight on what we are to expect from at least some complex disorder genes. Nevertheless only half of the LOAD patients carry the high-risk *APOE* allele ε4 and it has been argued that it is more likely an AOO modifier rather than a disease risk gene (36). It has been estimated that the proportion of patients with dementia attributable to the ε4 allele is only 20% and that *APOE* genotypes explain only 10% of the variance in age at the onset (37), with another estimated four loci with similar or larger effect (38). It appears therefore that the search for genes causing LOAD is far from over.

Linkage studies of multiple pedigrees that included late onset familial cases started as early as 1986 using phenotypic gene markers (39). The first linkage study using DNA markers and multiple families that included late onset cases appeared in 1988 and it was focused on chromosome 21 (40), as this chromosome was implicated by the AD pathology of trisomy 21 patients. Conflicting linkage results for both EOAD and LOAD was suggesting genetic heterogeneity (22,41) encouraging researchers to look beyond chromosome 21. As early as 1989 Dawson et al. (42) suggested nonparametric sib-pair linkage analysis for LOAD. In 1991, just a few months after the first APP mutations were identified, Pericak-Vance et al. (43) using a nonparametric method called the affected-pedigree-member method (44) reported a strong positive finding on chromosome 19 for later onset families, observing that any signal that was present on chromosome 21 (possibly from the soon-to-be discovered *APP* gene) came from early onset families. It only took two years for the gene responsible for this linkage signal to be identified. In 1993 Strittmater et al. (45) performing experiments to identify serine-protease inhibitors found the ApoE

protein to be a contaminant that remains tightly bound to Aβ. ApoE is a plasma protein that is involved in cholesterol transport and is secreted in the central nervous system by astrocytes at high levels. It was known that ApoE increases after nerve injury and in neurodegenerative disease such as AD. As the *APOE* gene is located in the chromosome 19 region of linkage and the authors were detecting strong interactions with Aβ and the senile plaques they considered the gene a prime candidate for AD. The ApoE protein shows three major allelic variants termed ε2, ε3, and ε4 resulting from two single nucleotide polymorphisms (SNPs) that are located in exon four of the gene and are in complete linkage disequilibrium (LD) with each other (Fig. 2). These T to C transitions in codons 112 and 158 change the two ancestral arginins to cysteins, with the change at position 112 creating the ε3 allele and a subsequent change at 158 creating the ε2 allele. Genotyping only 30 independent cases and 91 controls the authors were able to detect a significant association between AD and *APOE* genotype in what later proved to be one of the most consistently replicated association findings for a complex disorder. Confirmatory studies were very quick to follow (46,47). *APOE* has since been under intensive research. In addition to AD, it is known to be involved in abnormalities of blood lipids and in cardiovascular disease (48,49), although involvement in other neurodegenerative disorders, multiple sclerosis, and the response to acute brain injury have also been discussed (50). The specific mechanism of *APOE* involvement in AD remains poorly understood with the most favored hypotheses suggesting an involvement in the clearance of Aβ from the brain (51).

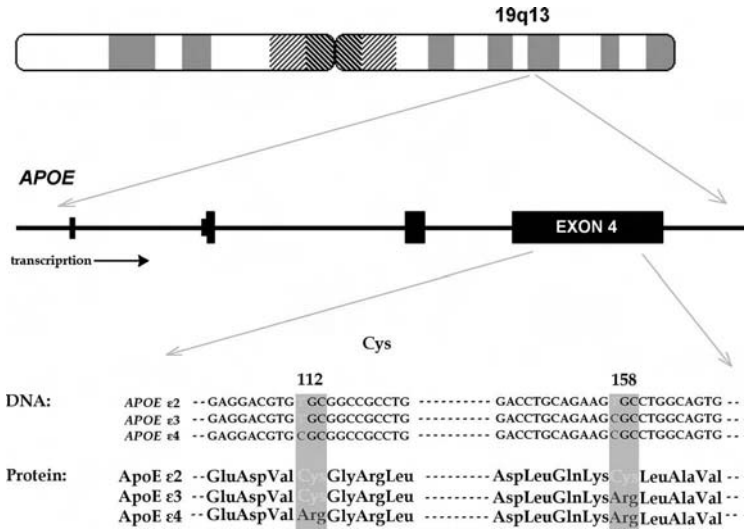

Figure 2 The APOE gene is located on chromosome 19q13 and is comprised of four exons. Two single nucleotide variants (*shaded*) in codons 112 and 158 lead to amino acid changes and the three different APOE alleles as shown.

The success in identifying a gene for LOAD, the form of AD that shows complex inheritance, was very encouraging to the field of neuropsychiatric genetics where other disorders also under study had not yet provided positive results. At the same time, during the 1990s, there were spectacular changes in the technologies available for linkage and association analyses as well as in the availability of patient and family samples. In part due to advances stemming from the human genome project, typing of DNA markers became a lot cheaper and more automated making it possible and affordable for individual laboratories to type hundreds of polymorphic markers across the genome. Computational advances made the analysis of such quantities of data possible even on personal computers. The development of new analytic methods allowed for speed and efficiency and for the best use of the available genetic information. Very accurate genetic maps became available allowing researchers to benefit from their superior accuracy and facilitating the comparison of results between studies. More and more publications of linkage scans for complex disorders began to appear in the literature, and LOAD was one of those. For most neuropsychiatric disorders, however, including LOAD, the results of this tremendous mapping effort did not meet the expectation that was set—possibly erroneously—by the quick results that AD and Mendelian disorders' research produced in the beginning of the decade.

Genome-Wide Linkage Scans for Late Onset Alzheimer's Disease

In 1998 Pericak-Vance et al. (52) reported on a complete genome scan of 280 microsatellite markers. They initially used a subset of their pedigree collection, 16 late onset pedigrees including 135 individuals of which 52 were affected, ascertained from clinical populations in the United States, reserving a subset of 38 smaller families for follow-up of positive findings. They set their follow-up criteria to a nonparametric p-value of less than 0.05 or a parametric LOD score of at least 1. Two-point parametric analysis using a dominant disease model and non-parametric analyses initially identified 15 regions of interest. The follow-up analyses including additional markers pointed to four regions that remained significant on chromosomes 4, 6, 12, and 20. Their best finding was on chromosome 12 with a multipoint LOD score (MLS) of 3.2. The prior knowledge of involvement of *APOE* in LOAD led them to stratify the families and analyzing those that included patients not carrying *APOE* they found that the LOD score on chromosome 12 increased to 3.7. They concluded that there is a LOAD locus on chromosome 12 and that chromosomes 4, 6, and 20 need additional follow-up. The finding on chromosome 12 was further followed up by Rogaeva et al. (53) on an independent sample of 53 families recruited from clinical populations in North and South America and Europe. Although the overall sample did not confirm the linkage they found significant evidence using an admixture test, estimating that about half their pedigrees were linked to this locus. In a follow-up paper on the original sample in 2000 Scott et al. (54) reported that data from additional genotyping on chromosome 12 confirmed the effect of *APOE* and suggested that this locus might be more specific to dementia

with Lewy bodies, a neuropathological hallmark of Parkinson's disease also found in 15% to 20% of autopsied individual with probable AD. Interestingly, in 2002 Funayama and colleagues (55) reported linkage of a large family with autosomal dominant parkinsonism to the same region of chromosome 12 suggesting the presence of a gene associated with both disorders. The chromosome 12 linkage finding was also tested by Mayeux et al. (56) in a study of Caribbean Hispanic pedigrees with familial AD extends that provided modest evidence in support of linkage. In a recent paper using an extended set of 585 multiplex families Liang et al. (57) performed dense genotyping on chromosome 12 using one marker every 5 cM and performed analyses taking into account linkage signals from other chromosomes. Although the entire data set did not detect linkage on chromosome 12 they reported significant results when accounting for linkage scores on chromosomes 9 and 10 as covariates.

In 1989 the National Institute of Mental Health (NIMH) launched the Human Genetics Initiative with the goal to establish a national scientific resource by funding collections of pedigrees containing multiple affected individuals, the generation of immortalized cell lines for indefinite availability of DNA and extensive collection of clinical information, all of which were to be available to qualified researchers for genetic studies. The initial focus of this initiative was on schizophrenia, bipolar disorder, and AD. Some of the pedigrees collected from this initiative were used in the more recent of the studies described earlier following up the chromosome 12 linkage finding. In 1999 Kehoe et al. (58) used 292 pedigrees from the ongoing NIMH collection to perform a whole genome scan at a density of 16.3 cM. They applied nonparametric analysis and in addition to analyzing the whole sample they stratified by *APOE* genotype. They found suggestive scores on chromosomes 1q, 5, 9, 10q, and 19q13. Stratified by *APOE* genotype, the 162 ε4 positive pairs showed linkage to chromosomes 1q, 2, 5, 6, 9q, 10q, 13, and 14, whereas the 63 ε4 negative pairs showed linkage to 1p, 9q, 10q, 12, 21, and X. The highest score observed was an MLS = 2.67 on chromosome 1. This scan was followed by a second-stage analysis by Meyers et al. (59,60) where the sample was extended to 451 sibling pairs and 91 additional markers were typed in regions of previous positive findings. The most significant finding was an increase of the MLS on chromosome 10 to 3.9 and a two-point LOD score reaching 4.1.

In 2000 Pericak-Vance et al. (61) performed a genome scan at a density of one marker every 10 cM, supplementing their sample with newly collected and publicly available samples to reach a total of 466 families with AOO $>$ 60 years including 286 families from the NIMH. Their best finding was in a region on chromosome 9q, followed by a region on chromosome 19, possibly representing the *APOE* locus, and a region on chromosome 7. Analysis of a subset of 199 families, each having at least one autopsy-confirmed case identified two additional regions on chromosomes 9p and 18q and enhanced many of the original findings, most significantly the finding at 9p22.1 where the MLS rose to 4.31. Notably no linkage signal was observed on chromosome 12.

In 2003 the NIMH Genetics Initiative Alzheimer's Disease Study Group, which includes the investigators involved in the collection of the NIMH sample, published a genome scan for the complete set of 437 pedigrees of the NIMH genetics initiative (62). The pedigree set included 994 affected individuals none of whom was diagnosed before the age of 50 years. Genotyping was performed at an average density of one microsatellite marker every 9 cM and both parametric and nonparametric analyses were performed. The families were analyzed together but also stratified to later and earlier onset based on the presence in the families of individuals with onset before the 65th year of age. The strongest linkage signal was observed on chromosome 19q13 in the earlier onset families with an LOD = 5.9, possibly indicating the *APOE* locus and its effect on the AOO. Another 12 regions showed suggestive linkage: 1q23, 3p26, 4q32, 5p14, 6p21, 6q27, 9q22, 10q24, 11q25, 14q22, 15q26, and 21q22. The authors noted the lack of linkage on chromosome 12 as well as the overall low level of significance observed on chromosomes other than 19. They attributed the low significance to the genetic complexity of AD but argued that at least some of the positive findings are expected to be real disease loci, especially those located at previously reported genomic regions including chromosome 10q and 9q.

Using a set of 86 multiplex pedigrees from a variety of sources not including the NIMH, in 2004 Wijsman et al. (63), genotyped microsatellite markers at a density of 10 cM to examine previous linkage reports on five chromosomes (9, 10, 12, 19, and 21). They applied an alternative analytical method based on a Bayesian Markov Chain Monte Carlo Method in order to allow multipoint linkage under oligogenic trait models. Their best finding was on the short arm of chromosome 19, a location distinct from the *APOE* locus. Regarding the previous findings they only found supportive evidence for the linked region on chromosome 10.

Using Other Phenotypes and Covariates in Late Onset Alzheimer's Disease Linkage Analysis

The relatively low significance of findings in linkage studies for LOAD has led many investigators to attempt to decrease the heterogeneity and thus improve the results by taking into account additional information or considering other related phenotypes. One example is a study by Ertekin-Taner et al. (64) where the authors used plasma Aβ42 as a surrogate trait and performed linkage analysis on five extended AD pedigrees selected through a proband with extremely high plasma Aβ levels. They reported linkage to chromosome 10 with a maximal LOD score of 3.93 at the same locus where more traditional analyses were also detecting linkage. This paper accompanied two other reports of linkage on the same chromosome (59,65) in the same issue of the journal Science making a strong case for an LOAD gene on chromosome 10. A major advantage of the approach used by Ertekin-Taner et al. was the use of an objectively measurable quantitative phenotype. This might account for the strong signal that was observed despite the relatively small number of families.

One prominent feature of AD is the frequent comorbidity of psychotic symptoms which include delusions and hallucinations [for a recent review, see (66)]. They occur in about half of AD patients and they are associated with more severe cognitive deficits, a more rapidly deteriorating course (67), exaggerated frontal lobe dysfunction (67,68), and neurochemical changes including reduced cortical and subcortical serotonin/5-hydroxyindoleacetic acid (69). Delusions and visual hallucinations appear to be associated with extrapyramidal signs (70) suggesting a subcortical mechanism in the etiopathology of psychosis in AD. It has been therefore suggested that the combination of AD with psychotic symptoms might delineate a distinct phenotype with reduced genetic heterozygosity (71). If this is true, the study of this phenotype would facilitate linkage mapping. Based on this hypothesis, Bacanu et al. (72), using data from the NIMH genetics initiative pedigrees, performed a genome scan using the combined phenotype (AD+psychosis) and including the *APOE* genotype in the analysis. This analysis identified one moderately strong signal on chromosome 2 for the *APOE* ε4 carriers and other signals on chromosomes 6 and 21. Our group, testing the same hypothesis using a different approach, reported on a genome scan of a subset of the NIMH pedigrees where the presence or absence of delusions or hallucinations was used as a covariate in the linkage analysis in an analytical method implementing a conditional logistic model proposed by Olson et al. (73). Our most significant finding showed a negative correlation with psychotic symptoms and it was located on chromosome 14 in the vicinity of the *PSEN1* gene. This finding was significant at the genome-wide level as determined by simulations and it was most pronounced among families that contained patients with AOO between 50 and 65. Positive findings of lower significance were also detected on chromosomes 1 and 3, whereas findings correlating positively with psychotic symptoms were on chromosomes 7 and 2, in agreement with the result of Bacanu et al.

Significant work has also been performed using the AOO and disease duration to reduce heterogeneity through methods more sophisticated than the stratification of pedigrees. Olson et al. (74) applied a conditional logistic model to re-examine the chromosome 21 data from the NIMH pedigrees. When age at last examination/death or the AOO plus disease duration were included in the linkage model LOD scores above five were observed. The authors concluded that the *APP* locus may also predispose to AD in the very elderly. In follow-up work (75) the authors reported on a similar pattern on a region on chromosome 20p using a model that included both current age and the number of ε2 alleles as covariates. Two-locus analysis provided evidence of strong epistasis between 20p and the *APP* region, limited to the oldest age group and to those lacking *APOE* ε4 alleles. The authors speculated that polymorphisms in both regions produce a biological interaction increasing susceptibility to a very late onset form of AD. Scott et al. (76) used a difference approach to the inclusion on AOO in the analysis, a method called ordered subset analysis (77). They ranked the pedigrees according to minimum AOO and performed linkage on subsets of increasing size until a change in score unlikely to be generated by chance was observed. Using

347 pedigrees from a variety of sources including the NIMH they found a statistically significant increase in the nonparametric multipoint LOD score on chromosome 2q34 in 31 families with a minimum age at onset between 50 and 60 years. The LOD score in the chromosome 9p region that they had previously reported (61) increased to 4.6 in 334 families with minimum age at onset between 60 and 75 years. No significant differences in LOD score were detected on chromosome 21, whereas a significant increase in LOD score was detected on chromosome 20 in the 61 families with mean age at onset more than 78 years. This result is 12 cM from the peak LOD score reported by Olson et al. (75) and it was driven by the NIMH pedigrees that were common in both studies. Recently Holmans et al (78) performed an affected sib-pair linkage analysis using AOO, rate of decline (ROD), and *APOE* genotype as covariates for linkage to LOAD. Their sample included 428 sibling pairs (277 from the NIMH). In agreement with Olson et al. when using mean AOO they observed linkage to chromosome 21 in the whole sample coming entirely from the NIMH sample, and appearing strongest in older pairs. A similar effect was observed on chromosome 2q, whereas suggestive evidence was observed for AOO difference to chromosome 19q in the vicinity of *APOE* and 12p for similar AOO. A significant effect of mean ROD was found on chromosome 9, which was more significant after allowing for *APOE* effects. Many other locations with positive findings were also reported.

Another approach to dissecting the disease into potentially more homogeneous groups is to examine the parental origin of the disease and analyze families with affected fathers or mothers separately, a strategy that has been applied in other neuropsychiatric disorders including bipolar affective disorder and schizophrenia (79–81). There are a number of different mechanisms that can produce a parent of origin effect observed through linkage including imprinted genes and interactions with maternally inherited mitochondrial sequences. Our group performed a parent of origin analysis for LOAD on a subset of the NIMH pedigrees (82). Despite the small number of families for which we had information on the parent of origin (49 maternal and 25 paternal) we detected significant increases in LOD score on chromosomes 10 and 12 for maternal families. The changes were not observed in paternal families suggesting a maternal effect rather than an increased genetic loading. The addition of more maternal pedigrees from the University of Alabama collection site further supported our findings (83), providing highly significant evidence for linkage (nonparametric LOD score 3.73) and for a parent of origin effect ($p=0.0016$) in a region of chromosome 10 that has been implicated by multiple linkage studies. Additional support was also provided by imaging studies (84) suggesting that the maternal families are a distinct subgroup.

In many of the reported linkage studies we described earlier there was significant sample overlap. The public availability of samples has allowed more researchers to get involved in studying AD, performing larger linkage and association studies, and often to applying different analytical approaches with very interesting results. Yet as researchers will usually supplement their own samples

with publicly available ones we end up with overlapping yet not identical collections of pedigrees analyzed in different studies. This makes comparing results and declaring replication very problematic. Nevertheless, because some genomic regions seem to consistently provide positive results for LOAD, such as, for example, on chromosomes 6, 9, 10, and 12, the linkage studies although not conclusive have provided some leads for follow-up research. It is not easy to decide on the best strategy to continue from here. Further linkage with increased sample sizes, genome-wide association studies, association studies focused on specific regions and/or genes accompanied by more sophisticated analytical methods or more intensive follow-up of the existing candidate genes are some of the options.

ASSOCIATION STUDIES

In 1997 Risch and Merikangas published a very influential paper on the future of genetic studies of complex human diseases (85) showing that the number of pedigrees required to detect linkage for genes with small or moderate effects on the disease risk is much larger than what was commonly used. They went on to show that a more powerful approach given the relatively small number of families is to look for association between allelic variants and the disease, as those would be more likely to give significant results. In addition to the increased power, another attractive characteristic of association studies is that because of the relatively short extent of LD they are likely to point to just one or a few genes, whereas linkage peaks for complex disorders even when they are of a significant magnitude are very wide including dozens of genes. Defining small segregating haplotypes as it was done for the positional cloning of *PSEN1* is usually not possible because of the intra and interfamilial genetic heterogeneity. This high resolution of association studies though comes with a price: for any given genomic interval hundreds or thousands of times more markers are needed for a comprehensive association study than a linkage study.

Three approaches have been commonly used for association studies: genotyping polymorphisms—most typically SNPs—in genes that are functional and/or positional candidates, genotyping across linked regions with or without a bias towards genes, and unbiased genome-wide screens for association. Either approach has advantages and limitations summarized by the following: for most diseases there are too many good candidate genes; almost no gene can be excluded as a candidate as we cannot claim to know all its functions; the more variants we genotype the more tests we perform necessitating higher correction of the required significance of a finding leading to reduced power. No genome-wide association screen for AD with sufficient and unbiased coverage of the genome has yet been published; neither are there published examples of densely screening entire linked regions, although both statements are likely not to be true by the time this book goes to press. There have been three attempts to detect association at the genome-wide level using microsatellites (86–88); yet based on our current

knowledge regarding LD in the genome (89) it is clear that the coverage of the genome they provided was extremely low. There have, however, been multiple studies on functional and/or positional candidate genes, one of the first being the one that detected *APOE*, which is also unfortunately the only one that has been consistently replicated. It is hard to choose results to present among the 100 or so genes have been reported to date. Instead we will briefly summarize some of the most studied genes that have been examined because of their localization on chromosomes with linkage evidence, focusing on chromosomes 6, 9, 10, and 12 and around linkage peaks. For a comprehensive list of association studies, the reader is referred to the Genetic Association Database (http://geneticassociationdb.nih.gov/) maintained by the National Institute for Health.

On chromosome 6, two genes close to linkage signals have received attention in association studies. (*i*) *TNFA*: Tumor necrosis factor-alpha was found to be associated with AD by Collins et al. (90) who detected significant association with a haplotype defined by three polymorphisms. One of the three polymorphisms was also examined by Alvarez et al. (91) showing an effect on AOO, whereas another study on an African American population detected an opposite effect (92). Typing a different polymorphism in 242 patients with sporadic AD and 235 normal controls, McCusker et al. (93) also detected an association and an interaction with *APOE*; two other studies, however, failed to detect such effects. Conversely, Ma et al. (94) did detect an association for *TNFA* using a different set of polymorphisms in a Chinese population. (*ii*) *HFE*: A class I-like major histocompatibility complex gene called *HFE* known to be associated with hereditary hemochromatosis was first implicated in AD by Moalem et al. (95) who based on 26 AD cases, 41 older and 50 younger controls suggested that among *APOE* ε4-negative individuals *HFE* mutations are predisposing to AD in males. In a subsequent study Sampietro et al. (96) suggested that the gene has an effect on the AOO of AD, a finding that was further supported by Combaros et al. (97) who detected an interaction with *APOE*, but was not supported by two other studies (98,99). Finally Robson et al. (100) suggested that there is synergy between variations in the *HFE* gene and the transferrin (*TF*) gene on chromosome 3.

On the chromosome 9-linked region we will briefly discuss three genes that have been implicated in AD. (*i*) *VLDLR:* The gene for the very low-density lipoprotein receptor (*VLDLR*) was examined in 1995 because of its functional relation with *APOE*. A Japanese population was genotyped for a trinucleotide repeat polymorphism and an association with the 5-repeat allele was reported (101). This study was followed by six studies reporting negative results mainly in Caucasian but also in Japanese and Chinese samples (102–107). These negative results were followed by another study supporting a strong association in a Caucasian population and noting allele frequency differences between Caucasians and Japanese (108). This was followed by more negative results (92,109) and a study showing the opposite effect for the 5-repeat allele but a higher risk for the 9-repeat allele in an Irish population (110). (*ii*) *ABCA1*: The *ABCA1* gene coding for a cholesterol transporter in central nervous system was

suggested to be involved in the secretion of Aβ (111), followed by a report of an association of a nonsynonymous coding SNP (R219K) with lower total cholesterol in cerebrospinal fluid and delayed disease onset (112). Another study also showed genetic variants of *ABCA1* to modify AD risk and Aβ metabolism (113); however, a negative result soon followed (114). Further studies on transgenic mouse models showed that lack of *ABCA1* increases Aβ deposition and decreases ApoE levels (115,116) keeping the interest in this gene alive. A more recent study of the R219K variant detected a strong association with AD that was gender specific (117). (*iii*) *UBQLN*: Recently Bertram et al. (118) reported an association between AD and a variant in the *UBQLN1* gene coding for ubiquilin, a protein involved in the degradation of the presenilins (119). This finding was partially supported by Slifer et al. who did not detect an association with the risk for AD but rather with the AOO of the disease (120). This has again been followed by negative reports (121–124), whereas Kamboh et al. (125) found a modest effect on risk, AOO and disease duration in a large case control study.

Chromosome 10 shows more than one reported linkage peak and a number of genes have been investigated showing positive—yet never unchallenged—association with AD. (*i*) *IDE*: The insulin-degrading enzyme had been functionally linked to Aβ degradation since 1994 (126) leading to a targeted linkage study by Bertram et al. (65) that showed linkage close to the corresponding gene *IDE* and association of the disease with a nearby microsatellite, and was quickly followed by another positive study (127). Soon after two studies reported negative results (128,129). A re-examination of this data however from Boussaha et al. suggested a possible effect modified by the *APOE* genotype (130) and further support for an association was provided by Prince et al. (131). Sakai et al. (132) examined seven SNPs and the microsatellite from the original study in a Japanese population of 240 cases and 163 controls but failed to replicate the associations. Later, further support for an association was provided by Blomqvist et al. (133) suggesting an effect on AOO for both AD and Parkinson's disease and by a study from Ertekin-Tanner et al. (113) who reported an association with plasma Aβ levels and risk for AD. (*ii*) *CTNNA3*: The gene for alpha-T catenin was investigated by Ertekin-Tanner et al. (134) following up on a linkage signal they had detected for Aβ levels on chromosome 10 (64), because its product is a binding partner for beta catenin a known interactor of presenilin. This has been followed by two negative findings (135,136) but functional studies have provided interesting potential links (136,137). (*iii*) *PLAU*: Plasmin promotes a cleavage of *APP* and also degrades secreted and aggregated Aβ blocking its neurotoxicity (138,139). The urokinase type plasminogen activator gene *PLAU* inhibits amyloid-beta neurotoxicity through plasmin (140). In 2003 Finckh et al. (141) reported an association of AD with a *PLAU* coding sequence variant, but Myers et al. (142) could not replicate the finding or find a correlation with the linkage on chromosome 10 and two more negative studies followed (143,144). Ertekin Taner et al. reported an association of *PLAU* with elevated Aβ and LOAD (145). (*iv*) *CHAT*: The gene for choline acetyl transferase is also located in a linkage

region on chromosome 10 and it includes in its first intron the vesicular acetylcholine transporter gene (*SLC18A3*). Given the strong links between cholinergic neurotransmission and AD these genes are strong candidates. Mubumbila et al. (146) first reported an association between AD and *CHAT* in 2002. Two following studies could not replicate the association (147,148) but a third also detected an effect (149). More recently three more studies have provided support for this gene (150–152). (*v*) *GSTO1* Based on gene expression differences Li et al. (153) performed an association test and reported that variation in the glutathione S-transferase omega-1 gene and the neighboring *GSTO2* gene modifies age at onset of AD and Parkinson disease. A subsequent study on a Japanese population did not detect an association; neither did a study on a German population which did however detect a possible effect on the AOO. This was followed by one more negative study by Ozturk et al. (154). (*vi*) *TNFRSF6* is a member of the tumor necrosis factor receptor superfamily encoding FAS, a cell surface receptor involved in programmed cell death (apoptosis) initiation. Feuk et al. (155) reported in 2000 an association between *TNFRSF6* and EOAD in *APOE* ε4 carriers. A negative report followed for LOAD (156) but in a subsequent publication Feuk et al. provided further evidence for the initial finding (157). That was followed by a report by Rosenmann et al. (158) who did not find the same association in their Jewish sample; however, that sample only included 19 EOAD cases, most likely a number too small to detect the effect.

Chromosome 12 contains one of the first reported strong linkage findings and despite the apparent nonreplication in larger scans it has attracted a lot of interest. We will only discuss five genes that have been subject to association studies on this chromosome. For a more detailed description of positive and negative findings for these and other genes we refer the reader to a recent review focused on this chromosome (159). (*i*) *A2M*: A long line of research shows an involvement of α2 macroglobulin in AD. Motivated by the functional evidence in 1998 Blacker et al. (160) reported a strong genetic association of a deletion near a splice site of the corresponding gene, *A2M*, with the risk for AD. This was followed by a report (161) for an association of a nonsynonymous variant (V1000I) with the disease. After these initial reports there have been a very large number of replication attempts. There are at least 27 studies reporting negative results and another 12 studies reporting positive findings although those are always weaker than the original study and on two occasions report an opposite effect (162,163). Overall there seem to be more replications in family-based studies than in case control studies. There is a very large volume of literature for this gene in relation to AD. Despite all the negative results it remains likely that this gene is genetically associated with AD; however, it does not provide the consistent evidence that would allow including it in the list of known AD genes such as *APOE*. (*ii*) *LRP1* The gene for the low-density lipoprotein (LDL) receptor-related protein coding for the human alpha 2-macroglobulin receptor was first studied for a genetic association with AD by Lendon et al. (164) who detected an association of the disease with a microsatellite marker. This was followed by a

study showing an opposite effect of the specified allele (165) and another showing an association with a synonymous SNP in exon 3 of the gene (166). Two subsequent studies were unable to replicate the association of the microsatellite (167,168) but the SNP finding was followed by four replications (169–172) More recently this has been followed by eight nonreplications and two studies supporting the finding, putting the *LPR1* gene in the same ambiguous position with the other candidates we discussed so far. (*iii*) *OLR1* The gene for oxidized LDL-receptor 1 was investigated and found associated to AD by Luedecking-Zimmer et al. in 2002 (173) as a positional and functional candidate. A replication study was published soon (174) followed by two nonreplications (175,176). One more replication followed (177) and a recent study of cholesterol-related genes found the gene to be included in a cluster that confers susceptibility to AD. (*iv*) *BDNF* The gene for the brain-derived neurotrophic factor was examined for association by Kunugi et al. (178), as multiple lines of evidence were connecting its product to neurodegeneration and AD. They reported a strong association of a noncoding SNP with the disease in a Japanese sample which was followed by a report on a different SNP by Ventriglia et al. (179), an SNP causing the amino acid change Val66Met. Both SNPs failed to replicate in subsequent studies (179–182), but were then both replicated by a study by Matsushita et al. (183) who also performed stratification by *APOE* genotype for the noncoding SNP but then again they both failed to replicate in another study (184). Another six studies that followed to date showed the same conflicting results. (*v*) *GAPDH* The gene coding for glyceraldehyde-3-phosphate dehydrogenase is a recent candidate for AD on chromosome 12 that has not yet been tested in multiple samples but an interesting association finding was recently reported (185). The gene is of interest because of prior evidence of involvement in neurodegeneration and AD independent of its well-documented glycolytic function (186). As chromosome 12 was the first to attract attention through genome scans for linkage, many more genes have been examined such as *NTF3*, *NOS1*, and *C1R* that we will not discuss here.

THE MITOCHONDRIAL HYPOTHESIS

Mitochondria are very interesting from the genetic standpoint as they represent semi-independent entities in the cell with distinct genetic properties. Each cell contains multiple mitochondria and each mitochondrion contains many copies of DNA, which are transcribed, translated, and replicated independently from the nuclear DNA. The mitochondrial genome is made of a circular double-stranded DNA molecule that contains 16.5 Kb of sequence tightly packed with genetic information including 13 protein-coding genes, two ribosomal RNA genes, and 22 tRNA genes. Some of the codons in the mitochondrial genome are translated differently than they are in other cells as the mitochondrion has its own genetic code. However, the majority of proteins functioning in the mitochondria are produced by nuclear genes. The mitochondria are believed to have started as a

symbiotic organism, useful for performing oxidative reactions and providing energy to the cell. Over the years transfer of genetic material to the nucleus has resulted in the generation of nuclear genes encoding mitochondrial products and elimination of the original mitochondrial genes. This genetic transfer has been very extensive also resulting in the presence of many nonfunctional copies of mitochondrial DNA fragments in the nucleus that have at times confounded experimental results. The mitochondria are transferred to the zygote almost exclusively from the ovum; thus their DNA is inherited almost exclusively from the mother and it does not undergo recombination, although a few reports have suggested the contrary (187,188). The mitochondrial DNA is subject to much higher mutation rates than nuclear DNA, which results in much more variation as well as the presence of multiple somatic mutations. The evolution of the human mitochondrial genome is characterized by the emergence of ethnically distinct lineages, which are called haplogroups. These have been the subject of research that has formulated the "out of Africa" hypothesis for the geographical origin of humans (189,190) and they have also been used for association studies in neurodegenerative disorders. Another interesting aspect of mitochondrial genetics is the possible existence of different copies of the same mitochondrial genes within a single cell which is known as heteroplasmy. This phenomenon can produce extensive phenotypic variability as it is often observed in known mitochondrial disorders.

The mitochondrial genome contains 13 protein-coding genes that together with many nuclear genes code for components of the electron transport chain (ETC). There is substantial biochemical evidence for an ETC defect in AD (191–194) and this defect may arise from mutated and/or oxidatively damaged mtDNA. Cybrids, which are cells created by the transfer of mtDNA from the cells of interest to clonal neuronal-like cells previously depleted of their own endogenous mtDNA, have provided further evidence (195,196). Therefore, inherited defective mitochondria or predisposition to mitochondrial DNA damage may be an important genetic determinant of AD. The resulting ETC defect could lead to increased free radical generation and oxidative stress leading to neuronal damage and death. The strong correlation between risk for AD or other neurodegenerative disorders and increasing age fits the hypothesis of mitochondrial damage accumulation well. The strong involvement of the mitochondria in programmed cell death (apoptosis), a mechanism of cell death involved in the neuronal death observed in AD also supports this hypothesis (197,198). Recently, the toxicity of Aβ has also been strongly linked to the mitochondria (199,200) (201) where γ-secretase activity has been shown to be present (202) . All the evidence suggesting the importance of the mitochondria in biological mechanisms involved in neurodegeneration including AD has led many investigators to test this involvement though screens for mutations, deletions (a common event in the mitochondria), and association studies for mitochondrial polymorphisms.

Compared to the biochemical evidence the research examining the involvement of the mitochondria in AD from the genetic standpoint is less extensive. The

detection of mutations in the cytochrome oxidase gene (203,204) attracted a lot of attention in 1997; however, it was later shown that the observed variants were derived from copies of the gene in the nuclear DNA that had no known functional significance (205). Other interesting findings include a mitochondrial variant at position 4336 (GenBank #J01415.0 gi:337188) that was first reported to be associated with AD and Parkinson's disease in 1993 (206). After the first report of the association of the variation at position 4336, an A to G transition on a moderately conserved nucleotide of a transfer RNA gene, a second study reported similar findings (207). Only one more study has provided support since (208), whereas many others have failed to support the finding (209–212). A study on a Japanese population (213) did not detect the associated allele in cases or controls, suggesting that this variant might not be involved in AD in the Japanese or it might not be the causative variant in either Japanese or Caucasians. The study of mitochondrial haplogroups has also shown some positive results both for AD and for Parkinson's disease (212,214–217); however, these have also not been consistently replicated (211,218). Other indications for possible involvement of mitochondrial DNA in AD include the presence and accumulation of deletions and mutations (219–224); however, the results of such studies have also not been consistent. The hypothesis of mitochondrial involvement in AD remains interesting and attractive. The small size of the mitochondrial genome together with the continuous development of faster and cheaper DNA sequencing and genotyping methods makes it likely that more detailed studies will address and potentially answer this question in the near future.

The presentation of the linkage and association results mentioned earlier including the examination of the mitochondria is not meant to be a comprehensive review either in terms of covering all the studies or in terms of providing enough information to assess the significance of each study, positive or negative. It is rather an attempt to provide a general overview of the state of the genetics research on AD, as the problems encountered in this disease are very representative of other neuropsychiatric disorders. On one hand there are a number of promising results and it is quite likely that some of the genes mentioned here and some of the many other candidates we did not mention are true susceptibility genes. On the other hand nonreplications are the rule rather than the exception. It is becoming increasingly clear that we are very likely looking for genes with small effects, allelic heterogeneity, and genetic heterogeneity. This might be further complicated by gene–gene interactions, by haplotypic rather than allelic effects, by the study of markers in partial LD with the causative variants, and by population differences. Often we might be examining phenotypic modifier genes rather than genes directly involved in risk and such effects can give inconsistent results under slightly different study designs. Such complications can explain the lack of replication even for genes that are truly involved in the disease. The field of AD's genetics started out with a period of great successes, but it is now facing the same problems as the other neuropsychiatric disorders where multiple genes show promising results but none is a proven susceptibility gene. However as many

investigators are intensively studying these disorders, as the sample sizes are continuously growing, as high throughput technologies are constantly improving, and as new analytical methods are becoming available the future is starting to look more hopeful.

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2

Gene-Mapping Studies for Schizophrenia: How Useful Are They for the Clinician?

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INTRODUCTION

This is a period of tremendous excitement in the schizophrenia genetics field and the next few years may enable discoveries that impact directly on the clinician's work. In this chapter, we have first provided the background for such research that should usefully complement the concepts provided in other chapters. An update on current gene-mapping efforts for schizophrenia follows. We have highlighted recent successes with selected "candidate genes." We have also summarized efforts to map genes for traits that usefully complement the conventional diagnostic phenotype. We conclude by summarizing the implications of this work for the practicing clinician.

BACKGROUND TO GENE-MAPPING STUDIES

The role of genetic factors in the etiology of schizophrenia has been substantiated by classical genetic studies. The heritability of schizophrenia ranges from 70% to

90% (1). Heritability is the proportion of phenotypic variance attributable to genotypic variance in a population. In other words, it denotes the fraction of the etiology attributable to inherited factors, *at the population level*. It does not indicate the role of genes as etiological factors for individual patients. Moreover, heritability does not indicate the number of genetic factors likely to confer susceptibility; nor does it explain the type of interaction between genetic and nongenetic etiological factors. Indeed, the inability to accurately predict the number of genetic factors a priori, and, hence, the risk conferred by an individual genetic variant has made it very difficult to identify individual genetic factors. This difficulty is shared with other common disorders (2).

The conventional approach to mapping genes for diseases is called "positional cloning" (3). It is greatly aided by prior knowledge of the mode of inheritance of the disease of interest. Like common disorders such as hypertension, classical gene-mapping studies for schizophrenia have been difficult because these disorders do not follow classical Mendelian inheritance pattern (4–6). The possible interaction of as-yet-unidentified environmental risk factors with these genetic factors is another hurdle. In addition, schizophrenia has so far defied establishing clear phenotypic boundaries from a genetic perspective (1).

UPDATE ON GENE-MAPPING EFFORTS

Two broad approaches are being used: linkage and association. Recent studies have yielded encouraging linkage results, as described in the following text. Because linked chromosomal regions are typically very extensive (spanning millions of nucleotides), they may not be of great interest to the clinician. Therefore, we have focused more extensively on association studies in the present chapter. Such studies focus on much smaller genomic regions and typically involve selected genes, also called "candidate" genes. We provide critical analyses regarding these findings.

Linkage Studies

Early studies were modeled on the assumption that a single gene conferred significant risk in selected families. Such a model would predict a Mendelian pattern of inheritance, as was noted in certain early onset forms of Alzheimer's disease (AD, Chap. 1). Hence, investigators ascertained families that appeared to segregate for schizophrenia in a Mendelian fashion and applied classical linkage designs for the mapping efforts. Results from such studies are difficult to replicate, because the linkage results may be unique to the sample identified. Hence, investigators have increasingly resorted to analytic methods that do not presuppose a particular mode of inheritance. This "model-free" approach also enables recruitment of a wider array of multiply affected families.

Linkage for schizophrenia (SZ) has now been reported in several regions, for example, 1q32-q41 (7), 5q31 (8), 6p24-p22 (9,10), 6q25.2 (11), 6q13-q26

(12), 8p21 (13), 8p (14), 10p15-p11 (15,16), 10q 23 and 12q24 (17), 13q32 (13), 5q11.2-q25 (18,19), and 22q12-q13 (20,21). Although some independent samples have detected linkage in the same or overlapping regions (e.g., 6p22-p24, 6q13-q26, 10p15-p11, 13q32, and 22q12-q13), many other samples have failed to detect linkage in these regions (22–26) (27) (21) (28). The lack of overlap may be due to false-positive results or genetic heterogeneity (the possibility that several genomic regions may harbor genetic risk factors for the same disorder). Efforts are in progress to synthesize these data. A recent meta-analysis of twenty published genome-wide scans revealed significant genome-wide evidence for linkage on chromosome 2q (29). Linkage was observed over a 30 cM bin; so localization of the susceptibility gene is likely to be a daunting task.

Although most groups have analyzed linkage at the whole genome level, others focused on particular chromosomal regions. For example, a large pedigree identified in Scotland has been investigated extensively (30). Several individuals in this family inherited a translocation that involved portions of chromosomes 1 and 11 (1;11)(q42;q14.3) (30,31). There appeared to be linkage between this inherited anomaly and psychoses, as well as electrophysiological variation (31). These interesting findings are discussed in detail in the section relating to the Disrupted in schizophrenia 1 (*DISC1*) gene.

Association Studies

Association studies rely on linkage disequilibrium (LD), the nonrandom association of alleles at linked loci in the population. This phenomenon enables the researcher to scan limited genomic regions among unrelated cases and controls by analyzing polymorphisms known to be localized to that region. If case-control differences are noted with regard to the distribution of a particular polymorphism, this suggests that the polymorphism itself or another polymorphism in "LD" with it could be the disease susceptibility variant. Association studies can be used to study relatively small genomic regions because LD dissipates over relatively small genomic regions (thousands of nucleotides). Several factors influence LD, such as recent population admixture, inbreeding, recent drift effects, as well as the number and recency of disease mutations. All these factors can potentially complicate gene-mapping efforts because they can be difficult to estimate for a given population (32). It is also necessary to correct for multiple comparisons, because association studies may compare dozens of polymorphisms.

The choice of a control group is a critical element in the design of genetic association studies, as with case-control studies in general (33). Most studies use either unrelated population-based controls or family-based control subjects. There are strengths and weaknesses in each approach (34). Because ethnic admixture can confound disease association studies, researchers have increasingly relied on family-based controls. As the family-based controls are drawn from the same ethnic background as the controls, confounds due to ethnic admixture are avoided. In addition, some family-based tests are significant only in the presence

of linkage and association (35). Originally termed the "Transmission Disequilibrium Test," such analyses are considered more rigorous than the conventional case-control analyses involving unrelated individuals. However, it is usually necessary to expend more effort to gather family-based samples. Hence, attempts have been made to correct the likely impact of ethnic admixture on associations detected, using unrelated cases and controls (36,37).

A Critical Appraisal of Genetic Association Studies

The human genome comprises millions of variations and analyses of all variants pose a formidable logistical and statistical challenge. The likelihood of false-positive results is increased as more variants are analyzed. To maximize the chances of detecting "true" associations, researchers typically focus on genes that encode proteins that are implicated in the pathogenesis of the disorder of interest. Because the pathogenesis of schizophrenia is uncertain, this is a daunting task and has led to the analysis of hundreds of polymorphisms (38). Selection of candidate genes that are localized to linked regions would narrow the choice and would increase the chances of detecting meaningful associations. This principle is borne out by recent success in association studies of SZ. Here, we review some of the genes that have attracted serious attention in the past three years. Our choice is necessarily biased, but is tempered by a desire to illustrate some of the pitfalls in such studies and the success of this approach. In a recent review, we had raised the following questions regarding the candidate gene association studies in psychiatry (39): (*i*) Once an association with a gene has been reported, do replicate studies need to provide associations at the same polymorphisms in order for the association to be credible? (*ii*) How many replicate studies are required? (*iii*) How much credence should be given to adequately powered studies that fail to detect associations? (*iv*) Is it necessary to demonstrate associations using both familial and case-control samples? We review recent candidate gene association studies in the light of these issues.

Neuregulin 1

Neuregulin 1 (*NRG1*) is localized to chromosome 8p22. Neuregulins are a family of proteins that signal through erbB2, erbB3, and erbB4 receptor tyrosine kinases. *NRG1* is widely expressed in the brain (40). *NRG1* has also been implicated in several neurodevelopmental processes of relevance to schizophrenia, such as neuronal migration and synapse formation (41,42), oligodendrocyte development (43), and $GABA_A$ and glutamate receptor expression (44,45). Association studies of *NRG1* polymorphisms began when Stefansson et al. (46) detected linkage to a locus on chromosome 8p at D8S278, an anonymous microsatellite marker in an Icelandic population-based sample (46). Suspecting that the linkage result might indicate the presence of a susceptibility gene, the authors conducted an extensive search in this region. In a clear exposition of the utility of the positional cloning effort, they detected association with one single nucleotide polymorphism (SNP)

in the 5' region of *NRG1* and a seven-marker haplotype. The same authors also noted that *NRG1*-mutant mice were behaviorally abnormal and showed decreased functional *N*-methyl D-aspartate (NMDA) receptors that mediate glutamate signaling. Following this, the same group examined a Scottish cohort of cases and controls and detected association with three SNPs and the seven-marker haplotype (47). Follow-up studies in relatively large Caucasian (48,49), Japanese (50), and Chinese samples (51) did not detect association with the same markers that were reported in the Icelandic and Scottish populations. However, Williams et al. (48) found association with a three-marker haplotype in familial cases. (A haplotype denotes a short chromosomal region "tagged" using a set of polymorphisms as opposed to analysis with just one polymorphism; haplotypes are thus more polymorphic than SNPs and may even have functional significance). Using another Chinese sample (51), a novel haplotype upstream of the Icelandic haplotype was reported to be associated with schizophrenia and another associated haplotype overlapped the haplotype associated in the Icelandic sample. Other studies have also reported haplotypic associations but the pitfalls of such associations have already been discussed. The associations at the markers have been variable and some of the studies may have lacked power to detect the original associations (52–54). The results from Caucasian subjects appear to be more consistent, whereas the results from the Asian samples are variable, suggesting genetic heterogeneity. Functional assays to elucidate the significance of these variations and a thorough examination of the LD architecture of *NRG1* among these groups may shed more light on this possibility. Overall, the associations at *NRG1* are promising, but a survey of all representative polymorphisms, also known as "tag SNPs," followed by meta-analysis, will clarify some of the discordant results.

Dysbindin or Dystrobrevin-Binding Protein

Dysbindin is an evolutionarily conserved 40-kD protein that has been detected in the axons of corpus callosum, mossy fiber terminals of cerebellum and hippocampus, neuropil of the neocortex, substantia nigra, and hippocampus. Dysbindin binds to β–dystrobrevin and is believed to be a part of the dystrophin protein complex found in the postsynaptic densities. Through complex mechanisms, dysbindin may be involved in nicotinic and glutamate receptor clustering and may also participate in signal transduction (55,56). The gene encoding dysbindin is localized to chromosome 6p22. Straub et al. (56) first reported linkage to this locus in Irish multiplex families and then went further to identify 36 SNPs in the linked short tandem repeat polymorphism (STRP) markers, of which eight SNPs showed significant association with schizophrenia. There have been impressive replications with individual markers in several large studies. However, the associated markers differ in these studies. Williams et al. (57) did not detect significant association with the initial markers, but found association with unique markers and a three-marker haplotype consisting of a risk haplotype and two protective haplotypes. Hall et al. (52) examined five markers

and reported no significant association with any of the markers or haplotypes. Tang et al. (58) reported an association with a five-marker haplotype but did not find a significant association with any marker that was tested. Another study involving Caucasian subjects of Polish, German, and Swedish descent (59) did not find association with individual markers or haplotypes in Polish and German samples, whereas Swedish samples showed association with one of the markers (rs1011313). A five-marker haplotype, including this marker, showed a stronger association with familial schizophrenia cases. These results are very intriguing, but suggest that the allele primarily associated with schizophrenia has not been identified yet. Indeed, the association only among the familial cases in the study mentioned suggests that some of the discrepancies may also have occurred because the "true" association may be present in a subgroup of the patients. The numerous analyses of a large gene (140 kb) such as dysbindin or dystrobrevin-binding protein (*DTNBP1)* also raise the possibility of false-positive results. As with *NRG1*, identification of representative polymorphisms and analysis in a set of adequately powered samples may help clarify the association further. It would also help if the data from the different reports could be synthesized through meta-analysis.

Regulator of G-Protein Signaling

A group of GTPase-activating proteins (GAPs) for heterotrimeric G-protein subunits that negatively regulate G-proteins are collectively called regulators of G-protein signaling (RGS). Such regulation may modulate the function of dopamine (60), glutamate (61–63), and serotonin (64). Using DNA microarrays, reduced expression of the gene encoding the regulator of G-protein signaling, subtype 4 (*RGS4*), but not other members of the RGS family of proteins, was initially noted in the dorsolateral prefrontal cortex (DLPFC, Brodmann's area 9), visual and motor cortices of schizophrenia patients compared to the matched control subjects, and patients with major depressive disorder (65). *RGS4* has been localized to 1q21–22, a locus linked to schizophrenia in a recent study (21). Chowdari et al. subsequently reported association with individual SNPs and a four-marker haplotype in two independent samples ascertained at Pittsburgh and by the National Institute of Mental Health (NIMH) Collaborative Genetics Initiative (66). Although associations were noted with the same SNPs, the associated alleles and haplotypes were different in the Pittsburgh and NIMH samples. Suggestive association with the same haplotype as the NIMH sample was noted in a third, independent Indian sample (66). Several other studies have reported associations at the same SNPs of this gene (67–70). The associated SNPs/haplotypes have mirrored the Pittsburgh or the NIMH-associated alleles/haplotypes. One recent United States study did not report any association with schizophrenia (71). These results can be interpreted in different ways. The most parsimonious explanation is that there is no significant association, although the discordant results could also be attributed to association with an unidentified polymorphism. Else, more than one variation could confer risk. To evaluate these possibilities, we

conducted one of the largest meta-anlysis of genetic data in schizophrenia using both published and unpublished family-based and case-control samples (n = 13,807). The data suggest that an association could not entirely be ruled out and that more than one variant could confer risk (72). The possibility of more than one risk variants at the gene, are thus similar to those already discussed for *NRG1* and *DTNBP1*. A comprehensive examination of the entire gene is feasible because *RGS4* is a small gene (7.5 kb), and this is currently underway in our lab.

Disrupted in Schizophrenia 1

Over two decades ago, during the course of a study of cytogenetic abnormalities associated with schizophrenia, an unusual Scottish pedigree was identified (30,31). In this large family, a significantly higher frequency of major mental disorders was noted among individuals with a balanced chromosomal translocation t(1:11)(q43, q21). After painstaking analyses, the breakpoints were identified and were noted to disrupt two genes, aptly named Disrupted in Schizophrenia gene 1 (*DISC 1*) and gene 2 (73,74). Another gene, translin-associated factor (*TRAX*) within the translocated region on chromosome 1q42 has also been identified recently (75). The translocation interrupts the coding sequence of *DISC1* gene that results in the loss of C-terminal 257 amino acids for DISC1 protein. In a mutation analysis on a group of schizophrenia probands, a small deletion at the 3' end of exon 12 that is suspected to cause a frameshift has been reported (76). Animal studies suggest that this mutated form of DISC1 protein may not bind appropriately to binding partners such as NUDEL and Lis-1, proteins that regulate cortical development (77). Furthermore, the DISC1 protein has been reported to regulate neurite outgrowth (78), mitochondrial function, modulation of actin cytoskeleton, neuronal migration, glutamate transmission, and signal transduction (75). Recent studies suggest that DISC1 stabilizes the dynein protein complex, contributing to the microtubular dynamics that, in turn, plays a role in the neurodevelopment of the cortex (79). The mutant form of DISC1 (mutDISC1) functions as a dominant negative mutation that disrupts the process, possibly leading to the cortical abnormalities observed in schizophrenia. Further, interaction of DISC1 with phosphodiesterase 4B (PDE4B) has recently been demonstrated (80). Phosphodiesterase is the only enzyme that inactivates cyclic adenosine 3'-5' monophosphate (cAMP) that has been implicated in learning, memory, and mood. DISC1 binds PDE4B and sequesters PDE4B within the cell. These two proteins dissociate with increasing cAMP levels. PDE4B then activates phosphodiesterase, thus inactivating cAMP. The authors propose that the variations in these genes may affect the normal interactions between DISC1 and PDE4B that may lead to abnormalities in learning, memory, and mood. Sawa and Snyder (81) have proposed that the major psychiatric disorders may share a common signaling mechanism. Thus, DISC1 participates in two major pathways in animal models. Through cAMP signaling, it is proposed to affect learning, memory, and mood; through dynein-NUDEL pathway, it may affect the neurodevelopment.

It would be of interest to know if polymorphisms in these genes confer risk for schizophrenia. An association study of Scottish patients and controls did not find an association of multiple SNPs and a microsatellite at DISC1 (82). However, another study of Finnish families revealed linkage at chromosome 1q42 and the *DISC* genes were suggested as promising positional candidates (83). In a replicate study on an independent Finnish nuclear family sample, the investigators found an associated haplotype spanning exon 9 to intron 9 of DISC1 (84). A common haplotype was reported to be undertransmitted in schizophrenia subjects from a larger Finnish sample, especially in female patients (85). Undertransmission of the common haplotype at the 5' end of the *DISC1* gene was also reported in a case-control study of North American Caucasian individuals (86). Further, the investigators found an association between multiple haplotypes and schizophrenia, schizoaffective disorder, and bipolar disorder. Interestingly, these investigators found an association of a missense variant with schizoaffective disorder. Five markers showed modest association with schizophrenia. Another study from Scotland also reported nominally significant associations (87). These results are important because of the initial report of a translocation in a Scottish family. Another analysis suggested associations with certain haplotypes among bipolar women (88). Associations with reduced hippocampal size (89) and neurocognitive functions, such as working memory (90), have also been reported. Overall, the evidence suggests that *DISC1* gene codes for products involved in neurodevelopmental processes that underlie schizophrenia, schizoaffective disorder, and mood disorders. Further studies, particularly synthesis of the reported samples, are needed.

G72 and D-Amino Acid Oxidase

G72 and D-amino acid oxidase (DAAO) may be functionally related in the oxidation of D-serine, which is an allosteric activator of NMDA receptor (91). NMDA receptors have modulatory sites for glycine and D-serine, which need to be stimulated by these amino acids for the ionotropic action of the receptor (92). Glutamate dysfunction has been suggested to be of pathogenic importance for schizophrenia (61). Therefore, these genes are interesting functional candidate genes. Chumakov et al. (93) first reported significant associations with SNPs at the *G72* locus. These results are intriguing not only because of the suggested role for G72 in schizophrenia pathogenesis, but also because this gene is localized to chromosome 13q34, a region with suggested linkage with schizophrenia (13,94). The investigators also found that G72 interacts with DAAO, and three SNPs at DAAO were significantly associated with schizophrenia in the same study. DAAO is localized to 12q24, which was not a linked region in earlier studies. Several groups reported association of other polymorphisms of these genes (52,95–98). As is the case with the other candidate genes, the associated markers differed between the studies. Addington et al. (97) reported that three of the G72 SNPs were associated with childhood onset schizophrenia, but did not find any significant associations with the marker on DAAO. Wang et al. (99) found

gender-based associations. One marker (rs2391191) was significantly associated with men and women, whereas rs3916965 was significantly associated only with women. It is notable that the association with rs2391191 was noted even in relatively small samples, suggesting replicability. However, Mulle et al. (100) did not find any significant associations. Further studies are warranted to ascertain LD patterns, ethnic variations in the association of markers/haplotypes with schizophrenia, and to elucidate the biological significance of these polymorphisms.

Catechol *O*-Methyl Transferase

Catechol *O*-methyl transferase (COMT) is an enzyme that catalyzes the transfer of a methyl group to dopamine (DA), resulting in the inactivation of DA (101). DA dysfunction has been implicated in the pathogenesis of schizophrenia (102); so inactivation of DA is a key step for investigation. *COMT* is localized to chromosome 22q11, a genomic region that has been implicated in several linkage studies (29). Deletions in this region could also lead to the Velocardiofacial syndrome (VCFS), which may be associated with increased risk for schizophrenia (103). Schizophrenia susceptibility has also been associated with interstitial deletions in this region (104). Thus, several lines of evidence suggest *COMT* as a plausible functional and positional candidate gene for schizophrenia. The majority of published studies have examined one marker (Val108/158Met polymorphism; rs4680). Overall, studies that examine samples of less than 200 cases failed to detect significant associations individually, suggesting the possibility that they may have lacked power (105–110). However, three successive meta-analyses did not find a significant association at the val/met polymorphism (111–113). Association studies using larger samples have reported differing results. Shifman et al. (2002) investigated a large sample of Jewish cases and unrelated controls (114). These investigators reported association with the Val/Met polymorphism and two other SNPs in the upstream and 3' UTR regions. A haplotype comprising these SNPs was significantly associated with schizophrenia. However, Williams et al. did not find association with any of these three SNPs in adequately powered Caucasian case-control and case parent trio samples recruited in Europe (115). Thus, it appears that significant associations with the Val/Met polymorphism may not be present among Caucasians. The Shifman study suggests that the association may be restricted to Jewish individuals and merits replicate analysis. However, a recent study on Ashkenazi Jewish population failed to detect the associations with *COMT* variants, including the Val/Met polymorphism (17). These uncertainties highlight the vicissitudes of detecting associations even in a highly favored positional candidate gene.

An interesting aspect of this gene is its association with working memory (116,117)—a cognitive function that has been reported to be impaired in schizophrenia (118–120). Brain regions involved in working memory processing have been shown to be more efficient in met/met individuals compared to the val/val individuals (116). Association of *COMT* gene variations with cognition and brain activations continues to fuel interest among investigators.

Dopamine Receptor, Subtype 3

We have included discussion of this gene to illustrate the fallacy introduced by inadequate evaluation of genetic polymorphisms in earlier genetic association studies. Dopamine receptor, subtype 3 (*DRD3*) is a promising candidate gene. Postmortem studies of brain tissue from schizophrenia patients and controls have reported increased *DRD3* density in the mesolimbic regions (121) and decreased levels of *DRD3* mRNA in the cortical regions (122–124) of patients with schizophrenia. Furthermore, it may mediate the therapeutic effects of some antipsychotic drugs (125). On the other hand, *DRD3* maps to chromosome 3q13.3, a region not commonly reported in linkage studies. Despite this, more than 40 association studies of a common, nonsynonymous coding polymorphism in exon 1 that codes for either serine or glycine at the ninth amino acid in the N-terminal extracellular domain (Ser^9Gly; rs6280) have been published (126,127). More than a decade ago, it was reported that individuals with schizophrenia were more likely to be homozygous for *either* allele at this polymorphism compared with controls (128). In other words, there was an excess of heterozygous individuals among the controls. Such heterozygote advantage is well known in plant genetics. Indeed, expression studies of *DRD3* in vitro suggest that this variant leads to differential affinity for DA (129). This intriguing genetic association was investigated extensively, with predictably disparate results. Successive meta-analyses (127,130–134) suggest a significant, but modest association with the serine variant ($n = 8{,}761$; estimated OR = 1.10, 95% CI = 1.01 – 1.20) (127). However, the latest analysis did not detect significant association ($n = 11{,}066$) (131). Thus, a set of mutually exclusive conclusions is possible: either there is no significant association, or the Ser^9Gly may be in LD with an unidentified liability locus. Therefore, polymorphisms flanking rs6280 have been investigated. Three studies have reported significant associations, but two others did not (135–139). These studies focused mostly in the 5' upstream regions of the gene, but did not investigate the 3' end of the gene comprehensively. We evaluated both regions in two independent United States and Indian samples using both case-control and family-based designs. Our studies of 13 SNPs revealed several individual SNPs that were associated in these samples (140). Intriguingly, a common haplotype spanning intron 1 to the 3' region of *DRD3* was overtransmitted in the United States and Indian samples, suggesting some consistency. We are now evaluating all representative common SNPs of *DRD3* in replicate samples.

MAPPING GENES FOR TRAITS THAT USEFULLY COMPLEMENT THE CONVENTIONAL DIAGNOSTIC PHENOTYPE

Another approach that is useful in examining psychiatric diseases, including schizophrenia, is the quantitative trait locus (QTL) approach. Quantitative traits are continuous variables that may be more tractable to linkage analyses compared with the traditional studies using categorical variables such as diagnoses (141). If

there are heritable quantitative traits that underlie the pathogenesis of schizophrenia and occur at an intermediate step between genetic variation and psychopathology, mapping such traits may usefully complement traditional gene-mapping studies (142). Such traits are also called endophenotypes (143). Several such endophenotypes are being actively pursued by investigators; for example, brain morphology, brain activation in response to cognitive tasks, and cognitive function. The association of *COMT* Val/Met polymorphisms with alterations in working memory (117) and DLPFC activation in response to working memory tasks (116) illustrates a potentially rewarding approach to studying endophenotypes. Similarly, association between *RGS4* genotypes and DLPFC volume (144) has also been reported. It is likely that attention will focus on these variables in the coming years.

Space considerations preclude a review of genetic associations with treatment response. Ongoing work in several laboratories suggests that genetic polymorphisms may predict treatment response, as well as deleterious side effects (145,146).

CONCLUSIONS AND PROSPECTS FOR THE FUTURE

The gene-mapping studies to date have provided initial evidence for genetic variants that may confer risk for schizophrenia, but their interpretation may not be straightforward. Variations in the noncoding regions pose considerable challenges because the functional implications of such variations are not entirely understood. Further, a marker that is reported to be associated with the disease may not be the etiological factor. Instead, it may be in linkage disequilibrium with another polymorphism in the same gene or another gene localized physically close to it. It can be extremely difficult to tease out the primary risk allele if there is strong LD in a genomic region. Like any epidemiological study, control selection can have considerable impact on the putative associations. Furthermore, the functional impact of such variations needs to be examined. At an even more complex level, it is unclear if and how the associations at the different genes interact to increase risk. Indeed, conclusions about individual genetic associations could require reconsideration as other ongoing studies are published. To enable synthesis of the disparate findings and to facilitate studies of interactions between risk alleles, genome-wide association studies are being considered. Such studies would simultaneously evaluate associations across the gene, but there are daunting logistical and statistical issues that have not yet been resolved (2).

How does this research impact on the busy clinician's work? There is no immediate utility of these findings in choosing appropriate treatment, predicting prognosis, or for identifying at-risk individuals. Even the most consistently associated alleles do not confer risk that could be considered meaningful clinically. Indeed, the associated marker may be neither necessary nor sufficient to cause a complex trait such as schizophrenia.

Given this relatively low "yield" for the clinician, is it beneficial to continue such studies? We believe it is, primarily because the pathogenesis of schizophrenia is uncertain. Hence, rational therapeutics remains a distant dream. Although there is considerable value in studying brain function among patients using a sophisticated range of neuroscience techniques, it is difficult to identify the primary pathogenic events using such approaches. Studies aimed at identifying etiological factors are thus necessary and will complement research in other domains. Conventional epidemiological research has yielded several risk factors in the environment, such as the risk due to low socio-economic status (147) and the excess risk due to birth in winter months (148). Unfortunately, it has proved difficult to understand the biological processes mediating such risk factors. Genetic studies have a dual advantage in this situation. First, it is difficult to dispute the primacy of a proven genetic risk factor. Second, understanding the biological function of a genetic risk factor may be relatively facile. In addition, approaches using integrated techniques to define and refine the endophenotypes associated with the illness are coming to the fore. For example, neuroimaging and electrophysiological studies examining the neurobiological correlates of candidate gene variations could illuminate the brain structural, functional, and chemical alterations associated with risk gene variations. Transgenic, knockout, or knockin animal studies to characterize behavioral phenotypes associated with them could also prove very valuable. Pursuing such integrated efforts could help in characterizing endophenotypes specific to a disease, paving the way for more etiologically tractable nosography.

How can the busy clinician contribute to such studies? First and foremost, it is important to clarify the precise traits associated with the various polymorphisms listed earlier. We believe that much of the inconsistency in the genetic association studies may be due to differences among the individuals diagnosed with SZ by different researchers. The clinician can usefully contribute to a dialogue about meaningful clinical subgroup subsumed under the DSM IV diagnostic rubric. Second, an astute clinician may identify subtle congenital abnormalities that may help ascertain families with rare, but highly informative, chromosomal abnormalities. The series of studies leading to the ongoing exciting work with *DISC1* highlight the key role played by clinical colleagues.

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3

Rare Genes of Major Effect in Neuropsychiatric Diseases

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INTRODUCTION TO COMPLEX NEUROPSYCHIATRIC DISORDERS

In comparison to the early and rapid successes with single-gene, Mendelian disorders, research into complex genetic disorders has seemed interminably slow, contradictory, and intractable (1). Neuropsychiatric disorders have demonstrated this recalcitrant behavior well and, indeed, to researchers in the field it sometimes feels like they are even more "complex" than other "complex genetic disorders" such as diabetes, cancers, asthma, coronary artery disease, and autoimmune conditions. The aspects of disorders, which make them genetically "complex" can be thought of in two ways. First, the underlying genetic risk factors are numerous and often without a direct genotype-to-phenotype correlation. Second, the complex disorder phenotype may be broader and more diffuse—certainly at a clinical level where it is often a clinical diagnostic end point (e.g., obesity, blood pressure, stroke, behavior, and communicated cognitions) rather than a unifying pathophysiology (e.g., cystic fibrosis lung pathology).

These issues have led some to base their research into complex disorders on a "worst-case scenario" of quantitative trait genetics. Here, complex disorders are hypothesized to result from the combined actions of many genes of small effect. This "death by a thousand cuts" approach lends itself to mathematical modeling of prevalence, risk, and transmission at the epidemiological level and may be valid for some traits, but can overlook the fact that relative genetic simplicity may underlie some apparently complex conditions.

For the analysis of complex disorders, it has been necessary to scale up approaches traditionally applied to simpler genetic conditions by recruiting larger sample sets for association and linkage studies, genotyping with dense, carefully selected markers, and applying statistical methodologies that take into account the qualitative nature of diagnostic criteria and the increasing variety of non-Mendelian as well as Mendelian modes of inheritance (2). For neuropsychiatric disorders, the goal is to separate the faint signals of genuine causative factors from a genome's worth of noise produced by locus and allelic heterogeneity coupled with differences in ethnic origin, population history, and sampling strategies. It is perhaps not surprising then that such approaches are only beginning to yield results that stand the test of independent replication.

This chapter will present a research strategy that permits a first-line, direct detection of potential genetic risk factors. This approach takes advantage of the "mutation screen" offered by naturally arising, microscopically visible chromosomal abnormalities. Where these are associated with a disease, they provide an observable physical indicator of a potential candidate gene locus to guide the researcher. Although cases where a chromosomal abnormality is responsible for the genetic risk may only form a tiny subset of the total number of people with a particular disorder, it is important to realize that they appear to differ only in the underlying genetic mechanism: at a clinical level their illness can be indistinguishable from karyotypically normal patients.

Historically, the idea that genes could be disrupted by chromosomal abnormalities was initially applied to somatic chromosomal rearrangements in cancers (3) or apparently Mendelian disorders such as neurofibromatosis type 1 (4–9), retinoblastoma (10,11), Wilms tumor, Duchenne muscular dystrophy (12) and tuberous sclerosis (13). With recent studies of chromosomal abnormalities in complex neuropsychiatric disorders, however, we have an opportunity to identify key central nervous system genetic pathways that may confer risk to these conditions. This chapter will describe what chromosomal abnormalities reveal about the underlying genetic architecture of neuropsychiatric disorders and will examine the biological relevance of several recent candidate genes identified through this route.

CHROMOSOMAL ABNORMALITIES: BALANCED AND UNBALANCED FORMS

Studying naturally occurring chromosomal abnormalities in patients mirrors in many ways large-scale mutagenesis screens (especially enhancer/gene-trap

approaches) in laboratory fly and mouse populations that attempt to link a scorable phenotype to a discrete mutation: a process made easier by the fact that the physical cause of each phenotype is used as the means to expose the causative gene. Obviously, for human disorders we have to rely on naturalistic events rather than direct mutagenesis to provide those rare, but survivable, instances of errors in the usually tightly controlled chromosomal events that underlie meiosis or mitosis (14,15). As an aside, it should be noted that such events are not always deleterious and may have an evolutionary importance as a mechanism to maintain genomic variation and hence adaptability.

As a very rough guide, chromosomal abnormalities (also known as aberrations or rearrangements) can be classed as "balanced" or "unbalanced." In balanced re-arrangements, material is switched between (reciprocal translocations) or within chromosomes (peri and paracentric inversions) (16). Unbalanced rearrangements show a net loss (deletion) or gain (duplication) of genetic material such that regions of the genome may deviate from the normal disomic state (two copies of each gene, excepting the sex chromosomes in males). The extreme is seen in polyploidy (multiple copies of the entire chromosome set) but more common are partial or full monosomies or trisomies (one or three copies of a given chromosomal region resulting from partial or full chromosomal loss or gain).

It is becoming increasingly apparent that even apparently simple balanced rearrangements can have an underlying complexity with sometimes multiple breakpoints and small deletions (17). However, overall, it can be appreciated that the consequences of balanced and unbalanced abnormalities associated with disease are going to be very different with clear implications for their study by (and value to) the human geneticist. Chromosomal dosage imbalances can be pathological because the genome has evolved to provide the cell, organ and body with a functional protein (or in some cases a functional RNA) encoded by the tightly regulated expression of two copies of each gene (biallelic expression). All rules have exceptions as shown in mammals by the dimorphism of the sex chromosomes XX and XY with random inactivation (save for a few pseudoautosomal regions) of one complete X chromosome in females balancing the state in males where the Y chromosome has little coding material. A second exception is in the (increasing) number of genes that have been shown only to be expressed from one chromosome of the pair (monoallelic expression) often on the basis of a parent-of-origin imprint. For autosomes, however, monosomy will result on average in 50% of the usual "dose" of a gene and resulting protein, and trisomy, 150%. These gene expression alterations may have no discernable phenotypic outcome, but in other cases there are clear pathologies. Broadly, the more genes involved in the particular dosage imbalance, the more likely a pathology will develop. The classical example is trisomy 21 where around 250 genes are overexpressed resulting in the clinical phenotype of Down syndrome. In addition to the common full trisomy chromosome 21, the study of partial trisomies associated with a full or partial Down syndrome phenotype has led to the concept of a chromosome 21 minimum "window" [Down syndrome critical region (DSCR)] that in trisomy

will replicate the phenotype. It is postulated that the DSCR contains a subset of genes that are the major risk effectors for all, or important parts, of the clinical phenotype, and such information has been used in association with animal syntenic trisomies to study possible phenotype–genotype correlations (18). However, such animal models of the DSCR have yet to add much mechanistic substance to our understanding of the precise gene–phenotype correlations in Down syndrome (19–21). A critical region approach using information from chromosomal abnormalities has also been applied to the study of monosomies, for instance, in the mapping of the Wolf-Hirschhorn syndrome on the short arm of chromosome 4 (22). A critical window approach on the basis of cytogenetic abnormalities has been demonstrably fruitful—one clear example being the study of chromosome 17 abnormalities that result in the Smith-Magenis syndrome, with its clear but complex behavioral and cognitive phenotype (23). Genomic disorders (or contiguous gene syndromes) can also encompass deletions or duplications intermediate in size between classical rearrangements and disorders of single genes (24).

A genomic disorder with neuropsychiatric correlates is the 22q11 deletion syndrome (22q11DS) associated with the clinical velocardiofacial (VCFS) and diGeorge syndromes (25). VCFS is associated with a reported 25-fold relative risk of developing schizophrenia and has also been associated with other psychiatric and childhood behavioral conditions (26). Its genetic definition has been completed with at least 20 genes thought to comprise the minimal window. Among these, *COMT* and *PRODH* have emerged as potential candidates for influencing the psychiatric outcomes although their role in nonsyndromic forms of psychiatric illness is still far from clear cut (27).

Chromosomal abnormalities that give rise to clinical disorders are often at the limits of detection by light microscopy. A new generation of array-based genomic screening methodologies is already uncovering a wide variety of smaller scale copy number changes within the genome, and although some of these are clearly common variations in the population, others will likely be pathological in nature (28–31). Their smaller size may be especially useful in highlighting causative genes in these instances.

Balanced translocations share aspects of dosage changes mentioned earlier but in a much more restricted sense. Generally, inversions and translocations result in two breakpoints. In the simplest case, if a breakpoint falls within a gene locus, defined as the region between the start of the first exon and the end of the last exon, then a full transcript will not be generated from the allele of the gene on this particular chromosome. A cellular mechanism, “nonsense-mediated decay,” exists to detect and degrade such prematurely truncated transcripts as a way of preventing pathogenic protein product formation (32). Normally, this mechanism acts on the products of stochastic failures in the transcription of normal genes, but in the case of chromosomal abnormalities it is likely to act and degrade breakpoint-induced truncated transcription products. Thus the breakpoint has created a dosage effect with respect to the normal protein and the overall

result will be a haploinsufficiency of gene expression and reduction in protein level to around 50% of normal.

By definition, balanced rearrangements do not result in a net loss or gain of genetic material; so techniques described earlier for unbalanced rearrangements, including current CGH methodologies, will not detect them. In their place, we can employ fluorescent in situ hybridization (FISH) with fluorophor-conjugated probes whose physical localization on the chromosome and genomic sequence is known. Taking as an example a reciprocal translocation, successive rounds of FISH enable the user to "home in" on the breakpoints because the fluorescent probe signals will initially be located on the normal chromosome and just one or other of the two derived chromosomes. This gives a direction for the selection of further FISH probes until the situation is reached where there is a three-way "split signal" between the normal and two derived chromosomes—an indication that the probe consists of genomic DNA sequence containing the site of the breakpoint. Now that the Human Genome Project (33) has resulted in the annotation of the entire genomic sequence, the user knows precisely where the breakpoint lies with respect to the genes—and most likely the gene's individual exons—at that region.

It can be immediately seen that the study of breakpoints can be an incisive tool for the gene hunter, especially where disruptions occur without chromosomal imbalance. It is much easier to form a hypothesis regarding the cause of a condition—and go on to test it—when only a single gene is involved. For this reason, researchers studying a number of neuropsychiatric conditions have exploited instances of balanced translocations in order to pinpoint potential candidate genes. Examples of the results of this approach are listed in Table 1.

PHENOTYPES ASSOCIATED WITH TRANSLOCATION EVENTS

A simple breakpoint-mediated disruption of a single gene may not result in a unitary phenotype, and comorbidity is common. Reported comorbidities include physical dysmorphisms (facial, dermatoglyphs etc.), mental retardation (in U.K., this is equivalent to learning disability), and epilepsy. The association of schizophrenia with mental retardation was first described by Kraepelin when he discerned the diagnosis "dementia praecox," and there is a well-reported three-fold increase in the rate of schizophrenia in people with mild mental retardation (34). Moreover, the association seems strongly familial with a high rate of chromosomal rearrangements (35).

Two possible explanations exist for the observed comorbidities. Firstly, a candidate gene may have two entirely different functions in the body/cell, a phenomenon known as pleiotropy. For instance, individual mutant alleles of *GJB2*, a member of the connexin gene family of gap junction subunits, can give rise to either neurosensory deafness or a skin condition (hyperkeratosis), or a syndrome, which includes aspects of both disorders (Vohwinkel syndrome) (36). This phenomenon is not restricted to simple Mendelian genetics. A missense polymorphism in the *PTPN8/PTPN22* gene is present at twice the frequency in

Table 1 Genes Contributing to Neuropsychiatric Disorders Identified at the Sites of Chromosomal Translocations

Gene	Alternative	Disorder	Domains and function	Ref. Seq.	Chromo-some	Refs.
NTNG1	Netrin G1/Laminet-1	Rett syndrome	Peptide with axon guidance role. SCZ association?	NM_014917	1p13.3	100,101
USP6	Ubiquitin specific protease 6/TRE2 oncogene	Asperger	TBC domain (RAB interaction), protease domain, hominoidspecific	NM_004505	17p13.2	102
NBEA	Neurobeachin/BCL8B	Autism	A kinase anchoring protein (AKAP) function, PH-BEACH-WD40 domains—protein interaction/ subcellular localization	NM_015678	13q13.3	103,104
ST7	Suppressor of tumorigenicity 7, RAY1/FAM4A1/TSG7	Autism	No domains but mouse/drosophila/ nematode orthologues identified	NM_013437	7q31.2	105,106
AUTS2	ARG1/KIAA0442	Autism	PY motif. Mouse orthologue and other related sequences identified	NM_015570	7q11.22	107
SSBP1	Single-stranded DNA binding protein 1	Autism	Mitochondrial single-stranded DNA-binding protein (replication function)	NM_003143	7q34	108
GRPR	Gastrin releasing peptide receptor	Autism/multiple exostoses/mental retardation/ epilepsy	Gastrin-releasing peptide G-protein-coupled receptor (disruption has position effect on syndecan 2 gene?)	NM_005314	Xp22.13	109
IMMP2L	Mitochondrial inner membrane peptidase subunit 2 like	Gilles de la Tourette syndrome	Mitochondrial inner membrane peptidase subunit 2 like	NM_032549	7q31.1	110
CNTNAP2	Contactin-associated	Gilles de la Tourette	Neurexin family, potassium	NM_014141	7q35	111,112

	protein-like 2, CASPR2, NEUREXIN4	syndrome/OCD	channel interaction, membrane protein locate near axonal nodes of Ranvier			
DYX1C1	DYXC1	Developmental dyslexia	3 tetratricopeptide domains—protein interaction	NM_130810	15q21	113
FOXP2	Forkhead box P2, TNRC10, CAGH44	Speech and language development	Transcription factor, winged helix type	NM_014491	7q31.1	114–116
NPAS3	Neuronal PAS domain protein 3	Schizophrenia	Transcription factor, basic-loop-helix and PAS domain type	NM_022123	14q13.1	117–119
DISC1	Disrupted in schizophrenia 1	Schizophrenia	Globular N-terminal, helical c-terminal—protein interaction	NM_018662	1q42.2	120
MGAT5	mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyl transferase	Schizophrenia and learning disability	N-linked glycosylation pathway	NM_002410	2q21.2 - 2q21.3	121
GRIK4	KA1	Schizophrenia and learning disability	Ionotropic glutamate receptor, KAINATE type	NM_014619	11q23.3	122
PDE4B	Phosphodiesterase 4B	Schizophrenia	Regulatory and catalytic domains. cAMP catabolism.	NM_002600	1p31.2	123
DIBD1	Disrupted in bipolar disorder 1, ALG9, AL9	Bipolar affective disorder	N-linked glycosylation pathway	NM_024740	11q23.1	124
GRIA3	AMPA3, GLUR3	Bipolar affective disorder and learning disability	Ionotropic glutamate receptor, AMPA type	NM_181894	Xq25	125

Note: Disrupted genes and their gene symbols are grouped according to the resulting condition. Also listed are the genes' domain structures, database accession numbers, chromosome loci, and key references. Several reviews have been written describing these findings in more detail (126–130).
Source: Adapted from Ref. 130.

Caucasian cases with rheumatoid arthritis than Caucasian controls (37,38). The very same polymorphism has also been associated with an increased risk of type I diabetes (39). The underlying biology of the polymorphism—a change in amino acid sequence which prevents PTPN8 from associating with the CSK protein in the negative regulation of T cell activation—points to modulation of the immune system as an explanation for the pleiotropic actions. In the same fashion, it can be appreciated that deficits in, for example, synaptic function could impact on both cognitive and behavioral/psychiatric aspects in an individual with a comorbid diagnosis of schizophrenia with mental retardation.

Secondly, disrupted genes may have roles not only in adult brain function but also in the processes involved in development. One interpretation for comorbidity could be that dysfunction during development could lead to intellectual impairment, but the same gene may have a different role in the adult brain, leading to a psychiatric illness. This two-stage deficiency has been well documented in mouse gene knockout experiments where earlier developmental events can cloud the interpretation of the adult phenotypes. The adoption of inducible and tissue-specific knockouts using Cre-LOX technology has permitted the formal separation of such compound phenotypes. Many psychiatric illnesses, even if late onset, are now thought to have developmental origin and there are well-known examples of genes whose function alters at different stages of brain development. One example is *SHH*, the human form of the Sonic Hedgehog gene, whose disruption leads to holoprosencephaly. In early neural tube development the SHH protein is involved in the induction of proneuron proliferation and interacts with various Hox and other factors to control somite differentiation. Later in development, it is involved with the control of nerve cell formation in specific areas of the cerebellum (40).

CAVEATS FOR CYTOGENETICS RESEARCH

It may seem from the preceding description that the analysis of breakpoints is a perfect panacea for the clean definition of disease genes. Unfortunately, there are a number of issues that hamper the identification and interpretation of disrupted genes: some of these are technical and others biological.

Many instances exist where translocations have not been associated with any obvious clinical phenotype, even though a gene has been disrupted. Thus cell physiology may be robust enough to withstand haploinsufficiency of particular proteins. Because clinically well individuals are not routinely screened for chromosomal abnormalities, this number is likely to be underestimated. So it is quite possible that a chromosomal abnormality in an individual with schizophrenia is not the cause of the condition, even if gene disruption is shown to occur. Cosegregation of the abnormality with illness in an extended family [see disrupted in schizophrenia 1 (*DISC1*) in the following section] can provide the statistical confirmation for a genuinely causative role, as can multiple independent translocations affecting the same gene. Many of the individuals we have studied have either de novo mutations

or lack family information. Without further lines of evidence, there must always be a level of caution in linking breakpoint genes to illness.

Breakpoints that do not directly disrupt genes can also have a clinical outcome if gene regulatory mechanisms are disrupted (41,42). Enhancers are regulatory elements that interact with gene promoters to determine the level, location, and timing of gene transcription. Many instances have been described where enhancers are located at considerable distances up or downstream of the main protein-coding regions of the gene. The presence of a breakpoint outside the main transcription unit of a candidate gene (or even a break in a neighboring but uninvolved gene) has been observed in several instances in association with a definite clinical effect—the mechanism here seems to be a spatial separation of important enhancer elements from the gene itself, leading to regulatory alterations. In an analogous process, translocations can occasionally result in the chance apposition of heterochromatic (gene-silencing) and euchromatic (gene transcription-permissive) blocks of chromosomal material. In such well-described instances, where a translocation marries part of an inactive (heterochromatized) X chromosome to an autosome (43), there can be a spreading of heterochromatin into euchromatin such that local gene expression is silenced.

Finally, we have encountered a number of situations where breakpoints are located within highly repetitive or heterochromatic genomic regions. These are interesting genomic environments where the similarity and repetitiveness of the underlying DNA may increase the likelihood of rearrangements. In the special case of acrocentric chromosomes, the short arms are full of repetitive domains without much coding material and this is likely to be a reason for the relatively high frequency of Robertsonian translocations between acrocentrics. These usually have no clinical effect—such as the very common t(13;14), but some can lead to increased risks of trisomies in the offspring. However, these stretches of DNA can make precise definition of breakpoint positions very difficult—mostly due to repeat-mediated false-positive triple probe signals in FISH experiments. As these regions are generally within or proximal to heterochromatic regions, the gene density is usually very low and, as a consequence, the other breakpoint of the pair is more likely to be the location of the disrupted gene.

COMPLEX PHENOTYPES RESULTING FROM TRANSLOCATIONS: A PARADOX OR AN INSIGHT?

There remains a paradox resulting from the identification of a candidate complex disease gene through cytogenetics: how a *single* mutation can produce a condition that is typically thought to require *multiple* genetic interactions. At one level, this is fairly easily resolved. We would subscribe to the Garrodian view (44) that a clinical disorder related to a particular gene mutation (however caused and however extensive) will only develop when the effects of the mutation exceed the buffering capacities of the person's internal (genetic and metabolic) and external (social and cultural) environments to adapt to the adverse consequences. Although allelic variation in other genes (buffering potential) may account for a

large part of clinical variance, it can probably be assumed that the greater the functional consequence of a mutation, the more likely the deficit will exceed the buffering capacity of the person and the more pronounced and predictable (perhaps, more "Mendelian") the phenotypic outcome will be. Gene disruptions (or nonsense mutations, which are equivalent at a protein level) are likely to have greater functional consequences than other classes of mutations (Fig. 1). In short, the model proposes that the more deleterious the mutation, the greater the chance of destabilization (as opposed to perturbation) of the biological pathways and processes underlying the clinical condition.

Dementia of the Alzheimer's type is an example (and is described in much greater detail elsewhere in this book) of a disorder, in this case with clear pathophysiology (Alzheimer's disease), demonstrating independent modes of inheritance. Families with an early-onset presentation were tractable to the approach of linkage mapping, which resulted in the identification of the gamma-secretase activity gene Presenilin 1 (*PSEN1*) on chromosome 14 and, by homology, *PSEN2*. Amyloid precursor protein (encoded by the *APP* gene) was identified because it aggregates in the

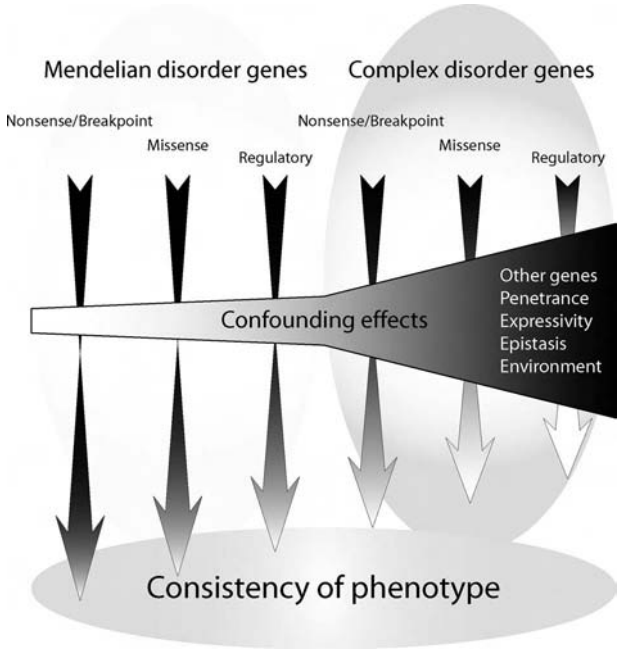

Figure 1 The paradox of single genes of major effect in complex disorders. Hypothesis: the nature of the mutation event affects the genotype-phenotype correlation (shown decreasing in strength from left to right–*large arrows*). This is particularly the case for complex genetic disorders where breakpoints may highlight potential candidate genes by amplifying their link with a disease phenotype.

pathological lesions found in Alzheimer's but also through the extremely common comorbidity of early onset dementia (and Alzheimer's pathology) with trisomy 21. However, from a numerical point of view, very few cases/families (258) have been described with *PSEN1* mutations and 39 and 15, respectively, with *APP* or *PSEN2* mutations (45). Late onset dementia of the Alzhemier's type has been much more refractory to analysis. Molecular variation in the *APOE* gene on chromosome 19 is common in the general population and has been very extensively investigated. It is the key example where a common variant of a gene significantly alters risk to a neuropsychiatric disorder, perhaps, at least partly in this case, by altering the age at onset for the condition (46). The *APOE4* variant has been shown to be a clear risk factor in many populations, including people with Down syndrome. Through modulation of lipid transport activity, the APOE4 protein isoform predisposes towards dementia in a dose-dependent fashion (3-fold risk when heterozygous, 15-fold when homozygous).

Thus, the dementias of the Alzheimer's type provide a variety of important models—a common codominant and weakly penetrant variant of one gene predisposes the carrier to dementia, and rare, familial, deleterious mutations in three other genes guarantee the carriers a clinically similar condition.

A number of other recently discovered disorder genes can also support and extend this model whereby mutation type determines the classification of the disease as either Mendelian or complex. We present four of these in the following text and in Figure 2.

The nuclear receptor peroxisome proliferator-activated receptor gamma gene, *PPARG*, is strongly implicated in type II diabetes/insulin resistance/hypertension through the study of a number of rare families, some with mutations that lead to a dominant negative action of the resulting protein and another with a frameshift mutation resulting in a premature stop codon (comparable with breakpoint disruptions) (47–49). In the latter family, not all carriers were affected, but all affected individuals were carriers—an example of a dominant pattern of inheritance with reduced penetrance, which is mirrored in the studies of the *DISC1* translocation family (see following text). It is now also reported that a relatively common missense polymorphism in *PPARG* is responsible for a modest protective effect in the Finnish population: decreased PPARG receptor activity, lower body mass index, and improved insulin sensitivity.

Missense mutations in the nucleotide-binding domain of the *CARD15/NOD2* gene have been identified in families with the autosomal dominant arthritic condition, Blau syndrome (50). However, a relatively common (and, presumably, less deleterious) frameshift/premature stop codon polymorphism in the leucine-repeat region of the same gene predisposes individuals to Crohn's disease, especially when inherited in the homozygous form (51,52). This represents an instance where the functional outcome of the mutation is the key factor dictating not only the predictability but also the precise nature of the phenotype. The apparent discrepancy between the clinical phenotypes of the two forms of mutation in this single gene has

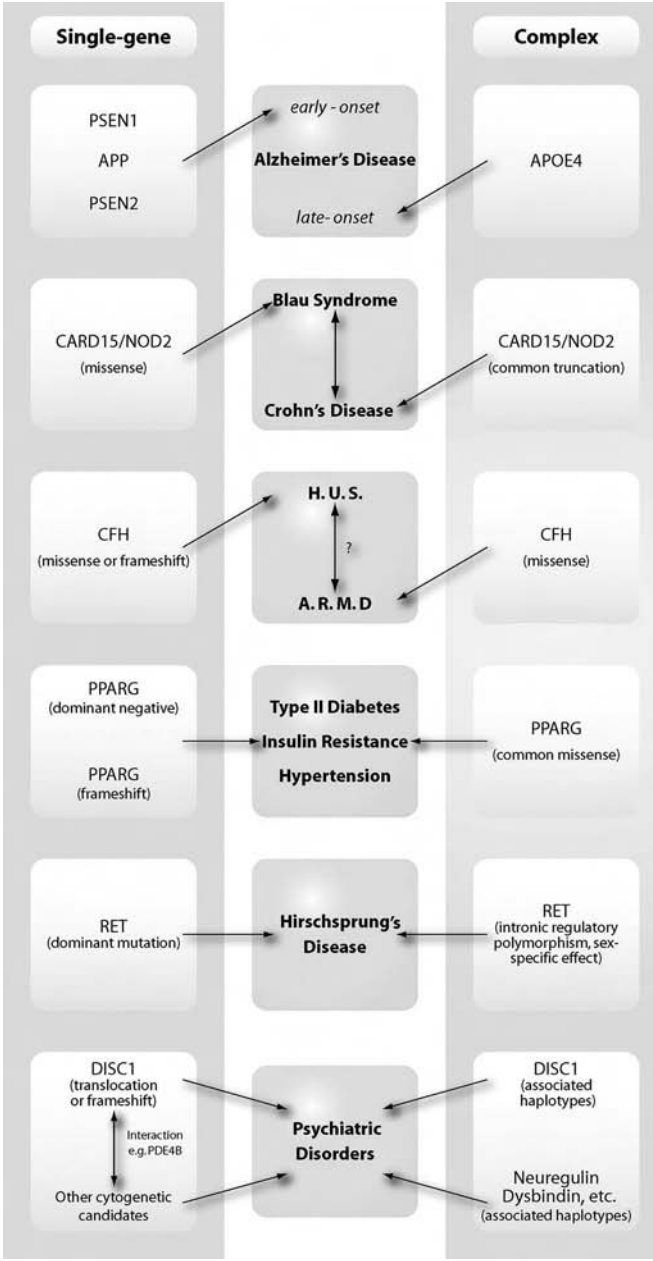

Figure 2 Several examples of genetic disorders where the nature of the mutation (genotype) affects the correlation to phenotype. An example of how this model may act in the study of psychiatric disorders is shown in the bottom panel.

been partially resolved through the appreciation that both conditions display an inflammatory response and involve epithelioid cell lineages.

The earlier-mentioned *CARD15* example is entirely analogous to findings in another gene involved in immune response. One missense mutation and one frameshift/premature stop mutation in the complement factor H gene, *CFH*, have been reported in patients with hemolytic uremic syndrome (HUS) (53). In addition, *CFH* has particular functional polymorphisms, which seem to increase risk to the eye condition, age-related macular degeneration (ARMD) (54). In this case, the common biological link—or presence of true pleiotropy—has yet to be discovered. Interestingly, a similar finding has been reported for the gene, *ABCA4*, in which some mutant alleles lead to the severe eye disease, Stargardt syndrome, whereas other variants contribute to the risk for ARMD.

Dominant mutations within the *RET* gene receptor tyrosine kinase have been described in Hirschsprung disease (HSCR; aganglionic megacolon). Recently, however, a sex-dependent susceptibility effect of an intronic enhancer element polymorphism has been reported (55). These findings also closely fit a model whereby the severity of the effects of a mutation correlate with its power to predict the phenotype. HSCR is also a well-described feature in a percentage of children with Down syndrome suggesting that increased dosage, or particular pattern, of alleles of a chromosome 21 gene may also predispose to this condition.

To summarize these examples, it appears that there is a link between the properties of the phenotype (strongly versus weakly penetrant, Mendelian versus complex) and the consequences of the underlying gene mutation on the functionality of its encoded protein. As a corollary, it can be appreciated that mechanisms such as direct disruptions by chromosomal breakpoints are more likely to lead to more severe consequences for protein function than missense polymorphisms—and these consequences, exceeding the cell's buffering capacity, may result in more consistent phenotypes. In terms of the paradox of the disrupted genes, perhaps the particular examples highlighted here may bring about a blurring of the distinction between simple versus complex disorders from an appreciation that such extremes exist, in part, because of the effects and prevalences of the underlying gene mutations.

TESTING CANDIDATE GENE ALLELES IN THE POPULATION: AN UNHELPFUL DOGMA?

We have addressed the issue of whether single, chromsomally disrupted genes can contribute significantly to a complex disorder. The next issue is whether or not these genes increase risk of neuropsychiatric disorders in the karyotypically normal population. To answer this invariably requires that the gene's candidacy be tested in an association study where marker alleles and their associated haplotypes are examined in case and control groups to search for biases in frequency. A statistically positive association implies the existence of a nearby causative mutation in linkage disequilibrium with the selected markers—a trigger

for resequencing studies of that region to identify and characterize the responsible mutation(s).

However, this approach has a somewhat arbitrary air to it—the set of contributory gene alleles giving rise to "noncytogenetic" complex neuropsychiatric conditions existing solely because of random ancestral mutations and their historical passage through diverging populations. This set is merely a subset of all the genes that could potentially give rise to the conditions. Thus, the set of human disease genes is an accident of chance distilled by history (especially relatively recent genetic history) and long-term selection. This has practical implications for those investigating disrupted genes in association studies and theoretical implications for the model we proposed earlier.

If the candidate gene shows association with the condition in a case-control study, then that represents an instance not dissimilar from the *RET* and *PPARG* examples described earlier—different mutations with different functional consequences give rise to the same disorder but with differing certainty. If the association proves negative (in the face of exhaustive and careful experimental design and implementation), this does not indicate that the gene is irrelevant to the disease. Although it does exclude an epidemiologically important role for the candidate gene, it does not exclude its involvement in the biology of the condition (e.g., *APP* in Alzheimer's disease). Supporting evidence for the latter role could come from three potential directions. Firstly, the existence of statistically significant cosegregation between the presence of a translocation (or rare deleterious mutation) and disease status in a large pedigree as has been observed for the gene *DISC1* (in the following text). Secondly, multiple cytogenetic "hits" on the same gene or locus in the same condition: for example, cleft palate and the *SATB2* gene (56), the *PAX6/WT1* region in aniridia/WAGR syndrome (41), and Beckwith-Wiedemann syndrome and the "imprinting centre" on the short arm of chromosome 11 (57,58). Thirdly, it may be possible to relate the function of the disrupted gene directly into pathways and processes known to be central to the disease etiology (see *GRIK4* in the following text). This is in keeping with a model that disruption of many different individual genes may have the potential to give rise to the same clinical condition (in a manner akin to a set of genes encoding multiple enzymes present in a linear metabolic pathway) but only one or a few have actually picked up pathological polymorphisms in human genetic history, or conversely only a few have survived selection through recent history to permit their current detection.

In the context of the search for novel therapeutic strategies for neuropsychiatric disorders, there may presently exist an unhelpful bias of attention toward culprit genes rather than culprit pathways and a reluctance to pursue rare cytogenetic candidates that do not obviously contribute to population risk. In other words, are we so convinced, as a body of researchers, that the next generation of therapeutic targets for novel antipsychotic drugs, for example, will only be found using methods with an epidemiological bias?

TOWARD A MECHANISTIC UNDERSTANDING OF NEUROPSYCHIATRIC DISORDERS?

What have the genes identified through cytogenetics told us to date about the underlying biological pathways of neuropsychiatric disorders? Table 1 lists the genes identified in the common neuropsychiatric disorders and it can be seen that studies on autism and schizophrenia predominate. Although there may be a bias toward karyotyping patients with autism due to the clear association with mental retardation, the situation for schizophrenia and bipolar disorder is less clear and many of the described genes are in individuals who do not have mental retardation (although an increased interest in karyotyping people with schizophrenia occurred during the 1970s when an association with sex chromosome aneuploidies was postulated). At present, progress on the characterization of cytogenetic origin autism genes is limited, both at the level of association studies and also in the functional analysis of the encoded proteins. For this reason, the remainder of this section will detail findings from schizophrenia and bipolar disorder.

As mentioned previously, *DISC1* is the most studied example of a gene candidate identified through cytogenetics in the neuropsychiatric disorders. Its discovery owes a great deal to the large-scale cytogenetic screens carried out by the Medical Research Council in Edinburgh in the 1960s. The presence of a t(1;11) chromosome translocation was observed in a person in a young offenders institution and in the proband's extended family (59). Later clinical diagnosis, blind to karyotype status, revealed significant linkage between the translocation and major mental illness (LOD score 7.1) (60). After considerable effort in the pregenome era, a gene, *DISC1*, located on the long arm of chromosome 1 (1q42.2) was shown to be disrupted by one of the translocation breakpoints (54,61). Although expressed in regions of the brain implicated in schizophrenia (62–65), this gene displayed no significant homology to other genes in the database and required a multidisciplinary approach to place it in within a clear biological and pathological context. In just five years, however, the results of these studies have placed *DISC1* at the forefront of the various candidate genes for psychiatric conditions.

The Primary Disrupted in Schizophrenia Mutation and Association with Disease in the Population

A number of groups have carried out case-control association studies on *DISC1* both in schizophrenia and bipolar disorder populations (66–74). As has been seen with other noncytogenetic-derived candidate genes for schizophrenia such as neuregulin and dysbindin, associations have been detected in many of these studies, but there is no clear consensus positional evidence from haplotype analyses where the causative mutation lies within the gene. For example, results from our laboratory suggest that a region of the *DISC1* gene confers nominal risk to both sexes for schizophrenia but strong risk to females for bipolar disorder (73). Results from the Finnish population show association with a different haplotype

(HEP3) with a protective effect against schizophrenia; again a sex-specific observation (75). Clearly, reasons must be identified for these study differences. At present, explanations range from the practical (differences in SNP choice between studies) to epidemiological (population differences highlighting locus and allelic heterogeneity). Another feature of these studies is the broad nature of associated conditions/phenotypes; schizophrenia and bipolar disorder appear to be associated with *DISC1* variation in a number of studies, but so too is visual working memory in people with schizophrenia, and neurocognitive function (verbal working memory) and cognitive aging in the non-ill population (76–80). These reports complement earlier studies on subjects from the translocation family itself, which demonstrated an association between the *DISC1* disruption and decreased amplitude of the P300 event-related potential (of complex cognitive origin, but may depend on short-term memory efficiency) (60). Importantly, amplitude reductions were observed in translocation carriers even if they did not have psychotic symptoms. This suggests that the psychiatric illness might be the secondary (if highly probable) consequence of primary *DISC1* disruption effects on a number of cognitive and/or developmental domains. A second rare form of *DISC1* mutation has recently been described in members of a small family with schizophrenia and schizoaffective disorder (although cosegregation between mutation and illness is not entirely clear in this instance). The frameshift mutation causes an altered reading frame for nine amino acids followed by a premature stop codon, resulting in the loss of the extreme DISC1 C-terminus (81). In parallel, recent publications (82,83) have described the identification of a deletion within all "129"-derived strains of laboratory mouse. This has implications not only for transgenic work into behavior (as embryonic stem cells are frequently derived from this strain) but also for the testing of *DISC1*'s biological properties in vivo. These discoveries, together with the original translocation and the association findings, suggests that *DISC1* fits the *RET* gene paradigm of complex disease—it can act either as a "quasi-dominant" factor (subject to the vagaries of penetrance) or as a general risk/protective factor in the population.

Protein Interaction Studies Suggest Disrupted in Schizophrenia Functions as a Hub for the Assembly of Distinct Protein Complexes

The study of lower organisms such as Drosophila and yeast has repeatedly shown that functionality can be frequently ascribed to novel gene products by defining the set of proteins with which their own product interacts. This has been the goal of several DISC1 yeast two-hybrid studies since 2000. In fact, the studies all point to DISC1 as an "organizing hub" for a number of cellular processes taking place in a variety of subcellular locations (mitochondrion, centrosome, nucleus, cell membrane etc.). A unifying feature of these complexes seems to be the involvement of the microtubule cytoskeleton implying that DISC1 may be involved in trafficking and/or assembly of functional multicomponent complexes at structurally defined regions of the cell as well as neuronal migration (84–86). The

proteins that have been identified as interactors with DISC1 include DISC1 itself (homodimerization), NUDEL, NUDE, MAP1A. MIPT3, ATF4/5, FEZ1, KENDRIN, α-TUBULIN, γTUBULIN, dynactin, dynein intermediate chain, LIS1, ARHGEF11, AKAP9, GRIPAP1, eIF3(p40), and PDE4B (57,85,87–93).

Emerging Pathways and Biology

Two recent publications have further clarified the roles of DISC1 in two biological systems. When at (1;16) translocation carried by an individual with chronic schizophrenia was studied, the breakpoint on the short arm of chromosome 1 was found to directly disrupt a gene coding one transcript form of *PDE4B*. Subsequently, PDE4B was identified as a DISC1 interactor through a yeast two-hybrid screen (57). *PDE4B* encodes a member of the phosphodiesterase enzyme family responsible for the breakdown of the secondary messenger, cAMP. In essence, PDE enzymes act as an "off switch" for neuronal signaling through G-protein-coupled receptors. Historically, PDE4 proteins have been studied as the targets of the antidepressant Rolipram and as the molecular basis for the "dunce" learning and memory mutant in Drosophila. Thus, there is an established biological functionality that can be readily integrated into the DISC1 system via its interaction with PDE4B. The DISC1–PDE4B interaction appears to be dynamically correlated to PDE4B activity and modulated by phosphorylation events (57). Hence, this is an example where independent cytogenetic findings have demonstrated convergence at the level of protein biochemistry. It is to be hoped that other disrupted genes will show similar shared functional connectivity in the future. Questions still remain as to the physiological location of DISC1–PDE4B interactions and the manner in which this influences the cognitive underpinnings of psychiatric disorders.

Another recently explored aspect of DISC1 function has centered on DISC1–NUDEL interactions at the centrosome (86). An experimentally produced truncated form of DISC1 appears to act in a dominant negative fashion through dimerization with wild-type DISC1 and acts to destabilize the dynein–microtubule complex at the centrosome. In vivo, the developmental consequence in mouse of transfection of the mutant form of DISC1—and, alternatively, RNAi species directed against mouse *Disc1* transcripts—is abnormal neuronal migration from the ventricular zone to the cerebral cortex during embryogenesis. Similar patterns of disorganization have been seen in milder forms in postmortem brains of individuals with schizophrenia and in a much more severe form in the lissencephalies (neuronal migration disorders resulting in abnormal cortical lamination that involve another DISC1 interactor, LIS1) suggesting that DISC1 may play an important role in developmental aspects of schizophrenia and bipolar disorder.

We have also studied two other translocation-disrupted genes and investigated their roles in the emerging biology of schizophrenia and bipolar disorder.

The chromosome 14 breakpoint of a balanced translocation between chromosomes 9 and 14 in a mother with schizophrenia and daughter with psychosis and

mental retardation disrupts a neuronal transcription factor, *NPAS3* (51–53). This gene is expressed in inhibitory interneurons and has been knocked out in a transgenic mouse strain, animals from which show deficits in behavioral tests including prepulse inhibition as well as alterations in social recognition and locomotor activity (94,95). Furthermore, Npas3 deficiency appears to attenuate Fgf receptor expression in the hippocampal dentate gyrus and increase expression of the Sprouty 4 inhibitor of the Fgf signaling pathway (96). Deficits in this pathway might explain the observed decreased neurogenesis and smaller hippocampal volume in these mice. Interestingly, these same morphological changes have direct counterparts in the brains of individuals with schizophrenia, suggesting possible NPAS3 involvement in the aberrant neurodevelopmental pathways that lead to the psychiatric disorder.

We have also identified an ionotropic glutamate receptor, *GRIK4* (*KA1*), disrupted in an individual with schizophrenia and mild mental retardation (56). Another glutamate receptor, *GRIA3*, has also been described by others as disrupted by a translocation in a family segregating bipolar disorder (59). Glutamate receptors are prime candidates in contributing to the etiology of psychiatric illness, in part, from the clear psychotomimetic effects of phencyclidine and ketamine—glutamate receptor antagonists (97). Our case-control association study on *GRIK4* in karyotypically normal populations revealed two distinct regions of association within the gene, one with schizophrenia and another with bipolar disorder—a finding, which, just like the *DISC1* findings already described, supports the belief that these two conditions may in some cases share common genetic origins (98,99).

SUMMARY

The Human Genome Project with its associated technologies such as single nucleotide polymorphism (SNP) genotyping has brought within grasp the prospect of high-density, genome-wide linkage and association studies. Although this strategy may bring us closer to the holy grail of disease genetics—a definitive set of risk-causing alleles in biologically relevant genes—it is not without difficulties and will require an immense effort in terms of clinical resources and marker genotyping. In this review, we have shown a very powerful complementary strategy—identifying and characterizing candidate genes disrupted by cytogenetic rearrangements. This approach has yielded *DISC1*, arguably the best genetically replicated and biologically confirmed candidate gene for severe psychiatric illness and, in addition, identified *PDE4B*, *NPAS3*, *GRIK4* and others, each of which integrates into and makes sense within the framework of our current knowledge and understanding of schizophrenia and each of which provides a concrete starting point for further genetic and biological validation.

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4

Neurotransmission

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INTRODUCTION

Schizophrenia and bipolar disorder, two of the most prevalent psychiatric disorders, have been considered to be nosologically and etiologically distinct. However, both "lifetime" disorders have striking similarities in prevalence rates and risk factors: their lifetime prevalence rates are similar (~1%) and stable across countries and cultures, and male-to-female ratios have a broad overlap for patients 18 to 30 years old (1). The causes of schizophrenia and bipolar disorder are unknown, but multiple lines of evidence suggest that both genetic and environmental factors contribute to their pathophysiology (2–5). Furthermore, both disorders have been implicated in multiple neurotransmission abnormalities involving the dopamine (DA), serotonin [5-hydroxytryptamine (5-HT)], acetylcholine (ACh), glutamate, and γ-aminobutyric acid (GABA) systems (Table 1) (6–24).

The DA hypothesis of schizophrenia has been well established for half a century. Drugs acting at the DA D_2 receptors are commonly used to alleviate symptoms in bipolar disorder and other psychiatric disorders as well as schizophrenia, suggesting the common role of DA neurotransmission in their pathophysiology (Table 1) (6). Psychostimulants (indirect DA receptor agonists),

including amphetamine and methamphetamine, induce psychosis in human subjects. Recent studies have demonstrated that in the two disorders, the catechol-*O*-methyltransferase (*COMT*) gene, which encodes for an enzyme involved in DA metabolism, is located in the 22q11 candidate region (2–4). 5-HT has a wide variety of effects on proliferation, migration, neurite outgrowth, and synaptogenesis, and 5-HT neurotransmission is also involved in the pathophysiology of schizophrenia and bipolar disorder (13,20). Atypical antipsychotic drugs such as clozapine have high affinity with 5-HT receptors (Table 1). GABA is the major inhibitory neurotransmitter in the brain, and is synthesized in a single step from L-glutamate by the enzyme L-glutamic acid decarboxylase. Much of the glutamate and GABA used in neurotransmission is derived from glial storage pools of glutamine. It has been shown that changes in glutamatergic and GABAergic neurotransmission might be implicated in the pathophysiology of both schizophrenia and bipolar disorder (21–24). Benzodiazepine/GABA receptor agonists such as diazepam are useful adjuncts to antipsychotic medications in the acute psychosis of schizophrenia and bipolar disorder (Table 1).

This chapter provides an overview of the roles of neurotransmission (especially glutamate and ACh) in the pathophysiology of schizophrenia and bipolar disorder. The potential therapeutic approach for these disorders is also addressed. We do not cover the roles of DA, 5-HT, and GABA in the pathophysiology of these disorders; this information is reviewed elsewhere (6,13,21–24).

GLUTAMATERGIC NEUROTRANSMISSION

Glutamate

L-Glutamic acid (glutamate) is accepted as the major excitatory neurotransmitter in the nervous system. It plays a major role in brain development, affecting neuronal migration, neuronal differentiation, axon genesis, and neuronal survival. Multiple lines of evidence have suggested that dysfunction in glutamatergic neurotransmission via the *N*-methyl-D-aspartate (NMDA) receptors might be involved in the pathophysiology of schizophrenia (Table 1) (7–15). The NMDA receptors are modulated by glycine, D-serine, kynurenic acid, polyamines, and specific divalent cations including magnesium and zinc (Fig. 1), as well as by glutamate. Taken together, it seems that abnormal levels of these endogenous substances might potentially lead to decreased activation of the NMDA receptors, and that the endogenous dysfunction of NMDA receptor-mediated neurotransmission might contribute to the pathophysiology of schizophrenia (15). Furthermore, it is also suggested that dysfunction in glutamatergic neurotransmission might be implicated in the pathophysiology of bipolar disorder (16–19, 25–27).

Kim et al. (28) first reported that the cerebrospinal fluid (CSF) levels of glutamate in schizophrenic patients were significantly decreased compared with normal controls. However, several studies found no changes in CSF glutamate levels of patients with schizophrenia (29,30) and of first-episode and drug-naive

Table 1 Role of Neurotransmitters in the Pathophysiology of Schizophrenia and Bipolar Disorder

Neurotransmitter	Drug	Mechanisms of action and effects in human subjects
DA	Amphetamine, methamphetamine	Increase synaptic DA levels; induce psychosis
	Antipsychotic drugs	Antagonists of DA D_2 receptors; block psychosis
Serotonin	Antipsychotic drugs (atypical)	Binding to 5-HT_{2A} receptors; block psychosis; decrease side effects
Glutamate	Phencyclidine, ketamine	NMDA receptor antagonists; induce psychosis and cognitive deficits
	D-serine, D-cycloserine, glycine	Agonists of glycine modulatory sites on NMDA receptors; improve psychotic symptoms including cognitive deficits
	Sarcosine	Glycine transporter-1 inhibitor; improve psychotic symptoms including cognitive deficits
Acetylcholine	Nicotine	Agonist of nicotinic receptors; improve cognition
	Tropisetron	α7 nicotinic receptor (α7 AChR) agonist and 5-HT_3 receptor antagonist; improve deficits of auditory sensory gating P50
	DMXB-A	α7 AChR agonist; improve deficits of auditory sensory gating P50
GABA	Diazepam (benzodiazepines)	Benzodiazepine/GABA receptor agonist; improve psychosis (in adjunction to antipsychotic drug)

Abbreviations: DA, dopamine; GABA,γ-aminobutyric acid.

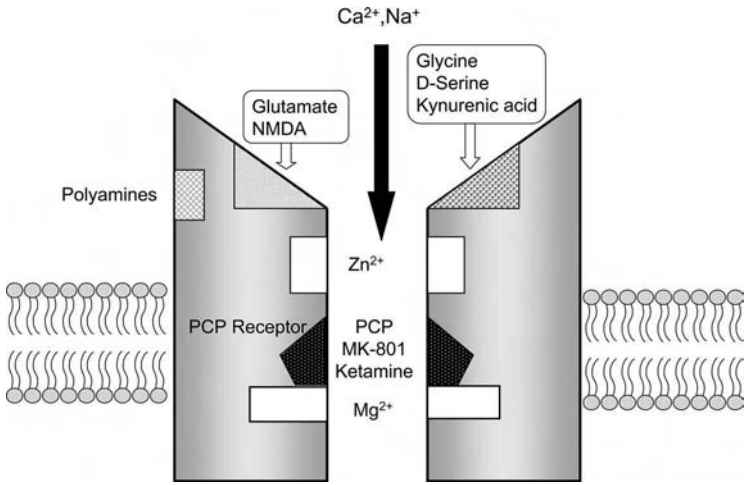

Figure 1 The NMDA receptor complex. Glutamate and NMDA bind to the agonist site on NMDA receptors. PCP, dizocilpine [(+)-MK-801], and ketamine bind to the PCP-receptor on the inside of the NMDA receptors. Glycine and D-serine bind to coagonist sites (glycine modulatory sites) on NMDA receptors, and kynurenic acid binds to coagonist sites as a noncompetitive antagonist. *Abbreviations*: NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine.

patients with schizophrenia (31). In postmortem studies of schizophrenia, the brain levels of glutamate in the hippocampus and frontal cortex of schizophrenia demonstrated significant alteration (32). Other studies showed no differences between the glutamate levels of schizophrenic patients and normal controls (29,30,33). Hashimoto et al. (33) found that the levels of glutamate in the postmortem brains of bipolar disorder patients were significantly higher than those of normal controls, suggesting that abnormality of glutamatergic neurotransmission might be implicated in the pathophysiological features of bipolar disorder. It is shown that mood stabilizers such as lithium and valproate exert neuroprotective effects against glutamate-induced excitotoxicity (34,35). Therefore, these mood stabilizers might, in part, exert therapeutic effects via neuroprotective action against glutamate-induced excitotoxicity.

In the CNS, glutamine synthesis from glutamate and ammonia occurs exclusively in the astrocytes. Glutamine plays major roles in nitrogen and carbon homeostasis, in the detoxification of ammonia, and as a precursor for the synthesis of neurotransmitter glutamate and GABA in specialized excitatory and inhibitory neurons (36). Glutamate is released from presynaptic neurons, and this amino acid can interact with postsynaptic glutamate receptors, including kainite, γ-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and NMDA receptors. Released glutamate is taken up by surrounding astrocytes via the glutamate transporter, where it is converted to glutamine, transported back to the presynaptic

neurons, and reconverted to glutamate (36). A magnetic resonance spectroscopy (MRS) study demonstrated that levels of glutamine were significantly increased in the medial prefrontal cortex of never-treated schizophrenic patients, whereas the levels of N-acetylaspartate (NAA) and glutamate were not altered in these patients (37). A recent 4.0 T MRS study has shown that the levels of glutamine are significantly increased in the left anterior cingulate cortex and thalamus of never-treated schizophrenic patients, whereas the levels of other substances (NAA, glutamate, taurine, choline, creatine, myoinositol) are not altered in these patients (38). These findings of increased glutamine in schizophrenic patients may indicate an abnormality in the cycle of conversion of glutamine to glutamate in the human brain. Recently, we found that the ratio of glutamine to glutamate in the CSF of first-episode and drug-naive patients with schizophrenia was significantly higher than that in the controls, although the glutamine and glutamate levels in the patients were not significantly different from those of the controls (31). These findings suggest that the increased ratio of glutamine to glutamate in the CSF sample may reflect impairment of the glutamate-glutamine cycle in the brains of schizophrenia (15,31).

D-Serine

Research during the past decade has consistently revealed significant levels of D-serine in mammal brains. The distributions of D-serine and NMDA receptors in the rat brain are similar, suggesting that D-serine might be an endogenous agonist on NMDA receptors (39,40). Immunohistochemical studies have revealed that endogenous D-serine is localized in the astrocytes of the forebrain grey matter, near or ensheathing the NMDA receptor synapses (40,41). Agonists of the non-NMDA subtypes of the glutamate receptors promote the release of D-serine from cultured astrocytes (42). The origin of D-serine in mammals was unclear until serine racemase (SRR), which catalyzes direct conversion of L-serine to D-serine, was isolated from the brain (43–45). Very recently, it has been demonstrated that knockout mice for the *SRR* gene show decreased levels of D-serine in the mouse brain (46), suggesting the role of SRR in the production of D-serine in the brain. Taken together, these findings suggest the role of D-serine as an endogenous agonist at the glycine modulatory sites on NMDA receptors (47).

Treatment with D-serine revealed significant improvements of positive, negative, and cognitive symptoms in schizophrenic patients treated with antipsychotic drugs (48), suggesting that levels of D-serine may be decreased in the brains of schizophrenic patients. It has been reported that serum levels of L-serine in patients with schizophrenia were significantly increased compared to normal controls, and that serum levels of D-serine and the ratio of D-serine to total serine in patients with schizophrenia were markedly decreased in comparison to those of normal controls (49,50). At the ultrastructural level, D-serine staining appears patchy, and is most abundant around the blood vessels in the forebrain (41). Furthermore, no changes of D-serine in the forebrain of mice lacking D-amino acid oxidase (DAO) were demonstrated (51,52), suggesting that DAO

does not regulate D-serine levels in the forebrain where NMDA receptors are abundant. It is currently unclear how D-serine is metabolized or excreted in the forebrain. It seems that astrocytic D-serine might activate glycine modulatory sites of NMDA receptors on the spines of neurons near blood vessels to regulate excitatory neurotransmission. These lines of evidence suggest that D-serine may be swept away by the blood vessels, and that the resulting D-serine in the blood may originate from the brain (15).

Recently, we found that the ratio of D-serine to total serine in the CSF of first-episode and drug-naive patients with schizophrenia was significantly lower than that of the controls (53), suggesting that the synthetic and/or metabolic pathway of D-serine may be impaired in the brains of schizophrenic patients. In the well-characterized set of autopsied brains (Brodmann area 6) obtained from the Stanley Foundation, Hashimoto et al. (33) reported that D-serine levels in the postmortem brain samples of schizophrenia were not different from those of controls. One possible explanation may be the difference in brain regions studied. It was reported that changes in glutamate and N-acetylaspartylglutamate (NAAG) were selective and restricted primarily to the prefrontal cortex and hippocampus (32). Therefore, it is of interest to study whether D-serine levels are altered in the prefrontal cortex and hippocampus of schizophrenic patients. Another explanation may be the different effects of D-serine during development. It has been suggested that D-serine may play different roles in the adult brain than in the developing brain, which may be mirrored in distinctive localizations at different ages (54). A recent study has demonstrated that D-serine plays an important role in neuronal migration (55), suggesting that D-serine serves as a coagonist for NMDA receptor-dependent cell migration at the development stage. Therefore, it is possible that the levels of D-serine in the brain may be altered at the developmental stage or at the onset of schizophrenia.

G72, D-Amino Acid Oxidase, and Serine Racemase

The G72 gene was identified in a genomic region where association with schizophrenia had been significant (56). Because G72 protein was shown to interact physically with DAO and to regulate its activity in vitro, G72 has now been renamed D-amino acid oxidase activator (DAOA).

Although D-serine metabolism thus presents a promising candidate pathway, few studies have systematically tested its multiple components for genetic association. In the analysis of a German case-control sample set, association of DAO with schizophrenia was replicated, whereas interaction between G72/G30 and DAO genotypes was not (57). In a case-control analysis of a Japanese cohort, no evidence of association was detected for either DAO or serine racemase (SRR), and serum concentrations of D-serine did not correlate with DAO or SRR genotypes (50). Further studies are needed for sufficient interrogation of genomic variation in the analysis of larger samples, and possibly for testing of additional genes involved in D-serine metabolism.

The G72/G30 locus was found to be associated with bipolar disorder as well (58). The associations of G72/G30 with schizophrenia (57,59,60–64) and with bipolar disorder (57,58,65) have both been replicated in multiple populations, and constitute evidence that the same gene locus may predispose individuals to both psychoses as previously predicted from the linkage findings (66,67). A recent meta-analysis (68), which gave significant results for both illnesses, adds to credibility of this notion, although effect of publication bias cannot be ruled out. There are unanswered questions, though. As is the case for other promising positional candidate genes, associated genomic variants of G72/G30 are not always consistent among studies (68). It is unclear whether causal mutations are shared by the two distinct disease categories or by distinct populations. Also, one can ask whether any clinical domain is more specifically associated with G72/G30 locus than either schizophrenia or bipolar disorder. This issue has been addressed but no consensus has been reached yet. One study attributed association of G72/G30 with bipolar disorder to persecutory delusion rather than to the general disorder category (69), whereas another demonstrated that G72/G30 locus primarily affects susceptibility to mood episodes rather than psychotic symptoms in both schizophrenia and bipolar disorder (70).

Potential Therapeutic Approach

As already described, the hypofunction of the NMDA receptors might be implicated in the pathophysiology of schizophrenia. Therefore, the NMDA receptor antagonists [phencyclidine (PCP), dizocilpine ((+)-MK-801), ketamine] have been used widely in animal models of schizophrenia (71–73). The NMDA receptor antagonists such as PCP and ketamine are known to induce schizophrenia-like symptoms including cognitive deficits in healthy subjects (7,12). Therefore, NMDA receptor antagonists such as PCP have been used in animal models of cognitive deficits because cognitive deficits in patients with schizophrenia are a core feature of the illness, resulting in vocational and social disabilities (74). Recently, Hashimoto et al. (75) reported that repeated administration of PCP (10 mg/kg/day for 10 days) caused cognitive deficits in mice for a long time (more than six weeks after a final administration of PCP), and that PCP-induced cognitive deficits could be improved by subsequent subchronic (two weeks) administration of clozapine, but not haloperidol. Therefore, reversal of PCP-induced cognitive deficits may be a potential animal model of atypical antipsychotic activity in relation to amelioration of cognitive deficits in schizophrenia (75,76). In this model, we recently found that treatment with D-serine or (R)-(N-[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl])sarcosine (ALX-5407), a selective glycine transporter-1 (GlyT-1) inhibitor, could attenuate PCP-induced cognitive deficits in mice (77). Interestingly, in a recent six-week double-blind, placebo-controlled trial of the GlyT-1 inhibitor N-methylglycine (sarcosine: 2 g/day), schizophrenic patients who received sarcosine treatment showed significant improvements in their positive, negative, cognitive, and general psychiatric

symptoms (78). Sarcosine was also well tolerated, and no significant side effects were noted (78). A recent double-blind, placebo-controlled study has demonstrated that sarcosine, superior to D-serine, can benefit not only patients with long-term stable disease but also acutely ill persons with schizophrenia, suggesting that a GlyT-1 inhibitor may be more efficacious than NMDA-glycine site agonists for adjuvant treatment of schizophrenia, at least during the acute phase (79). In contrast, sarcosine produced no greater improvement when coadministered with clozapine than placebo plus clozapine at weeks 2, 4, and 6 (80). Thus, unlike patients treated with other antipsychotiocs, patients who received clozapine treatment exhibit no improvement by adding sarcosine (80). These findings suggest that pharmacological modulation of glycine modulatory sites on the NMDA receptors by GlyT-1 inhibitors might be beneficial in the treatment of cognitive deficits as well as psychosis in several psychiatric diseases including schizophrenia (81–85). In addition to approaches aimed at modulation of glycine by GlyT-1 inhibitors, modulation of D-serine has been proposed as a possible target. It is reported that inhibition of DAO may enhance D-serine-mediated activity at the NMDA receptor, and that mutant mice lacking DAO show enhanced NMDA receptor-mediated electrophysiological responses (86). Therefore, it seems that DAO inhibitors or agents that promote the conversion of L-serine to D-serine by enhancing SRR activity would be potential therapeutic drugs for schizophrenia (83). In addition, agents that modulate the serine racemase-interacting proteins, including the glutamate receptor-interacting protein (GRIP) (55,87,88) and protein interacting with C kinase 1 (PICK1) (87,89), may also be potential therapeutic drugs for these psychiatric disorders.

CHOLINERGIC NEUROTRANSMISSION

Acetylcholine

ACh has been implicated in cognitive processing, arousal, and attention in the brain. Neurotransmission by ACh can occur through muscarinic (G protein-coupled) or nicotinic (ionotropic) receptors, and is terminated by the action of cholinesterases. At present, five different subtypes of the muscarinic receptors (mAChRs) and 17 different subunits of the nicotinic receptors (nAChRs) have been cloned, and a majority of those are known to be expressed in the brain (90,91). Among the many subtypes of mAChRs and nAChRs, we focus here on the $\alpha 7$ subtype of nAChRs.

$\alpha 7$ Nicotinic Acetylcholine Receptors

The $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ nAChRs) are assumed to comprise five $\alpha 7$ subunits that are highly permeable to Ca^{2+} and may serve a distinct role in regulating neuronal plasticity. By elevating intracellular Ca^{2+} levels in discrete neuronal locations, the $\alpha 7$ nAChRs may influence numerous physiological processes in the developing and adult CNS (90–93). Several lines of evidence

suggest that both pre-and postsynaptic α7 nAChRs modulate transmitter release in the brain through Ca^{2+}-dependent mechanisms, and that the α7 nAChRs play a role in regulating neuronal growth and differentiation in the developing CNS (92–94). Furthermore, a hypothesis on the possible coregulation of intracellular Ca^{2+} by NMDA receptors and α7 nAChRs in the brain has been proposed (92). Together with NMDA receptors, postsynaptic α7 nAChRs may serve to regulate intracellular Ca^{2+} levels in neurons, whereas presynaptic α7 nAChRs could serve as a feedback mechanism for modulating glutamatergic transmission (92,94). Thus, it is possible that a close interaction between cholinergic and glutamatergic pathways, mediated by α7 nAChRs and NMDA receptors, may play a role in the pathophysiology of schizophrenia and bipolar disorder.

α7 Nicotinic Acetylcholine Receptors and Auditory Sensory Gating P50

Deficits of sensory gating in schizophrenia derive from the clinical observation that patients report failures of information processing characterized by poor sensory gating (95–97). The underlying problem is evident in the inability of people with schizophrenia to adequately filter their response to incoming sensory stimulation, as measured by inhibitory processing of the P50 auditory-evoked potential. The P50 auditory-evoked potential is a positive electroencephalographic waveform that occurs 50 msec after presentation of an auditory stimulus. When pairs of auditory stimuli are presented at a 500-msec interstimulus interval, schizophrenic patients fail to adequately inhibit the P50 response to the second stimulus. Furthermore, it has been demonstrated that P50 suppression differs between bipolar disorder patients with a lifetime history of psychosis and healthy subjects (98). Normal subjects, however, have significantly reduced responses to the second stimulus (95,96). The reduced response to the second stimulus reflects inhibitory processing of the information that may function to protect the individual from being overwhelmed by incoming, repetitive sensory information. Thus, it is likely that diminished suppression of the P50 may represent a common physiological mechanism associated with the vulnerability to psychosis in people with bipolar disorders as well as schizophrenia. It is known that nicotine transiently normalizes the P50 auditory-evoked potential deficits in schizophrenic patients (96). Postmortem human brain study demonstrated decreased expression of hippocampal α7 nAChRs in schizophrenic patients (99), suggesting that schizophrenic patients have fewer α7 nAChRs in the hippocampus, a condition which may lead to failure of cholinergic activation of inhibitory interneurons, manifesting clinically as decreased gating of responses to sensory stimulation (99,100).

Genetic linkage analysis of the P50 auditory-evoked potential deficit in families of patients with schizophrenia found a peak LOD score at 15q13-q14, and the LOD score was 5.3 ($\theta = 0.00$) at the D15S1360 marker, which is located at intron 2 of the gene for the α7 nAChR subunit (CHRNA7) (101). The

CHRNA7 gene is located on chromosome 15q13-q14, a region linked with schizophrenia in several earlier studies. D15S1360 microsatellite repeats in intron 2 of the *CHRNA7* gene cosegregate with an auditory gating deficit in family linkage studies of schizophrenic patients (101). Furthermore, it has been reported that mutation screening of the *CHRNA7* gene from schizophrenia and controls identified the promoter polymorphisms associated with schizophrenia that decreased the subunit transcription and P50 inhibition (102). Taken together, it is likely that the *CHRNA7* gene is susceptible to the deficits of sensory gating P50 in schizophrenia (96,97,100–103).

Animal Models of Auditory Sensory Gating

The hippocampal P20-N40 wave in DBA/2 mice has been used to model the neurobiology and pharmacology of the human P50 processing deficit (104-106). Inhibition of the P20-N40 response and expression of α7 nAChRs in the hippocampus were found to be significantly correlated across nine inbred strains of mice (104). This correlation showed that mouse strains with the fewest hippocampal (α7 nAChRs had the least inhibition of P20-N40 responses to the second of paired stimuli (104). In particular, the DBA/2 strain of inbred mice was shown to fail to attenuate their response to the second stimulus and to have significantly decreased expression of the α7 nAChRs in their hippocampus (104). This finding parallels those of postmortem human tissue studies documenting decreased expression of hippocampal α7 nAChRs in schizophrenic patients (99,100). Interestingly, subcutaneous or intragastric injection of α7 nAChR agonist DMXB-A [GTS-21; 3-(2,4)-dimethoxybenzilidine anabaseine] has been demonstrated to normalize deficient P20-N40 inhibition in DBA/2 mice (105,106). Tropisetron, a potent 5-HT3 receptor antagonist widely used in the treatment of patients with chemotherapy-induced or postoperative nausea and vomiting, is a partial agonist at α7 nAChRs with a high affinity (107). Hashimoto et al. (108) found that tropisetron improves deficient inhibitory processing of P20-N40 in DBA/2 mice, and that tropisetron's effects could be antagonized by coadministration of a selective α7 nicotinic receptor antagonist methyllycaconitine (MLA) (108), suggesting that tropisetron improves abnormal auditory gating P20-N40 in DBA/2 mice via α7 nicotinic receptors. In addition, Koike et al. (109) have found that tropisetron improves deficits of P50 suppression in schizophrenic patients (Table 1), suggesting that agonist activity at both α7 nicotinic receptors and 5-HT3 receptors for tropisetron might be implicated in the therapeutic action of normalization of P50 suppression by tropisetron (94,109). Recently, Olincy et al. [110] have reported the effects of DMXB-A (3-[2,4-dimethoxy]benzylidene)anabaseine, a natual alkaloid derivative and a partial α7 nAChR agonist on neurophysiological and neurocognitive deficits in schizophrenia. Significant improvement in P50 inhibition and neurocognition on the Repeatable Battery for the Assessment of Neuropsychological Status total scale score were found for DMXB-A-treated group. Therefore, agonists at α7 nAChRs are drug candidates

that may prove efficacious in normalizing deficient P50 processing in schizophrenic patients (94,111–113).

CROSS-TALK BETWEEN GLUTAMATERGIC AND CHOLINERGIC NEUROTRANSMISSIONS

Kynurenic Acid

Kynurenic acid is synthesized via kynurenine from the essential amino acid L-tryptophan, and kynurenic acid is produced and released by the astrocytes in the brain (Fig. 2) (114,115). Interestingly, it has been reported that the levels of

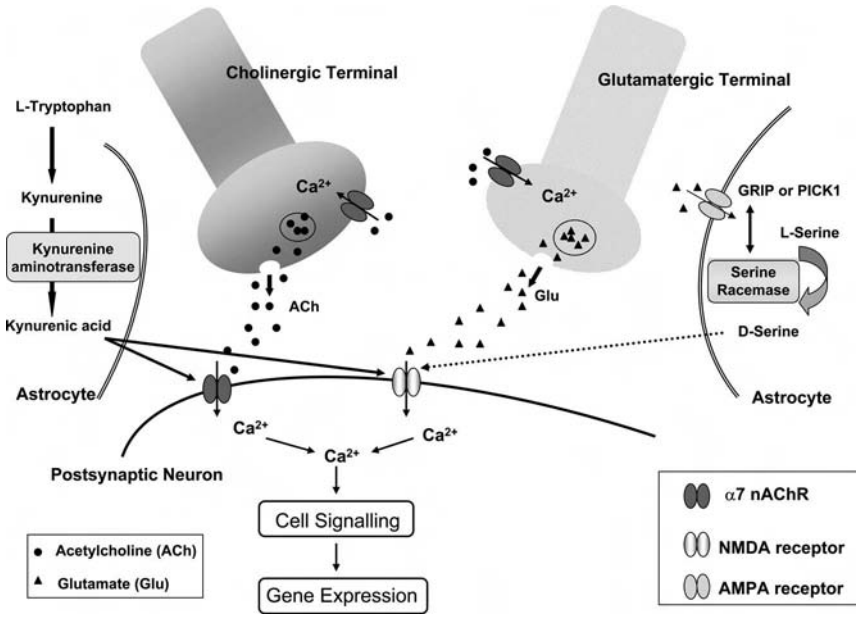

Figure 2 Role of NMDA receptors and $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ nAChRs) in the brain. Acetylcholine (ACh) released from the nerve terminal of cholinergic neurons binds to $\alpha 7$ nAChRs on postsynaptic neurons. By stimulation at $\alpha 7$ nAChRs on the presynaptic terminals, glutamate released from the nerve terminals of glutamate neurons binds to NMDA receptors on the postsynaptic neurons. The increase in intracellular Ca^{2+} arising from activation of $\alpha 7$ nAChRs and NMDA receptors leads to cell signaling and gene expression. Kynurenic acid is synthesized from L-tryptophan in astrocytes, and, as a noncompetitive antagonist, binds to both $\alpha 7$ nAChRs and glycine modulatory sites on NMDA receptors. D-serine is synthesized from L-serine via serine racemase in astrocytes. Glutamate binds to α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors on astrocytes, stimulating the release of D-serine. Released D-serine binds to glycine modulatory sites on the NMDA receptors. Thus, $\alpha 7$ nAChRs and NMDA receptors can exert a wide range of influences through Ca^{2+} signals, due to changes in the synaptic plasticity of the brain.

kynurenic acid are increased in the CSF (116,117) and postmortem brain (118) of schizophrenic patients. In addition to its well-characterized action as a competitive antagonist of the glycine site on NMDA receptors, kynurenic acid also acts as a noncompetitive antagonist of the α7 nAChRs (94,119), which may be implicated in deficits of auditory sensory gating P50 in schizophrenia (Fig. 2).

The administration of L-kynurenine, a precursor of kynurenic acid, together with probenecid, an inhibitor of organic acid transport, increased the levels (500-fold) of kynurenic acid in the hippocampus of rats, and also disrupted their auditory sensory gating. In contrast, the administration of L-701,324, a centrally acting antagonist of the glycine site of the NMDA receptors, failed to disrupt auditory gating in rats, suggesting that elevated levels of kynurenic acid produce disruption in auditory processing through α7 nAChRs (120). Elevation of the endogenous brain levels of kynurenic acid by the administration of kynurenine (100 mg/kg), the precursor of kynurenic acid, or by the administration of PNU 156561A (10 mg/kg), a potent inhibitor of kynurenine 3-hydroxylase, increased brain kynurenic acid levels, and significantly reduced prepulse inhibition (PPI) (121). These disruptions of PPI were restored by administration of the antipsychotic drugs haloperidol or clozapine (121). These findings suggest that brain kynurenic acid serves as an endogenous modulator of PPI, and are consistent with the hypothesis that kynurenic acid contributes to the pathophysiology of schizophrenia (115,121). Taken together, it is likely that the blockade of α7 nAChRs in the hippocampus by elevated levels of kynurenic acid might lead to a deficit of auditory sensory gating P50 in schizophrenic patients, and that the disruption of reciprocal astrocyte-neuron signaling mechanisms between kynurenic acid and α7 nAChRs and NMDA receptors may play a role in the pathophysiology of schizophrenia (Fig. 2) (15,94). Therefore, agents that act by modifying endogenous kynurenic acid metabolism may be potential therapeutic drugs for psychiatric disorders such as schizophrenia.

CONCLUDING REMARKS

Several putative susceptibility genes for schizophrenia and bipolar disorder have been demonstrated: dysbindin-1 (chromosome 6p), neuregulin 1 (chromosome 8p), disrupted-in-schizophrenia 1 (DISC1) (chromosome 1q), and DAOA (G72) (chromosome 13q) (3,4). Neuregulin is present in glutamatergic synaptic vesicles and affects NMDA receptors via actions on ErbB receptors (122). It is also reported that neuregulin plays a role in the expression of several neurotransmitter receptors including GABA receptor subunits (123,124), NMDA receptor subunits (125), and α7 nAChRs (126). Furthermore, a recent postmortem brain study demonstrated that dysbindin-1 was reduced in intrinsic glutamatergic terminals of the hippocampal formation in schizophrenia, suggesting that such changes may contribute to cognitive deficits common in schizophrenia (127).

Thus, changes of the glutamatergic and cholinergic neurotransmission might be involved at several levels: in the neurodevelopmental stage that leads to illness vulnerability, in the expression of symptoms and deficits, and in the subsequent changes in brain structure and function. Taken together, understanding how these susceptibility genes mediate these neurotransmissions in the brain represents an important direction for future research into psychiatric disorders. Finally, gaining a further understanding of the role of these neurotransmissions in the pathophysiology of the two disorders should provide new perspectives for treating them.

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5

Molecular Genetic Study of Schizophrenia Based on Neurodevelopmental Hypothesis

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INTRODUCTION

The neurodevelopmental model of schizophrenia (1,2) is now a widely accepted hypothesis, and several investigations based on this model have been conducted. The actual pathophysiology of this disorder, however, is still not fully understood. The neurodevelopmental model suggests that schizophrenia is a behavioral phenotype, resulting from neurodevelopmental processes that start long before

the onset of clinical manifestations and are due to a combination of environmental and genetic factors (Fig. 1). Clinical studies have provided the following support for this hypothesis (3).

First, family, twin, and adoption studies of schizophrenia revealed that, in addition to environmental factors, multiple genetic factors contribute to the development of schizophrenia, and have a relatively high heritability of about 80%. Second, several adverse events or harmful stressors during prenatal and postnatal periods have been associated with development of schizophrenia. Third, clinical features (e.g., minor physical anomalies, minor deviations in motor and cognitive development) and abnormal magnetic resonance imaging (MRI) findings in individuals with high risk for schizophrenia suggest that brain abnormalities exist in those who later develop schizophrenia. Fourth, the psychosocial stressful life events in adolescence may trigger the onset of schizophrenia. Fifth, relapses following onset of schizophrenia induce not only deterioration of the condition but also the progress of abnormal MRI findings. Sixth, postmortem neuropathological studies of schizophrenic brains have revealed synaptic disconnectivity in this disorder.

Recent advances in molecular cell biology have also helped to clarify the mechanisms of neurodevelopment, such as formation of synaptic connectivity, myelination, and neuronal polarity. In the light of the neurodevelopmental hypothesis for schizophrenia, genes related to neurodevelopment would seem to be plausible candidates for schizophrenia susceptibility genes. In addition,

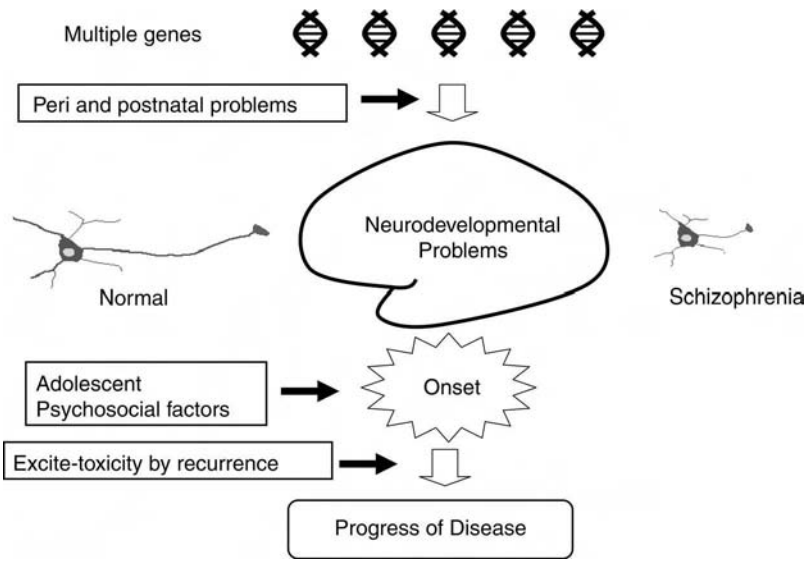

Figure 1 The neurodevelopmental model of onset and course of schizophrenia.

positional candidate loci for schizophrenia susceptibility genes have also been identified based on whole genome studies. As can be seen from the preceding text, several molecular genetic studies of candidate genes have been carried out based on neurodevelopmental models and a positional approach (4,5).

In this chapter, we review the recent findings in molecular mechanisms of neurodevelopment and molecular genetic studies of schizophrenia from the neurodevelopmental point of view.

MOLECULAR MECHANISIMS OF NEURODEVELOPMENT

Neuronal Migration

Several hypotheses of cortical malformations caused by migration defects exist (6,7). Studies of neuronal migration in the human neocortex demonstrate that directed migrations establish the neuronal layers. The development of neuronal layers, which have distinct morphological and functional identities, involves the migration of neurons radially to their exact positions. In the mouse brain, the formation of neuronal layers by radial migration occurs between embryonic days 11 (E11) and 18 (E18). Neurons migrate from the ventricular zone to the pial surface and then establish the preplate at E11. By E13, neurons migrate through the intermediate zone and split the preplate into the marginal zone and subplate to create the cortical plate. These migrating neurons subsequently expand the cortical plate in an inside-out fashion, as each wave of neurons passes its predecessors to settle underneath the marginal zone between E14 and E18. After the cortical plate has been fully established, the subplate degenerates and leaves behind a six-layered neocortex that persists throughout adulthood (8).

Genetic analyses has shown that impairment of several genes, including *lissencephaly-1* (*Lis1*) (9,10), *NudE-like* (*Nudel*) (11), *reelin*, *doublecortin* (12,13), *cyclin-dependent kinase 5* (*Cdk5*) (14), *Cdk5 cofactors p35/p39* (15), and *14-3-3ε* (16) causes migration defects. Among these genes, reelin was identified as one plausible candidate factor related to the neurodevelopmental hypothesis of schizophrenia (see following text). Lis1 and Nudel have been shown to form a complex with Disrupted-in-schizophrenia 1 (DISC1), which is a susceptibility gene for schizophrenia (17,18). DISC1 is also essential for maintaining the Lis1/Nudel/dynein complex at the centrosome and regulating neuronal migration (19).

Neuronal Polarization and Axon Elongation

Neurons contain two distinct types of processes, axons and dendrites, which allow them to receive, process, and transmit information. Neurons typically possess a single axon and multiple dendrites (20). Axons and dendrites differ from each other in their structural components and the composition of their proteins and organelles. Axons are typically long and thin, with a uniform caliber at

all distances from the cell body, and they branch at right angles. Many experiments using cultured embryonic neurons have revealed that neurons initially generate several equivalent neurites, and that neurons begin to polarize when one neurite becomes an axon; the other neurites then become dendrites. In this axon and/or dendrite formation, several key regulators have been reported: collapsin response mediator protein-2 (CRMP-2), partitioning-defective (Par) protein complex, small GTPases, and microtubule-associated proteins (MAPs) and their regulators. Interestingly, up or downregulation of these molecules forms multiple axons or prevents axon elongation in cultured hippocampal neurons (21).

For an example, overexpression of CRMP-2 causes multiple axons, whereas knockdown of CRMP-2 prevents axon elongation. CRMP-2 binds to tubulin heterodimers to promote microtubule assembly, thereby enhancing axon elongation (22). CRMP-2 associates with the specifically Rac1-associated protein-1 (Sra-1)/WAVE1 complex, and regulates actin filament stability at growth cone (23). CRMP-2 has been shown to link tubulin dimers or Sra-1 to kinesin light chain of Kinesin-1. Kinesin-1 acts as a molecular motor on microtubules that are essential for anterograde axonal transport (24,25). Thus, CRMP-2 links Kinesin-1 to tubulin dimers and Sra-1, serves as the cargo receptor, and regulates their transport to the growing axon (21). The activity of CRMP-2 is regulated by phosphatidylinositol-3-kinase (PI 3-kinase)/Akt/glycogen synthase kinase 3β (GSK3β) signaling pathways downstream of neurotrophic factors, neurotrophin-3 (NT-3) and brain-derived neruotrophic factor (BDNF). Activated Akt phosphorylates and inactivates GSK3β. Inactivation of GSK3β invokes the activation of substrates of GSK3β, thereby enhancing microtubule formation by CRMP-2 or MAPs. It is also reported that actin filament reorganization and membrane trafficking are essential for rapid axon formation in developing neurons. Because axon formation is closely regulated, so that an excellent network ultimately develops, a disorder in this step appears to work against proper neuronal and psychological development. Akt and GSK3β have been implicated in the pathogenesis of schizophrenia (see following text).

Recently, it has been reported that DISC1 plays an essential role in axon elongation. Several novel DISC1-interacting molecules, containing Kinesin-1, 14-3-3ε, and Grb2, are identified (26,27). 14-3-3ε forms a complex with Lis1 and Nudel, and regulates the localization of the complex into axons (16). DISC1 forms a ternary complex with Nudel and Kinesin-1. The reduction of DISC1, Lis1, and Nudel expression inhibit axon elongation. Thus, DISC1 links Kinesin-1 to the Lis1/Nudel/14-3-3ε complex and serves as the cargo receptor, thereby regulating the transport of the complex to axons for axon elongation (26). Grb2 acts as an adaptor molecule that links receptor tyrosine kinases and the extracellular signal-regulated kinase (Ras-ERK) pathway (28). DISC1 also forms a ternary complex with Grb2 and Kinesin-1, and regulates the transport of Grb2 to axons in a Kinesin-1-dependent manner (27). The transported Grb2 by DISC1 is involved in neurotrophin-induced axonal elongation. CRMP-2 is also is a candidate gene for susceptibility to schizophrenia (29,30). DISC1 and Kinesin-1 participate in the

transport of the Lis1/Nudel/(14-3-3ε) complex and Grb2, whereas CRMP-2 and Kinesin-1 participate in the transport of tubulin dimers and Sra-1. Although DISC1 and CRMP-2 have different partner proteins, they have similar modes of action that link motor proteins to transport proteins. Thus, it is possible that dysfunction of the cargo receptors, such as DISC1 and CRMP-2, may impair neuronal development, leading to psychiatric disorders.

Dendrite Formation

Dendrites are thicker and much shorter than axons and their caliber decreases with distance from the cell body. Dendrites possess dendritic spines, which are specialized membrane protrusions that exist on a majority of excitatory synapses in the mammalian brain (31). Spines have the machinery for neurotransmitter signaling, and compartmentalize the biochemical and cell biological events that regulate synaptic modifications. During neuronal development, dendrites extend filopodia, and those dendritic filopodia differentiate into mature spines capable of synaptic transmission through contact with presynaptic regions (32). Dendritic filopodia and spines are rich in filamentous actin, and remodeling of the actin cytoskeleton controls the formation and motility of filopodia and dendritic spines (33). Actin reorganization in dendritic spines is highly dynamic and responsive to synaptic signals, and is essential for the maintenance of synaptic plasticity (34). It has been reported that the number and shape of dendritic spines are regulated by several proteins that either directly or indirectly regulate the actin cytoskeleton, such as regulators of Rho family small GTPases (35). Several human mental retardation syndromes are linked to altered morphology and number of dendritic spines (36).

Synapse Formation and Maturation

Synaptogenesis refers to the phenomenon by which axons from different regions grow into their respective synapse. Various extracellular factors have been identified that serve as either attractive or repulsive guidance cues to induce extension or retraction of the axonal growth cones (37). Families of axon guidance molecules include semaphorin, ephrin, netrin, and slit, and their respective receptors plexin/neuropilin, ephrin receptor, DCC, and Robo provide both attractive and repulsive cues in a variety of neurons, depending on the intracellular and extracellular signals (38). For example, in the axonal targeting in cortical pyramidal neurons, Semaphorin 3A appears to serve as an attractive cue to dendrites, while also acting as a repulsive cue to axons (39). Neurotrophins can promote neuronal maturation including regional axon and dendrite branching. In other molecular mechanisms, members of the fibroblast growth factor (FGF), transforming growth factor-beta (TGF-beta), Sonic hedgehog, and Wnt families act as morphogens, which play important roles in the progressive patterning of embryos. Morphogens are secreted by characterized organizer centers in the central nervous system and have been shown to induce regional axonal branching as axon guidance cues (40).

In the pathogenic model, schizophrenia is a disorder of neuronal development related to abnormal synaptic activity or connectivity (41,42). During the phase of synaptic maturation, synapses expand in size and the number of synaptic vesicles increases (43). In this phase, synaptic maturation is regulated by excitatory, inhibitory, and moderating signals. At glutamatergic excitatory synapses, synapses initially contain *N*-methyl-D-aspartate (NMDA) receptor and then acquire alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the developing stage. At glycinergic and GABAergic inhibitory synapses, the synapses mature by switching receptor subunits. Many schizophrenia susceptibility gene products are expressed in the synapse and are involved in synaptic maturation and plasticity. For example, Neuregulin-1 regulates the expression of NMDA and $GABA_A$ receptors, Calcineurin is involved in synaptic plasticity, catechol-*O*-methyltransferase (COMT) modulates cortical dopamine signaling, and Dysbindin regulates glutamate release (4,5).

GENETIC STUDIES OF SCHIZOPHRENIA BASED ON NEURODEVELOPMENTAL HYPOTHESIS

The neurodevelopmental hypothesis assumes that before the onset of the illness, schizophrenic individuals are undergoing pathogenetic biological processes that are induced by multiple genetic and environmental factors. Thus, based on the neurodevelopmental hypothesis, it is very natural for investigators to search for candidate genes associated with these pathogenetic processes. Here, we review several promising candidate genes for schizophrenia, especially targeting those with neurodevelopmental aspects. Although there are many genes of interest, we limit our discussion to five genes for which there is strong evidence from genetic association studies (multiple positive replications), animal models, or functional assays (Table 1).

Dystrobrebin-Binding Protein 1 (Located on 6p22.3)

Dystrobrebin-binding protein 1 gene (DTNBP1) (44), which encodes dysbindin, is one of the most promising susceptibility genes for schizophrenia. Several genetic association analyses using independent samples and different population samples have replicated the original findings showing a significant association.

Table 1 Genetic Association Analysis of Promising Candidate Genes

Gene symbol	Positive findings	Negative findings	Evidence from positional cloning	Expression change
DTNBP1	++++++++++	+++	YES (Strong)	YES
NRG1	++++++++++++	+++	YES (Strong)	YES
AKT1	+++	+	YES (Weak)	YES
DISC1	++++	++	YES (Strong)	YES
RELIN	−	+++	NO	YES (hypermethylation)

In addition, two postmortem studies showed that decreased dysbindin expression was found in schizophrenics. In one, dysbindin mRNA was found to be significantly reduced in multiple layers of the dorsolateral prefrontal cortex (DLPFC) in schizophrenics (45). In the other, immunohistochemical methods were used to show that there was a significant reduction of presynaptic dysbindin-1 in the terminal field of intrinsic glutamatergic connections in the hippocampal formation (46).

Exciting as this evidence is, however, the fact remains that little is known about the function of dysbindin. Originally, using mouse beta-dystrobrevin in a yeast two-hybrid screen, dysbindin was cloned from adult mouse brain and myotube cDNA libraries. Dysbindin binds not only to dystrobrevins but also to muted and pallidin, which are components in the biogenesis of lysosome-related organelles complex-1 (BLOC1). BLOC1 plays important roles in trafficking proteins to lysosome-related organelles, and deficiencies in this complex are associated with Hermansky-Pudlak syndrome. Although the roles of BLOC1 are still unknown, it might be involved in vesicle docking and fusion (47).

A recent study of primary cortical neuronal culture showed that overexpression of dysbindin induced the expression of two presynaptic proteins (SNAP25 and synapsin I), promoted phosphorylation of AKT (described in the following text), and increased glutamate release. Alternatively, knockdown of dysbindin resulted in lower presynaptic protein expression, suppression of the phosphorylation levels of AKT, and a decrease in glutamate release. The authors of these studies speculated that dysbindin might play important roles in neuronal viability and vulnerability through AKT activation and other means (48).

As already noted, DTNBP1 is an exciting candidate gene for schizophrenia, based on evidence from genetic association analyses, postmortem studies, and cell biological studies. However, the findings of DTNBP1 are still far from conclusive, because no actual causal variants have been detected in schizophrenia patients. Thus, we cannot define DTNBP1 as a true predisposing gene, although one preliminary study has provided evidence for a relation between DTNBP1 and schizophrenia susceptibility. The authors examined the expression level of mRNA from specific haplotypes, and showed that the subjects with risk haplotypes had a lower level of DTNBP1 expression, whereas those with protective haplotypes had a high level of expression (49). These results indicate that the haplotypes in DTNBP1 contribute to schizophrenia through reduced expression.

Neuregulin 1 (Located on 8p22-p11)

The neuregulin 1 gene (NRG1) (50) was reported to be a plausible candidate gene for schizophrenia in the Icelandic population by the deCode Genetics group (51). Whole genome linkage and linkage disequilibrium studies showed an association between haplotypes and schizophrenia, and several replication studies supported this finding. However, some studies did not find such an association (52–54). In addition, studies showing a positive association indicated that different at-risk haplotypes were also associated with schizophrenia. These findings suggest that NRG1 is unlikely to

have a pathophysiological role in all cases of schizophrenia or in all populations. The differences in linkage disequilibrium (LD) among populations might also have introduced these inconsistent results, and the negative findings may only indicate a failure to identify the true predisposing variants due to the differences in populations owing to LD. Therefore, gene-wide replication analysis based on LD of NRG1 is essential to compare results stemming from different ethnicities.

A study using schizophrenic brains revealed that the type I isoform of NRG1, one of the major isoforms, was significantly increased in DLPFC of schizophrenics. The authors also showed that the NRG1 mRNA levels of the genotype had no effect at two single nucleotide polymorphisms (SNPs) previously associated with schizophrenia (55).

Neuregulins are epidermal growth factor (EGF)-like growth and differentiation factors that signal through tyrosine kinase receptors of the erbB family. The neuregulins constitute a family of proteins coded by four distinct genes (NRG-1 to NRG-4), and play important roles related to neurodevelopment (56) (OMIM: *142445).

First, NRG1-erbB signaling may affect neuronal migration. Abnormality of neuronal migration is considered to be a key process in the neurodevelopment hypothesis of schizophrenia. In vitro and in vivo studies suggest that NRG1-erbB signaling can induce the elongation of cortical radial glia fibers and result in an acceleration of neuronal movement. A recent study showed that gene-gene interaction between NRG1 and erbB4 increases susceptibility to schizophrenia (57).

Second, NRG1 regulates the expression of specific NMDA, $GABA_A$, and ACh receptor subunits. These neurotransmission receptors (and interacting molecules) can play a part in neurodevelopment. Therefore, NRG1 may also be related to neurodevelopment indirectly through these transmission cascades.

Third, NRG1 can modulate myelination. Several lines of evidence suggest that NRG has multiple roles in oligodendrocyte development, promotes survival and maturation of astrocytes, and affects interglial communication. Myelination is, of course, important for neuronal development, and may also be important in the pathogenesis of schizophrenia, as several studies have suggested that myelin-related molecules were altered in schizophrenia patients.

In the future, better knowledge of NRG1 function in the brain will be required to elucidate its roles in the pathogenesis of schizophrenia.

AKT1 (Located on 14q32.32)

AKT1 is a serine-threonine kinase that is a key component of many signal transduction pathways. In particular, AKT1-GSK3β signaling has a fundamental role in regulating cellular functions such as gene transcription, protein translation, apoptosis, and cell proliferation (58). Activation of AKT has also been linked to the GABAergic and glutaminergic systems. In addition, in an animal study with dopamine transporter knockout (KO) mice and wild type mice treated with lithium salts and amphetamine, it was shown that the AKT1-GSK3β signaling cascade partially mediated dopamine-dependent behaviors such as schizophrenia (59).

The AKT1-GSK3β signaling system has been discussed as a major target for lithium action, and it has been hypothesized that this system is involved in the pathophysiology of mood disorders. Recently, however, decreased AKT1 protein levels and phosphorylation of GSK3β at Ser9 in peripheral lymphocytes have been reported in schizophrenia. The authors also showed that AKT1 was lower in the hippocampus and frontal cortex of the postmortem brain in schizophrenics than in controls. In addition, a genetic association analysis using family samples showed significant association between schizophrenia and AKT1 haplotypes (60).

Following this study, two positive replication studies and one negative study were reported (61-63). In Caucasian samples, significant SNPs and haplotypes were located in the middle of AKT1, whereas in Japanese samples, they were on the 3' side. All SNPs positively associated with schizophrenia so far have been marker SNPs, and only intronic ones; therefore, actual functional predisposing variants will need to be identified in order to clarify the importance of AKT1 in the pathophysiology of schizophrenia.

Disrupted in Schizophrenia 1 (Located on 1q42.1) [L19]

A balanced translocation (1:11)(q42.1: q14.3) was strongly linked to several mental disorders in a Scottish family and led to the identification of Disrupted in Schizophrenia 1 (DISC1) (64). The biological function of DISC1 is not been understood; however, recent studies suggest that DISC1 plays roles in neurodevelopment, cAMP signal transduction, and other functions (65,66). This is described in more detail in other parts of this chapter.

The initial linkage study of the original Scottish family showed a high log of the odds (LOD) scores: 3.4 (the diagnosis was restricted to schizophrenia) and 7.1 (schizophrenia, bipolar disorder, and recurrent major depression) (66). Independent linkage studies also suggested linkage in this region in a Finnish population sample (67,68) and other populations (69,70). This indicates that this chromosomal region including DISC1 is a plausible candidate locus for a susceptibility gene for schizophrenia and mood disorders.

In genetic association analyses between DISC1 and schizophrenia, two nonsynonymous SNPs (Leu607Phe and Ser704Cys) and several haplotypes (HEP1, HEP2, and HEP3) were associated with schizophrenia (71-74). In particular, a positive association between HEP1 and HEP3 and schizophrenia was replicated in independent reports (however, risk haplotypes of HEP3 were different from those of the Hennah et al. study) (72,73). On the other hand, two negative associations between DISC1 and schizophrenia from a Japanese population have been reported (75,76). These results may be due to the difference in LD structure in study populations or the effect of ethnic backgrounds.

Endophenotypic approaches such as cognitive function and imaging studies were also carried out in consideration of the finding that unaffected t(1;11) carriers showed a reduced P300 amplitude (66). For example, the Cys allele of the

Ser704Cys mutation was associated with a reduction in hippocampal gray matter volume and altered engagement of the hippocampus during several cognitive tasks assessed with functional MRI (74). These endophenotypic approaches are reasonable (see discussion in the following section) and may elucidate the pathophysiology of schizophrenia by detecting the specific phenotype of DISC1.

Such consistent findings from molecular biological and population genetic studies suggest that DISC1 is a very good candidate for a susceptibility gene for schizophrenia. However, it is well known that DISC1 can interact with several proteins (77); therefore, not only DISC1, but also DISC1-related genes may predispose carriers to schizophrenia. Furthermore, multiple genes rather than a single gene are thought to be involved in the pathophysiology of schizophrenia. One study to date has examined the interaction among polymorphisms in DISC1 and COMT, and provided evidence for epistasis (78). In the future, comprehensive genetic association studies of DISC1-related genes will be required with consideration of gene-gene interactions.

Reelin (Located on 7q22)

Reelin is an extracellular matrix protein and plays important roles in neural migration, cortical lamination, neuronal synaptogenesis, and plasticity (79).

The mechanisms for these roles are introduced in the following. Reelin binds to transmembrane receptors including very low-density lipoprotein receptor (VLDL-R) and apolipoprotein E receptor 2 (ApoER2). Reelin also activates serine-threonine kinase (P35/Cdk5) and members of the Src-tyrosine kinase family (Fyn-kinase), leading to phosphorylation of disabled-1 (Dab1). Furthermore, phosphorylated Dab-1 can activate phosphatidylinositol-3-kinase (PI3K) and protein kinase B (PKB/Akt), which, in turn, affect molecules such as GSK3β and (β-catenin (discussed earlier). This signaling cascade plays critical roles involving cell proliferation, apoptosis, and neurodegeneration. In addition, Dab-1 interacts with neuronal Wiskott-Aldrich syndrome protein (N-WASP), and actin-related protein 2/3 (ARP2/3), and causes formation of microspikes or filopodia, which are important in processes of cell migration and synaptic plasticity (80).

In the adult mammalian brain, reelin is mainly localized to cortical GABAergic and hippocampal interneurons, located in regions considered to be involved in the pathophysiology of schizophrenics. In fact, several postmortem studies showed that reelin mRNA and protein were significantly decreased in the cerebellar, hippocampal, and frontal cortices of schizophrenic patients. In addition, decreased reelin was associated with decreases in glutamic acid decarboxylase (GAD) 67 kDa, 65-kDa proteins, and semaphoring 3A. These interactions may be susceptibility factors for schizophrenia. However, decreased reelin may not be a finding specific to schizophrenia, because other psychiatric disorders including bipolar disorder and depression also show decreased levels of reelin (80). Apart from these findings, recent investigations suggest that decreased expression of reelin in schizophrenic brains may be derived from hypermethylation of the RELN gene promoter (81,82).

To date, three genetic association studies of schizophrenia have been carried out. However, no significant association was found in these studies using relatively small samples (83-85. Although hypermethylation provides evidence that RELN is a candidate factor for schizophrenia, general genetic association analyses (e.g., case-control association analysis, family-based association analysis) cannot identify actual causal variants, because there are many regulators and effectors in reelin signaling, and epigenetic and environmental factors influence these processes.

FUTURE DIRECTIONS: SELECTION OF PHENOTYPE FOR NEURODEVELOPMENTAL HYPOTHESIS

The goal of genomic research is to elucidate the relationships between phenotypes and genotypes through both genetic statistical analysis and biological verification. In medical genomic research, investigators usually use diagnosis, with or without a disease as a phenotype. Thus, cellular or animal experiments connecting diagnosis and genotype are crucial for biological verification in this field. For example, animals that are genetically modified, based on the genetic findings of hypertension, are needed to create conditions similar to those in hypertensive patients.

Today, diagnostic criteria for most mental disorders, including schizophrenia, consist only of psychopathological symptoms such as delusions or hallucinations, without laboratory criteria. However, as it is impossible to examine whether or not animals have delusions, schizophrenic genomic research requires another phenotype that can be measured even in rodents for biological verification in addition to clinical diagnosis. Furthermore, the validity of psychiatric diagnosis has not been proven, although operational diagnostic criteria of mental disorders, such as Diagnostic and Statistical Manual of Mental Disorders Fourth Edition Text Revision (DSM-IV-TR), have demonstrated reliability (86). As a result, individuals sharing a diagnosis of schizophrenia can be heterogenous in terms of pathophysiology. Categorical classification such as DSM-IV-TR works best when all members of a diagnostic class are homogenous and there are clear boundaries between classes, and when the different classes are mutually exclusive. Therefore, it has been suggested that diagnostic classification should include dimensional criteria.

To solve the above-mentioned problems related to diagnosis, the concept of endophenotype has been proposed (87). Endophenotypes are measurable components unseen by the unaided eye along the pathway between disease and distal genotype, based on neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, or neuropsychological data. In addition to furthering genetic analysis, endophenotypes can clarify classifications and diagnoses, and foster the development of animal models. However, to be most useful, endophenotypes must meet certain criteria (Table 2).

For example, brain morphology measured by MRI-based morphometry was shown to have high heritability (whole brain (90%), gray (82%), and white (88%) matter volume) (88). Abnormality in brain morphology, which was reproducibly reported in schizophrenia (89), exists even before the onset of this condition (90).

Table 2 Endophenotype

The endophenotype is associated with illness in the population
The endophenotype is *heritable*
The endophenotype is primarily state-independent
Within families, endophenotype and illness co-segregate
The endophenotype found in affected family members is found in nonaffected family members at a higher rate than in the general population
Endophenotypes are quantitative traits

Source: From Ref. 87.

In addition, a recent twin study showed that genetic factors have a stronger influence on the shape of lateral ventricles than do the disease-related changes in schizophrenia (91). Abnormal brain morphology may, therefore, be one of the most plausible endophenotypes for molecular genetic studies based on the neurodevelopmental hypothesis for schizophrenia.

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6

Model Organisms and Neurogenetics

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WHAT ARE APPROPRIATE TRANSGENIC MODELS OF PSYCHIATRIC ILLNESS?

To date, there not been a single genetic lesion that has been linked unambiguously with any purely psychiatric disorder. This stands in marked contrast to the current state of neurological research, for which mutations in several genes have been clearly associated with familial forms of Parkinson's disease (PD), dementia, and Huntington's disease (HD). It is obviously difficult to construct bona fide transgenic models of psychiatric illnesses in the absence of clear-cut genetic candidates. As an alternative, although the relationship of neither the receptors nor transporters to the pathophysiology of psychiatric illness is known, their association with the treatment of schizophrenia, depression, and anxiety disorders has led a number of groups to consider transgenic models of these gene products as valuable models for the study of psychiatrically relevant behaviors in animals. In addition, molecular genetic studies such as microarrays have identified a number of genes that seem to be altered under some conditions relevant to psychiatric illness. Finally, one may study the endogenous behaviors of animals, or the behavior of animals

that have been surgically lesioned, or bred to enhance a psychiatrically relevant behavior. Indeed, many of the best-studied animal models in psychiatry are of the last type. We have restricted ourselves here to neurogenetic models in which a single gene or genetic locus has been manipulated either by deleting the endogenous gene(s) or introducing a transgene, and omitted models of the last type in which endogenous behaviors or behaviors in anatomically lesioned animals have been studied. Furthermore, we have restricted ourselves to animal models based on genetic lesions that have been identified in neuropsychiatric patients. We acknowledge that this excludes a rich literature of animal models that do not fit this restricted molecular-genetic paradigm.

DEVELOPING TRANSGENIC MODELS BASED ON HUMAN GENETICS STUDIES

In order to create transgenic models of psychiatric illness, it will be necessary to identify genes that are associated with that illness. Toward this end, human geneticists have employed both open-ended and candidate-based approaches. Although it is possible that candidate-based approaches may yield important results for schizophrenia and other illnesses, the consensus among at least some geneticists is that candidates based only on our current speculative models of pathogenesis can, in many cases, engender false leads. The genetics of PD offer a useful comparison in this regard. The obvious candidates for causing dysfunction of dopaminergic neurons are genes involved in DA synthesis and homeostasis such as tyrosine hydroxylose, the plasma membrane dopamine transporter, and the vesicular monoamine transporter. Although polymorphisms in these genes may generate some increase in risk for PD they are certainly not causative. Rather, recent data on rare genetic variants of the disease have implicated mutations in several genes that were not obvious candidates and would have been extremely difficult to predict a priori; many are ubiquitously expressed and for some, such as α-synuclein, their function remains unknown. These mutations are described in more detail in the following text.

With neurological examples such as this in mind, it is difficult to place too much credence in candidate genes for schizophrenia or other psychiatric illnesses. The identification of candidates is made even more difficult by our lack of even the most rudimentary knowledge about the neurons (or glia) in which cellular pathology occurs. Thus, because it so difficult to accurately identify candidates for psychiatric disease based primarily on theoretical concerns, the generation of transgenic models based on these candidates may be premature. Based on experience in neurology and other fields of medicine, it will be more fruitful to create transgenics based on genes that have been associated with disease phenotypes in open-ended genetic approaches. We focus on these models here. These include genes identified via both linkage and association analyses that are described elsewhere in this volume. A third approach exploits large, cytologically visible chromosomal deletions to identify potential disease loci. Although this approach is grossly open-ended in that it does not make any assumptions about the loci that may be

affected, the final choice of genes to be investigated is usually de facto candidate-based, because most deletions are quite large and include multiple genes. All of these genes will be deleted; yet it is likely that only one of them is truly associated with the disease phenotype, thus forcing geneticists to choose one or a few as candidates for further investigations. Another potential limitation of using large chromosomal abnormalities is that positional effects of the deletion on nearby genes can cause functional defects in genes that lie outside of the physically deleted portion of DNA. Positional effects can occur over long distances; thus, it is difficult to rule out the possibility that anonymous genes that lie far outside the apparent region of interest are responsible for the disease phenotype. These caveats aside, chromosomal deletions have been a factor in both ongoing neuropsychiatric studies and transgenic models of disease.

A third approach to identify disease-related genes employs essentially molecular rather than true genetic methods. In this case, techniques that have become generally available only over the past 10 years have been used to identify proteins and/or genes that show altered levels of expression in a given disease process. Both "genomic" and "proteomic" approaches are possible. That is, one may screen for changes in the expression of either genes in the form of altered mRNA levels or changes in protein expression. The details of the sophisticated technologies used in these approaches are beyond the scope of this chapter. Notwithstanding the tendency for some to overinterpret the results of these studies, the methods are quite powerful, and it is indeed possible to detect changes in one out of thousands of genes or proteins. However, the key difference between these approaches and the use of the genetic methods described earlier is that the molecularly identified genes are less likely to be dysfunctional themselves. Rather, their up or downregulation is more likely to reflect changes in associated biochemical pathways. That is not to say that this method of identifying disease-associated genes and their associated regulatory pathways is not useful. Nonetheless, because these effects can be direct or very indirect, the specificity of these findings often is unclear.

With these powerful methods and recent intense interest in neurogenetics, the paucity of transgenic models in psychiatry begs the question of why more genes relevant to psychiatric illness have not been identified. There are multiple answers to this question, and some of these will bear on the eventual analysis of transgenic models. First and perhaps most importantly, the genetics of most, if not all, psychiatric illness is thought to be "complex." That is, unlike classical Mendelian models such as Tay-Sachs disease, it is not possible to identify a single gene which can cause the disease. Rather, in complex genetic disorders, the disease phenotype is caused by a combination of either multiple genes and/or a gene-environment interaction. This aspect of disease pathogenesis will be important in the phenotypic analysis of transgenic models, because the vast majority of these animals are the result of mutating or overexpressing a single gene. For diseases in which the effect of a single gene is large or causative, single-gene transgenics can yield robust and very useful phenotypes. However, for complex genetic diseases this may not be the case. Rather, it is possible that these genes will best be

analyzed in combination. Unfortunately, it is currently time consuming as well as expensive to generate mouse models, and the genetic crosses to obtain double or triple mutants can take years. Additional genetic systems with shorter generation time such as the zebrafish and even invertebrates such as *Drosophila melanogaster* and *Caenorhabditis elegans* may be useful in this regard (Fig. 1).

The complex nature of neuropsychiatric genetics also spills over into a number of technical issues that may have precluded the identification of the relevant genes. These include the effect of limited sample size in complex genetic

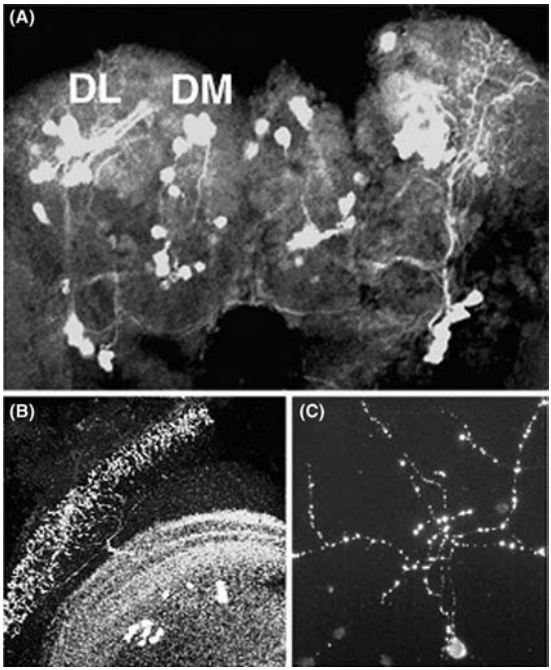

Figure 1 Invertebrates such as *Drosophila melanogaster* use many of the same neurotransmitters as mammals and are increasingly being used to model human neurodegenerative diseases. The identification of genetic risk factors for psychiatric disorders may, someday, allow the use of *Drosophila* to study psychiatric illnesses in the fly. (**A**) shows a fly brain immunofluorescently labeled to show the localization of dopaminergic neurons in the central brain. The indicated DM and DL clusters have been used to track the neurodegeneration of dopaminergic neurons in fly models of Parkinson's disease. (**B**) shows labeling with an antibody to the *Drosophila* vesicular monoamine transporter (DVMAT). VMATs in the fly as well as mammals are responsible for the storage of dopamine, serotonins, and other monoamine neurotransmitters. A cultured serotonergic neuron labeled with the DVMAT antibody is shown in **(C)**. Note the broad arborization of many of the aminergic processes in **(A)**, **(B)**, and **(C)**, also seen in aminergic projection neurons in the mammalian central nervous system.

diseases in which the effect of a particular gene is relatively small. Small effects will be missed unless the power of the sample is large, and few adequately large data sets have been assembled to date. Other issues relevant to both the identification of disease genes and their analysis in transgenics include the genetic concept of penetrance or the percentage of people/animals that harbor a mutation and also are affected. In addition, the "expressivity" of the mutation can vary, that is, some mutations can manifest a variable phenotype. Both penetrance and expressivity can be affected by other genes and the environment; in some cases variation may be purely stochastic.

For all of the reasons already listed, it is perhaps more useful to view genes that are related to psychiatric illness as susceptibility genes rather than genes that cause the illness or "the gene for x." This concept is important in light of the continuous stream of articles in the popular press regarding "the gene for x" and the potentially unrealistic expectations that these impart to clinicians and patients alike. The relevance of these expectations to clinical care is likely to increase dramatically when susceptibility genes for psychiatric illness are identified definitively enough for pharmaceutical companies to begin marketing drugs that target these genes. As with agents that are currently used to combat high cholesterol and stroke, it will be important for doctors to frankly discuss the relative levels of decreased risk that future medications will afford, as opposed to giving the impression that these drugs will essentially "cover" the patient for schizophrenia.

With these caveats in mind, one might ask whether it is indeed useful to identify susceptibility genes at all, much less study them in transgenic models. We suggest that to appreciate their importance, the identification and study of these genes should not necessarily be viewed as entrees to a cure or even potential drug targets. Rather, these genes and the animal models that are used to study them should be viewed perhaps as only a starting point to identify the cellular and molecular pathways that are affected by psychiatric illness and its treatment. It is only with such models that we will be able to enter the modern arena of experimental biology occupied by all other medical disciplines. We also caution that the path from starting point to clinically relevant treatment is likely to take a long time. Susceptibility and, in some cases, true causative genes have been identified for several neurological illnesses such as HD. However, the identification of these genes and the creation of relevant animal models have not yet led to clear treatments or even clearly defined pathogenic mechanisms. Nonetheless, these cellular and animal models have allowed, for the first time, informed experiments to address these issues. It is our hope that genetics of psychiatry will similarly allow us to some day study the biology rather than the phenomenology of psychiatry.

ALZHEIMER'S DISEASE

A hallmark neuropathological feature of Alzheimer's disease (AD) is the extracellular amyloid plaque. These plaques contain the Aβ peptide, which is formed by cleavage of amyloid precursor protein (APP). APP can be processed by two

pathways: the amyloidogenic pathway, which results in production of Aβ, or the nonamyloidogenic pathway. Dominant mutations of *APP* or *presenilins* 1 and 2 cause early onset AD (1,2).

Another hallmark neuropathological feature of AD is the neurofibrillary tangle (NFT). Neurofibrillary pathology is also seen in several other disorders referred to as tauopathies; these include frontotemporal dementia with parkinsonism (FTDP) linked to chromosome 17 and progressive supranuclear palsy (3). Tau associates with microtubules, and its interaction with microtubules is negatively regulated by phosphorylation. Abnormal regulation of tau phosphorylation is thought to take place in tauopathies.

A large number of mouse models of AD have been established. These have been engineered to express wild type or familial mutations in *APP* (4–7). Another approach has been to express Aβ peptides as fusion proteins (8). Mouse models generally develop plaques and diffuse Aβ deposits, accompanied by cognitive deficits. Coexpression of *APP* with mutant forms of *presenilin* results in more severe pathology and earlier onset of phenotypes (9). However, although there is substantial evidence of neuronal dysfunction in these models, frank neurodegeneration has been more difficult to demonstrate.

Other investigators have succeeded in establishing mouse models of tauopathy using wild type or FTDP-associated tau mutations (10–12). These demonstrate formation of NFT and cognitive decline, as well as frank neurodegeneration. In some instances, synergy in neuropathological features have been observed using combinations of amyloid and *tau* transgenes (13). A triple transgenic mouse expressing mutant forms of *tau*, *APP*, and *presenilin-1* has been described with particularly robust pathology (14).

Homologues of *APP* and *presenilin* are found in *Drosophila* (15–17). The fly APP homologue probably cannot be cleaved to generate pathogenic peptides. Several groups have reported fly models, using misexpression of Aβ or *APP* (18–20). These produce retinal and/or brain degeneration, motor and cognitive abnormalities, and plaque-like pathology.

β- and γ-secretase are responsible for generation of pathogenic Aβ peptides. Presenilin is a component of the γ-secretase complex. Mutations of the *Drosophila* presenilin homologue, *Psn*, result in phenotypes reminiscent of *Notch*, including embryonic neuroblast hyperplasia (21,22). *Psn* regulates proteolytic processing of Notch. Other components of the γ-secretase complex have been identified, including nicastrin, Aph-1, and Pen-2 (23,24). Homologues of each have been identified in *Drosophila*, and each component appears competent to serve as part of a γ-secretase complex (25). Mutations corresponding to human *Presenilin-1* mutations rescue fly *Psn* loss-of-function phenotypes to varying degrees, with the more severe mutations with regard to age of AD onset generally being more impaired in their ability to rescue the Psn mutant phenotype (26).

Transgenic *Drosophila* models of tauopathy have been reported. These have used both wild type and FTD-associated forms of *tau* (27,28). These may produce neurodegeneration without NFT. Tau kinases including *Shaggy/glycogen synthetic*

kinase (GSK)-3 beta and MARK/PAR-1 produce tau phosphorylation and affect tau toxicity in fly models (29,30). Tau misexpression in the fly also affects learning and memory (31).

Worm models also have begun to contribute to our understanding of AD. Worm homologues of presenilins, Aph-1, and nicastrin exist (24). Worm models expressing Aβ have been reported (32). A *C. elegans* model expressing wild type or FTD-associated forms of mutant human tau also has been characterized (33).

PARKINSON'S DISEASE

PD is an idiopathic disorder associated with degeneration of nigral dopaminergic neurons and formation of neuronal Lewy bodies, which are cytoplasmic inclusions containing α-synuclein. Although the true prevalence of all inherited forms of PD is unclear, rare cases of dominant PD have been associated with missense mutations in α-synuclein. Multiple systems atrophy (MSA), a "Parkinson's plus" condition, is also associated with α-synuclein-containing inclusions; however, unlike PD, these inclusions are glial.

Numerous investigators have created transgenic mice expressing wild type or mutant forms of α-synuclein (34–38). These may result in the formation of inclusions, although in most cases, these are diffuse and subtle neurochemical and synaptic abnormalities. Although many of these models display motor and other behavioral abnormalities reminiscent of PD, in most cases, frank neurodegeneration is not apparent. Attempts to create models of MSA using glial expression of α-synuclein have recently succeeded in generating fairly robust phenotypes (39,40).

The identification of mutations in parkin associated with autosomal recessive juvenile parkinsonism (AR-JP) (41) has provided new insights into the pathogenesis of both sporadic and familial forms of PD (42). Parkin is thought to function as a ubiquitin ligase. With the exception of the Dawson lab's model, which shows cell loss in locus ceruleus (43), the vast majority of loss-of-function studies of parkin in mouse have failed to phenocopy PD (44–46). A homologue of parkin exists in *Drosophila*. Pallanck and colleagues reported that parkin mutations in the fly cause cell death of sperm and indirect flight muscles (47). Marden and coworkers independently generated loss-of-function mutations and also found that these lead to reductions in cell size and increased susceptibility to oxidative stress (48). Initially, neither group was able to observe loss of dopaminergic neurons in mutant parkin flies; subsequent analysis by Pallanck and colleagues, however, using a more sensitive technique for analysis, did identify loss of dopaminergic neurons in homozygous fly parkin mutants (49). The failure of parkin knockouts in fly and mouse to phenocopy PD suggest that parkin mutations might give rise to disease by a mechanism other than simple loss of function, such as dominant negative activity, haploinsufficiency, or a toxic gain of function.

Mutations of DJ-1 also cause autosomal recessive PD (50). Although the true function of DJ-1 remains unclear, it appears to function as a redox-sensitive molecular chaperone. DJ-1 knockout mice show abnormal motor phenotypes, as

well as neurochemical and synaptic abnormalities. However, these mice generally have not shown frank neurodegeneration (51). Studies inactivating *Drosophila* homologues of *DJ-1* have highlighted importance of the protein in oxidative stress, but have not consistently shown a role in regulating survival of dopaminergic neurons (52–55).

Another recessive PD gene of interest is *PTEN-induced kinase-1 (PINK-1)* (56). Mouse models based on *PINK1* knockouts have not yet been reported. Four groups have reported loss-of-function phenotypes of *PINK1* in the fly. Half of these reports identified effects on dopaminergic neurons in the fly brain (57–60). Interestingly, like fly parkin knockouts, *PINK1* knockouts show male sterility and degeneration of indirect flight muscle. Moreover, *parkin* is able to rescue *PINK1* loss-of-function phenotypes, suggesting that the two genes somehow act in a common pathway.

TRINUCLEOTIDE REPEAT EXPANSIONS

HD is caused by expansion of unstable CAG repeat in exon 1 of the *huntingtin* gene; this results in expression of an expanded polyglutamine tract near the amino terminus (61). Other diseases in this class include spinocerebellar ataxias (SCA) 1, 2, 3 (also known as Machado-Joseph disease, MJD), 6 and 7, dentatorubral-pallidoluysian atrophy (DRPLA), and spinobulbar muscular atrophy (also referred to as Kennedy's disease)(62). Given that these disorders generally are thought to be due to a single mutation, which acts dominantly, a number of investigators have logically generated transgenic mouse models using full-length or truncated proteins using expanded repeats. For *huntingtin*, these have included models using exon 1 and longer repeat constructs, as well as knockin models (63-68). Conditional models also have been reported (69). Although the degree of neuropathology elicited in these models varies, in general, they have succeeded in recapitulating some aspects of the HD phenotype. Mouse models expressing full-length mutant *ataxin-1* also have been reported, which show ataxia and Purkinje cell degeneration (70). A mouse model of *ataxin-3* also has been described (71). An interesting feature of SCA7 is retinal degeneration accompanying cerebellar loss; of note, mouse models expressing mutant *ataxin-7* also have demonstrated photoreceptor degeneration (72,73).

Investigators have also succeeded in producing transgenic *C. elegans* models of glutamine repeat disorders. These have generally used either quasi-pure polyglutamine fragments or amino terminal fragments of huntingtin (74,75). Aggregation of polyglutamine and abnormal behaviors have been characterized. Worm models in particular have begun to prove their worth in compound screens.

Several glutamine repeat disorders have been modeled in *Drosophila*. These include a widely used model using a fragment of mutant *ataxin-3/MJD* (76); more recent studies have described phenotypes for full-length mutant ataxin-3 (77). Expression of polyglutamine-containing proteins in the eye in some cases produces a "rough" eye, which can be easily scored under the dissecting

microscope. Such phenotypes provide an attractive background for performing genetic screens. Other models have used amino terminal fragments of mutant huntingtin of various length, which tend to cause adult onset degeneration, but not rough eyes (78,79). Other investigators have reported robust phenotypes of quasi-pure polyglutamine peptides (80,81). Models using *ataxin-1* and the androgen receptor also have been reported (82–84).

The genetically tractable zebrafish (*Danio rerio*) may also prove to be useful in the study of neurodegenerative diseases. Thus far, a zebrafish model of polyglutamine pathology has been reported (85). It is anticipated that additional zebrafish models of neuropsychiatric disorders may appear in the near future.

BIPOLAR DISORDER, DEPRESSION, ANXIETY DISORDERS, AND ATTENTION DEFICIT DISORDER

A number of human loci have been suggested as potentially linked to bipolar disorder, but none to date have been generally accepted. Similarly, to date, no gene has been identified that clearly increases susceptibility to major depressive disorder, anxiety, or attention deficit disorder (ADHD). Many drugs used to treat depression, anxiety, and ADHD act on aminergic neurotransmission, and a large number of human genetic studies and transgenic models have been generated to test the hypothesis that changes in the biosynthetic enzymes, transporters, and receptors for these systems have something to do with psychiatric phenotypes. At the risk of being too dogmatic about our insistence on studying genes that have been associated at least tentatively with human disease loci, we will not discuss transgenic models of these candidates here. Similarly, we have omitted the discussion of transgenic models of depression that disrupt the hypothalamo-pituitary-adrenal axis or neurotrophin signaling pathways. The reader is referred to recent excellent reviews on these topics (86,87).

A large inversion on chromosome 13q has been reported to be associated with Tourette's syndrome, and additional mapping studies have suggested that a polymorphism in a potential regulatory region for the *Slit and Trk-like 1* (*SLITRK1*) gene may be the cause of the phenotype (88). In vitro studies suggest that *SLITRK1* may modulate dendritic growth, and if the genetic studies are confirmed, future mouse models may be very useful (88). However, the prevalence of this polymorphism is at present unclear, making further speculation difficult.

AUTISM AND RETT'S SYNDROME

Similar to schizophrenia, autism is highly heritable, possibly up to 90%, and a number of studies have been performed in attempts to identify genes associated with this disorder. At least one class of viable candidates, the neuroligins 3 and 4, have emerged from these studies, but mouse models are not yet available. In addition, patients with Rett's syndrome show behaviors similar to autism, and pathogenic processes involved in Rett's syndrome also may be relevant to autism,

despite the fact that Rett's patients have other symptoms, which include disruption of some fine and gross motor skills (89). Nonetheless, transgenic mouse models of methyl CpG binding protein (*Mecp2*) dysfunction are likely to be relevant to autism, both because of the shared clinical pathology and the molecular insights obtained from mice *Mecp2* knockouts (89).

The association of *MECP2* mutations with Rett's syndrome was first determined in 1999 (90), and since then, 90% to 95% of Rett's syndrome cases have been found to show mutations in this X-linked gene. The MECP2 protein binds to methylated DNA and functions as a transcriptional repressor in conjunction with other proteins that associate with MECP2 when it binds to DNA (91,92). The major downstream events are thought to be a modification of histone proteins responsible for chromatin structure (91,92). The mechanisms underlying chromatin remodeling and its effects on gene expression are a very active field, making the study of *MECP2* and Rett's a particularly exciting crossroads for neuropsychiatry and neuroscience (89). The key point here is that changes in the function of MECP2 should allow the aberrant activation of downstream genes. To study these changes, several *Mecp2* knockout and truncation models have been generated in mice (93–96). These have defects in spatial, contextual, and social memory (93–95,97). To identify the areas of the brain responsible for these changes, one group has performed a more restricted knockout in the forebrain and in postnatal animals (96). The behavioral phenotype of these animals was similar to that of the constitutive knockouts (96). A large number of additional tissue-specific promoter lines are now becoming available (98), and these may allow the fine mapping of the circuitry responsible for the Mecp2 knockout phenotype.

Another key use of transgenic models such as the Mecp2 knockout mice will be to determine how the underlying electrophysiological and neurochemical changes may contribute to the behavioral phenotype. Experiments using cultured neurons and slices have shown a decrease in excitatory neurotransmission but a relative preservation of inhibitory potentials as well as changes in cellular models of memory in *Mecp2*-knockout mice (99,100).

Molecular studies are beginning to determine the genes whose expression is regulated by MECP2 (101,102). These include the growth factor brain-derived neurotrophic factor (BDNF) (102). Importantly, additional studies have shown that expression of BDNF in the *Mecp2* knockouts can partially rescue the behavioral phenotype. This finding may be particularly relevant to the clinical treatment of Rett's, because it is unclear whether *MECP2* itself could be a target for pharmacologic intervention.

SCHIZOPHRENIA

To summarize the positive sentiments of some reviews on the genetics of schizophrenia, we have turned a corner and finally are obtaining reproducible data on disease-associated loci. To summarize less sanguine views, all of the genes may be false positives and even if they are real, they are not major risk factors. At

best, they show an odds ratio of 2.5. Thus, it is possible that the generation of transgenic models is premature. With this caveat, we note that transgenic models for several current candidates are yielding interesting findings, and the reader is referred to Chapters 2, 7, and 8 for additional details. We note that for some genes, transgenic models have been created for other reasons, before it was determined that they may be associated with schizophrenia.

As already discussed, the new candidates that may increase the risk of schizophrenia have arisen out of several types of studies. These include (*i*) using a positionally based approach and human genetics, either via linkage or association analysis. This is arguably the least biased method of identifying disease genes. For a discussion of animal models based on genes identified using positional/genetic approaches including the *neuregulin-1 (NRG1)* and *Dysbindin/Dystrobrebin-binding protein 1 (DTNBP1)*, the reader is referred to chapters 7 and 8, respectively. Additional methods include the use of (*ii*) chromosomal abnormalities, and (*iii*) molecular screens. Approaches (*i*) and (*ii*) are the most conservative and reflect the highly successful strategies used to generate transgenic models for neurologic diseases. In neuropsychiatric genetics, these approaches have led to candidate loci at chromosomal positions 1q, 2q, 3p, 6p, 8p, 13q, 14p, 20q, and 22q. There has been a spate of recent enthusiasm for these findings because, in contrast to earlier loci, the same sites are being identified in at least some replicate studies (103).

Approaches (*ii*) and (*iii*) require the selection of candidates after the initial screen and are inherently somewhat more biased that the first approach. Cytologically visible changes in chromosomal structure such as translocations and large deletions can disrupt the function of dozens of genes. In addition to eliminating the function of the genes within the deleted region, positional effects can act over long distances and alter the expression of genes flanking the actual deletion. These effects can make it difficult to determine whether genes in the deleted region or in the less clearly delineated flanking regions are responsible for a particular phenotype caused by the lesion. In addition, the deletion or disruption of multiple genes can, in turn, cause complex phenotypes that make if difficult to determine the contribution of each gene. In spite of these difficulties, chromosomal abnormalities have been used extensively in human genetic research, and their analysis is beginning to yield some potentially important genetic findings in the genetics of schizophrenia, and possibly other disorders (88). Importantly, for two chromosomal abnormalities associated with schizophrenia-like phenotypes, independent linkage results indicate risk factors for the same regions of the chromosome, thereby increasing the likelihood that risk loci are, indeed, somewhere in these areas (104). For a discussion of these findings, the reader is referred to Chapter 2 and the sections on 1q/*DISC1* and 22q/VCFS/*COMT*.

SUMMARY AND PERSPECTIVES

Animal models for neuropsychiatric illness have begun to be developed. For those disorders traditionally viewed as psychiatric, a relative paucity of single gene

defects that underlie disease has hindered development of models, but advances in psychiatric genetics have begun to address this. Several disorders with neuropsychiatric manifestations, such as HD and AD, have been successfully modeled in multiple model organisms including mice, worms, flies, and zebrafish. Given that transgenic primates have been produced, it seems to be only a matter of time until primate models of neuropsychiatric disorders are engineered. Genetically engineered animal models will allow detailed elucidation of pathogenic processes in vivo, as well as genetic and chemical screens for modifiers of disease.

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Toward a Unified Model of Neurogenetics

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INTRODUCTION

Schizophrenia (SZ) is a common psychiatric disorder affecting 1% of the population worldwide. It is now believed to be a neurodevelopmental disorder but its diagnosis remains a clinical challenge due to the lack of biomarkers that can aid in its recognition (1–3). Although the initial insults leading to SZ are thought to occur during fetal life, the symptoms do not usually appear until late adolescence and early adulthood. Schizophrenic symptoms include hallucinations, delusions, thought disorder, social withdrawal, and cognitive deficits such as working memory deficit.

The molecular mechanisms underlying the pathophysiology of SZ remain poorly understood. Current data from epidemiological surveys as well as genetic studies including family, twin, and adoption studies suggest that the etiopathogenesis involves the interplay of complex polygenic influences and environmental risk factors operating on brain maturational processes. Part of the reason why the pathogenesis of this disorder remains poorly understood is due to the unavailability of the diseased tissue (patient's brain biopsy) as well as the lack of animal models. The only patient samples available for research studies are postmortem brains; however, findings from postmortem brains are often difficult to interpret

because of confounding factors such as poor tissue preservation, long-term medications, drug abuse, and smoking.

Animal models for SZ are therefore needed to elucidate the pathogenesis of this disorder and to develop new and more efficacious pharmacological treatments. However, because SZ is a unique human condition, there is a tendency to negate the feasibility of developing animal models. Even though no one can deny that the perfect animal model will never recapitulate all the features of SZ, animal models may still be useful to study subphenotypes associated with the disease, such as enlargement of the lateral ventricle and working memory deficit (4).

Recently, a number of promising candidate genes for SZ have emerged and, as such, many researchers have characterized genetically engineered mice carrying mutated form of some of these genes and found that these mice displayed SZ-like behaviors such as a deficit in prepulse inhibition (PPI) (5–8). On the other hand, various environmental factors such as viruses have been proposed to contribute to some cases of SZ (9–13). These findings have encouraged several scientists to characterize mice or rats that were challenged with viruses such as influenza, cytomegalovirus, or herpes simplex virus, and they found that, in general, these mice also displayed behavioral, cognitive, and emotional abnormalities (14). At the present time, very little effort has been put into the generation of animal models that can be used to study how the interaction between genetic and environmental factors can contribute to the pathogenesis of SZ. Thus, what we would like to propose in this chapter is the design of animal models to understand how the interplay between genetic and environmental factors from the time of conception until adulthood can lead to SZ. Even though the focus of this chapter is on SZ, given that SZ and bipolar disorder share some susceptibility genes, a similar strategy can be used to generate mouse model for bipolar disorder.

GENETIC FACTORS IN SCHIZOPHRENIA

SZ is a multigenic disorder but we are still unclear on the nature of altered protein interactions that cause this disease. As an attempt to answer this question, we are interested in identifying possible interactions among SZ susceptibility gene products. We hypothesize that those proteins interacting together belong to the same pathways and that disturbance in these signaling pathways will confer increased susceptibility to SZ.

Many of the SZ susceptibility genes identified so far can be grouped into two categories: genes affecting neurodevelopment and genes affecting neurotransmission. SZ is now believed to be a neurodevelopmental disorder characterized by numerous neurotransmission abnormalities in adulthood. Two genes, Disrupted-In schizophrenia-1 (*DISC1*) and neuregulin-1 (*NRG1*), have emerged as very promising gene candidates for SZ partly because of their role in both neurodevelopment and neurotransmission. Because *NRG1* was described extensively in Chapters 2 and 6, we will focus our discussion on *DISC1*.

Role of Disrupted in Schizophrenia 1 in Neurodevelopment

DISC1, a susceptibility gene for SZ located on chromosome 1q42, was found to be mutated in a large Scottish family with a long history of SZ. Since then, genetic associations studies have shown that *DISC1* may play a role in major mental illness and cognitive functions (15,16–18) (see also Chapter 2). DISC1 has been shown to form a complex at the centrosome with lissencephaly-1 (Lis1) and NudE-like (NdelI), which are two genes that are known to be essential for proper neurodevelopment including neuronal migration (see Chapters 2 and 6). Kamiya et al. (19–21) reported that disruption of the interaction between DISC1 and its centrosomal partners causes cortical malformation resulting from delayed neuronal migration and improper dendritic arborization. In these studies, we reported that carboxy terminal-truncated mutant DISC1 (truncated mutDISC1), which could result from a translocation between chromosomes 1 and 11 has dominant negative effects. We found that truncated mutDISC1 causes a redistribution of wild type DISC1 through self-association and disrupts centrosomal DISC1–dynein complex. To confirm the dominant negative effect of truncated mutDISC1 in vivo, we electroporated truncated mutDISC1 or DISC1 RNAi in mice embryo and found that both groups displayed impaired neurite outgrowth and delayed neuronal migration in the cerebral cortex. Recent studies in our lab have also shown that DISC1–NdelI interaction is required for neurite outgrowth (22). In these studies, we showed that rat NdelI RNAi or overexpression of a fragment of DISC1 containing the NdelI binding site in differentiated PC12 cells inhibited neurite outgrowth. Overexpression of human NdelI in differentiated PC12 cells treated with rat NdelI RNAi restored neurite outgrowth.

Role of Disrupted in Schizophrenia 1 in Neurotransmission

The role of DISC1 in neurotransmission is not yet fully understood. DISC1 interacts with various known glutamate receptor interactors such as A kinase anchor protein 9 (AKAP9), alpha-actinin, and alpha-fodrin/spectrin (23–27). Furthermore, DISC1 has been reported to interact with a postsynaptic protein, citron, which is a neuronal Rho target interacting with postsynaptic density (PSD)-95/synapse-associated protein (SAP)-90 (23). Citron has been reported to form a complex with PSD-95 and NMDA receptor subunit at the synapse (28,29). Therefore, loss of DISC1 function may impair certain stages of NMDA receptor regulation via PSD-95 during neuronal morphogenesis, dendritic outgrowth, and synapse formation. Other studies found that rho guanine nucleotide exchange factor 11 (ARHGEF11), a DISC1 interactor, binds to ErbB2 and plexin B1 (30). DISC1 also interacts with activating transcription factors (ATF)-4 and ATF-5 as does the $GABA_B$ receptor, GABBR1 (24,31). Taken together, these studies suggest that DISC1 may modulate the function of NRG1 and $GABA_B$ receptors, which have also been implicated in SZ, as mentioned in Chapters 5 and 6.

ENVIRONMENTAL FACTORS IN SCHIZOPHRENIA

Various environmental factors are thought to be implicated in the pathophysiology of SZ. In this section, we will focus our discussion on the role of stress, immune response, and viral infection in the pathophysiology of SZ (Fig. 1).

Stress

Following exposure to any stress lasting longer than a few minutes, levels of cortisol released by the adrenal cortex increase. The hormone, corticotrophin-releasing hormone (CRH), which is produced by the paraventricular nucleus (PVN) of the hypothalamus in response to stress activates the pituitary gland to release adrenocorticotrophic hormone (ACTH). ACTH acts on the adrenal cortex to stimulate the release of cortisol. This cascade of events leading to the production of

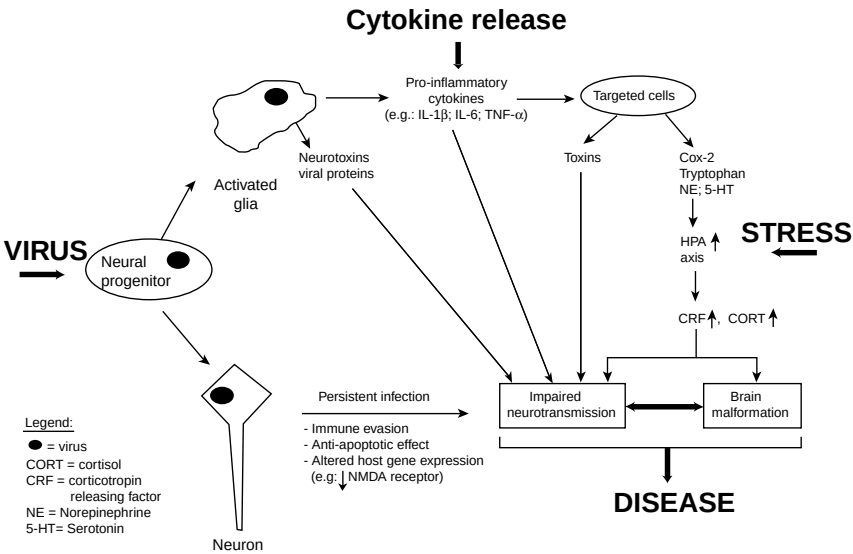

Figure 1 Viruses, stress, and cytokines are three environmental factors that are thought to be implicated in some cases of schizophrenia. Following viral infection of glia cells (activated glia), proinflammatory cytokines as well as toxins are released, which, in turn, will eventually lead to impair neurotransmission. Furthermore, cytokines released by activated glia cells can act on targeted cells to cause prolonged activation of the HPA axis. Persistent activation of the HPA axis can lead to brain malformation as well as impair neurotransmission. Viruses can persist in neuronal cells by triggering antiapoptotic pathways as well as downregulating host immune response. During this state of persistence, some viral proteins can interfere with host gene expression and cause, for example, downregulation of NMDA receptors (132). *Abbreviations*: HPA, hypothalamic pituitary-adrenal; NMDA, *N*-methyl-D-asparate.

stress hormone is usually referred to as the hypothalamic-pituitary-adrenal or HPA axis. The release of cortisol is inhibited by a negative feedback loop, which is regulated by the hippocampus (32).

Stress has been implicated in the onset of psychiatric disorders including schizophrenia (33–35). Some studies suggest that the onset and relapse of SZ are associated with environmental stress (36,37). Some epidemiological studies in humans have reported that psychological distress during pregnancy is associated with increased likelihood of giving birth to a child with SZ. According to these studies, the increase in the incidence of SZ during World War II was associated with increased exposure to stress during the second trimester of pregnancy of women who lost their husbands during the war (38). Other studies found that stress-reducing behavioral and pharmacological treatments may improve the symptoms of SZ (39–41).

Altered HPA axis activity has been proposed as a link between the psychological experience of stress and the development of psychosis (35,42). Studies in support of this hypothesis include reports of hippocampal and pituitary gland abnormalities, two brain structures that are integral to the HPA axis, in association with psychotic illness. According to these studies, patients with psychosis present with neuroanatomical defects including reduced hippocampal volume, glucocorticoids receptor number and cell size, as well as cognitive impairments. Some studies have reported decreased pituitary gland volume in SZ of at least five years' duration (50). Other studies revealed that patients with psychosis, including SZ patients, have elevated levels of cortisol as well as abnormal circadian rhythms (39,51–56). Furthermore, three other studies found that schizophrenic patients have a blunt response to cortisol, following exposure to various kinds of stresses (57). Parallel to these findings, some studies have reported that SZ patients failed to suppress cortisol release following administration of dexamethasone. These studies thus suggest that there is a failure of the HPA axis negative feedback mechanism in some schizophrenic patients, which will in turn lead to persistent activation of the HPA axis.

The mechanisms by which activation of the HPA axis could lead to psychiatric disorders such as SZ are poorly understood. What we know so far is that persistent activation of the HPA axis leads to prolonged increased levels of cortisol, which has been shown to cause neurodevelopmental abnormalities similar to those found in SZ patients, such as enlarged ventricles, cortical atrophy, hippocampal dysfunction, and cognitive impairment (58,59). The hippocampus is at increased vulnerability to elevated levels of cortisol because of its high expression of glucocorticoid receptors. Hippocampal dysfunction leads to decreased negative feedback response on the production of cortisol, which, in turn, will cause persistent elevated levels of cortisol (60).

Parallel to findings in human studies, studies in rats have shown that prenatal stress leads to hippocampal abnormalities similar to those found in patients with psychosis. These hippocampal defects include altered function of pyramidal neurons from Cornu Ammonis (CA1) and CA3, changes in cell morphology

characterized by decreased atrophy of the dendritic processes, impaired neurogenesis in the dentate gyrus, and prolonged activation of the HPA axis due to downregulation of the glucocorticoid receptors in the hippocampus of adult offspring (59–76). In addition, these mice showed altered gene expression, a deficit in PPI and auditory sensory gating, which are two neurobehavioral abnormalities seen in SZ patients (77,78). Recent studies have demonstrated that mice exposed to stress during the prenatal stage or adulthood display defects in the prefrontal cortex characterized by decreased spine densities and dendritic length, as well as reduced complexity of the dendritic trees, all of which have been reported in SZ patients (79–82).

Prenatal exposure to stress has also been reported to cause alterations in neurotransmission, characterized by changes in the activity of GABA receptors and increase in the affinity of benzodiazepine receptors located in the dentate gyrus and septal nuclei. In addition, there are also several lines of evidence of enhanced dopaminergic activity and dopamine release following prenatal stress or early exposure to glucocoricoids (83–85). Some studies have reported that prenatal stress is associated with increased binding to D2 receptors and decreased binding to D3 receptors in rats' nucleus occumbens (83). Taken together, these studies suggest that prenatal stress can induce neurotransmission abnormalities that are similar to those found in SZ patients.

Immune Response (Cytokine Release)

Cytokines are low-molecular weight proteins that are elevated during ischemia and infection, two events that have been associated with increased risk to SZ (86,87). Cytokines modulate systemic and central nervous system (CNS) response to injury, infection, and inflammation. Cytokines are actively transported through the blood–brain barrier and are also produced by glial and neuronal processes within the CNS. Cytokines are implicated in both normal and abnormal brain development.

Studies have shown that cytokines activate the HPA axis, leading to increased level of ACTH and cortisol (88). As discussed in the section on stress, persistent activation of the HPA axis has been associated with SZ. Althougth interleukin-1 (IL-1) is the most potent activator of the HPA axis, IL-2, IL-6, as well as tumor necrosis alpha (TNF-α) can also activate the HPA axis and, by doing so, enhance the effects of IL-1 (89).

It has been demonstrated that cytokines play a role in the regulation of many neuronal functions such as neurotransmission, neuronal survival, and synaptic plasticity (89,90). Studies have shown that cytokines can alter levels of noradrenaline, 5-hydroxytryptamine (5-HT), gamma-aminobutyric acid (GABA), and acetylcholine, all of which have been implicated in the pathophysiology of SZ, as discussed in chapter 5 (89). In addition, various cytokines have been reported to impair neuronal differentiation and growth as well as synaptic plasticity in brain slice culture (89,91). It has been reported that IL-1ß, IL-2, TNF-α, IFN-α, and IFN-ß all inhibit long-term potentiation (89). Studies in culture brain neurons

found that IL-5, IL-7, IL-9, and IL-11 can modulate the development of ion channels and action potentials in cultured brain neurons (89,91). TNF-α was found to be toxic to oligodendrocytes and to cause demyelination (89). Oligodendrocyte loss and demyelination have been associated with SZ.

Infection of pregnant women has been associated with SZ (87,92–95). Several viruses are thought to contribute to some cases of SZ, and this observation has led some scientists to propose that these viruses may be acting through a common pathway, possibly involving increased levels of cytokines, which could, as already described,impair several aspects of neurodevelopment (87,96). Support for these hypotheses include studies showing that cytokine levels are increased in pregnancies complicated by infection and altered levels of IL-6 and TNF-α in the blood of nenonates from infected mothers (87,92–95). Furthermore, it is known that maternally produced cytokines can cross the placenta and enter the fetal circulation; they can also cross the blood–brain barrier (87).

Studies in humans have revealed abnormalities of cytokines in schizophrenic patients. The levels of IL-1, TNF-α, IL-6, and IL-1 receptor antagonist were found to be significantly altered in plasma and cerebrospinal fluid (CSF) of patients with SZ. The TNF-α gene is located on chromosome 6p21.1–21.3, which is a region that has been linked to SZ (97). In addition, a TNF-α polymorphism (-G308A) as well as a polymorphism in its promoter region have been associated with SZ (98–100). Several IL-1 polymorphisms have also been reported to be associated with SZ (101).

Several studies have reported elevated IL-6 levels in SZ and other studies have shown that elevated IL-6 is associated with poorer outcome (102–104). Another study reported reduced levels of soluble gp 130 (sgp130) in the CSF of schizophrenic patients compared to depressed patients and healthy controls. sgp130 is an antagonist to the gp130 receptor and hence inhibits signaling downstream of IL-6 receptor (105).

One study reported an increase of IL-10 in schizophrenic patients compared to healthy controls (106). Another study found an association between IL-10 levels and negative symptoms in the CSF of 62 unmedicated schizophrenics (104,107). Furthermore, in schizophrenics treated with haloperidol, there is a correlation between IL-10 levels in the CSF and the severity of psychotic episodes as measured by the Bunney-Hamburg psychosis rating scale.

Several studies have found that CNS ischemia leads to increased risk of developing SZ (86). These studies have shown that birth complications involving hypoxia and ischemia are associated with increased risk to develop SZ. Because ischemia is known to enhance levels of cytokines, it is possible that insults associated with ischemia are, at least in part, mediated by cytokines.

Viral Infection

Numerous epidemiological studies have suggested that prenatal exposure to viruses may be involved in some cases of SZ (108–110). These studies have

reported that children born during the winter and early spring are at increased risk of getting SZ. Another finding in support of this hypothesis is that birth in urban areas (crowded areas) is associated with increased risk to SZ; individuals born in cities are twice more likely to get SZ compared to those born in rural areas or small towns.

Among the infections investigated in relation to SZ, influenza has received the most attention for three main reasons. First, there was an increase in the incidence of SZ following the influenza pandemic of 1918 and 1919. Second, the peak season of influenza is during the winter months, which coincide with the period associated with increased risk of SZ. Third, influenza is a relatively common infection affecting between 15% and 30% of the population each year and, thus, providing better statistical power for analyses.

Brown and colleagues (111) conducted a nested case-control study and found that prenatal exposure to influenza increased the risk to SZ of a large birth cohort that was followed up for psychiatric disorders for 30 to 38 years. These findings were validated by a study conducted by Strub and colleagues to compare the risk of exposure to influenza between SZ patients born between 1949 and 1981 and matched controls. They found that SZ patients were more likely to have been exposed to influenza during the fifth month of pregnancy than controls (112). As a follow-up to these findings, Patterson and colleagues phenotyped pups from pregnant mice infected with influenza and found that these pups displayed several behavioral and neuroanatomical deficits some of which have been associated with SZ (113,114).

A number of studies conducted by Dr. Yolken and colleagues have reported that untreated individuals with recent onset SZ have altered levels of antibodies to cytomegalovirus (CMV), Toxoplasma gondii, and human herpesvirus (9,11,115). They also reported that medication against CMV improve both positive and negative symptoms of CMV-seropositive schizophrenic patients (13). These studies thus suggest that active viral replication may be associated with some cases of SZ.

Some studies have suggested that retroviruses may also be implicated in the pathogenesis of SZ (116). It is known that retroviruses such as HIV can replicate within the CNS and cause neurological as well as psychiatric symptoms. Furthermore, our genome contains many endogenous retroviral elements (ERVs) bearing homology to known animal retroviruses. These ERVs are differentially activated at different stages during neurodevelopment and have been associated with several chronic human diseases (117–123). It is, therefore, not impossible that ERVs activation at various stages during brain development could lead to psychiatric problems later in life. As an attempt to come up with evidence in support of a role for retroviruses in some cases of SZ, Yolken and his group (124,125) conducted a study to compare levels of retroviral DNA sequence in the CSF of individuals with recent onset SZ and matched controls. They found retroviral DNA sequences in the CSFs of some SZ patients and these DNA sequences were absent in the CSFs of control subjects. Furthermore, they also reported

upregulation of the transcription of RNA homologous to that of the human endogenous retroviral family of retroviruses in the frontal cortex of SZ patients.

The mechanisms whereby viral infection can lead to neurodevelopmental disorders remain poorly understood. Currently, it is believed that the virus can act either directly or indirectly to affect neurodevelopment. Following viral infection, the immune system becomes activated and cytokines are released, which can, in turn, interfere with proper neurodevelopment as already mentioned. Viruses can also affect neurodevelopment directly through the interaction between viral and host proteins. Wataru and colleagues found several behavioral and neuroanatomical abnormalities in transgenic mice expressing Borna disease virus (BDV) phosphoprotein P in glial cells. Interestingly, these abnormalities were detected in the absence of immune activation, neurodegeneration, or reactive astrocytosis (126). Although the mechanism responsible for the neurobehavioral alterations in these transgenic mice is unknown, one can postulate that constitutive expression of BDV-P may impair neurotransmission, which, in turn, would lead to brain dysfunction (127,128).

ANIMAL MODELS TO STUDY GENE–ENVIRONMENT INTERACTIONS

Genetic mouse models are a very useful tool to identify key pathways in the pathophysiology of SZ as well as novel pharmaceutical agents for the treatments of SZ. Indeed, now that we have an extensive list of possible candidate genes for SZ, we can generate transgenic mouse expressing various mutated forms of these candidate genes, phenotype these mice at the neuroanatomical level, and compare to what extent the neuroanatomical defects present in these mice mimic those seen in SZ patients. We hypothesize that those genes that contribute to the pathophysiology of SZ will induce neuroanatomical alterations in mice that are similar to those seen in SZ patients.

SZ is a polygenic disorder and more studies are needed to determine what combinations of genetic alterations are required to cause SZ. Furthermore, genetic linkage and association studies have often yielded inconsistent results due to the heterogeneity of the disorder and/or limited sample size. For example, Margolis and colleagues (129) had reported that a *DISC1* frameshift mutation was associated with SZ in an American pedigree. Recently, another study showed that this frameshift mutation was not associated with SZ (130). Another example is the case of the FEZ1 gene that was found to be associated with SZ. A recent study found that FEZ1 is not associated with SZ (131). Animal models can help us identify what sets of genes are critical for the pathophysiology of SZ. This can be achieved by the generation of double, triple, quadruple, or more transgenic mice. Given that SZ is not a purely genetic disorder, the next step will be to expose transgenic mice expressing multiple SZ susceptibility genes to various environmental factors at different time points before and after birth so as to study the effects of gene–environment interaction on brain development.

Another advantage of genetic mouse models is that they can help us understand how insults during the early stages of neurodevelopment can lead to

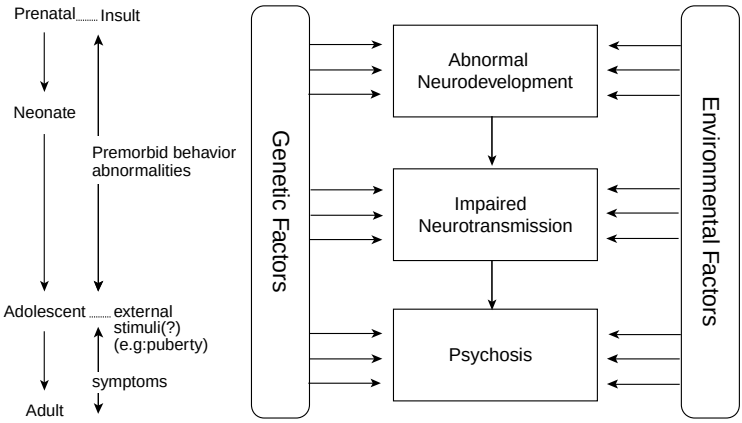

Figure 2 Schizophrenia is now believed to be a neurodevelopmental disorder where the primary insult occurs during the prenatal stage and the symptoms start from late adolescence to early adulthood. The primary insult can be caused by genetic alterations and environmental factors; this interplay between gene and environment can affect various neurodevelopmental stages, and this could eventually lead to abnormal neurodevelopment. These neurodevelopmental abnormalities can, in turn, cause neurotransmission abnormalities, which could eventually lead to psychosis.

neuropsychiatric problem later on in life. One of the unsolved mysteries in the pathophysiology of SZ is the gap between the time of insult and the onset of the symptoms (Fig. 2). The use of animal models can help us explain such a gap. For example, preliminary results from our lab suggest that loss of function of DISC1 in the cortex is associated with decreased dopamine level that was only noticeable after Postnatal day 56 (P56). Hence, from these studies, one can postulate that loss of DISC1 function may be associated with impaired maturation of dopamine neurons in the prefrontal cortex. Therefore, it is only after the maturation of dopaminergic synapses that DISC1 knockout mice will display decreased dopamine level as well as the deficit associated with it.

Finally, as our understanding of the key pathways involve in the pathophysiology of SZ becomes more clearly defined, genetic animal models carrying alterations in these pathways can be used to test novel therapeutic agents for the treatment of SZ. The current drugs for SZ have too many undesirable side effects and are not so effective against negative symptoms.

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8

Clinical Endophenotypes: Implications for Genetic and Clinical Research

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INTRODUCTION

The last 100 years have witnessed an unprecedented attempt by Western medicine to combine codified diagnostic systems with empirically derived treatment to identify and palliate disorders of mood, thought, and communication. Today, most individuals suffering from afflictions of mood or thought can be reliably classified into categorical "disorders" or "diseases," based on classification systems such as the Diagnostic and Statistical Manual for Mental Disorders (DSM) (1) or the International Classification of Diseases (ICD) (2). Yet, despite advances in illness classification and the wide variety of therapies available, persons suffering from severe mental illnesses (e.g., bipolar disorder and schizophrenia) continue to endure debilitating symptoms and experience occupational and social dysfunction accordingly (3,4). In a recent publication sponsored, in part, by the World Bank, several psychiatric disorders were listed as among the ten most disabling illnesses (5).

In this context, clinical psychiatrists are compelled to maximize benefits of available therapies by addressing the presence of both categorical illness (e.g., pharmacologic agents to treat "schizophrenia") and affiliated illness sequelae (e.g., disorganization or memory impairment). In this sense, most mental health professionals have already incorporated the concept of alternative phenotypes in their therapies. That is, we attempt to define and treat classifiable phenomena, which may be outside of the categories that have been reified in the DSM or ICD. Although private practice clinicians have been operating for some time at both the level of assessing and treating categorical diseases and particular symptoms and deficits, psychiatric genetic researchers have only recently begun to appreciate the impact of noncategorical behaviors. Genetic research promises to contribute to our understanding of the pathophysiology of psychiatric disorders, but only if genes for those disorders are indeed identified. Perhaps in response to the slow progress in identifying genes for the major psychiatric disorders, many psychiatric genetic researchers have turned toward redefining the "phenotypes" of psychiatric illness in order to more easily determine the genetic variants which underlie the complex phenomena of mental disorders.

In this chapter, we will discuss methods for using alternative phenotypes to find genes for mental illness. We will also provide a brief review of the search for the genetic basis of these alternative phenotypes as they interface with schizophrenia and bipolar disorder. Finally, we will discuss how alternate phenotypes are likely to be utilized in research and clinical treatment settings in the near future.

PHENOTYPES AND ENDOPHENOTYPES

In his treatise De Partibus Animalum, Aristotle (6) exhorted the natural philosopher to examine individuals at several levels: "the ultimate substances of which they are made," "the flesh, bone, and blood," the "face, hand, foot." The natural philosopher should "examine how each of these comes to be what it is, and in virtue of what force." Although written over 2300 years ago, this is essentially the task of the behavioral neurogeneticist of the 21st century. External behaviors (signs) and internal experiences (symptoms), and the observed "syndromes" in which these cluster together, have been the basis of how psychiatric disorders have been categorized in diagnostic manuals such as DSM-IV. In recent years, neuroscientists have gathered important information linking variation in anatomic, cognitive, and biochemical markers to these different categories of illness (7–10). With advances in the ability to correlate specific genes with both diagnostic categories and more discrete, quantifiable entities, neurogeneticists must face the challenge of explicating how one of the elemental "forces" of nature—the "ultimate substance" of DNA—operates at several levels within the human organism. Whether these efforts help to clarify our current conceptualizations and treatments of mental illness, or whether these efforts will further obscure an already complex endeavor, will depend on carefully designed research

studies and a scientific and medical field that is able to view psychiatric phenotypes from both a categorical perspective as well as from the viewpoint of continuity with nonpathologic behaviors and conditions.

One of the fundamental goals of neurogenetic research is to tie variation in specific genes to specific qualitative or quantitative phenomena. In genetic parlance, these phenomena are referred to as "phenotypes." Phenotypes can be directly studied and conceptualized as discrete diagnostic categories (e.g., idiopathic hypertension or schizophrenia) and/or as continuous variables (e.g., systolic blood pressure or a rating of negative symptom severity). Historically, physicians were concerned with the classification and treatment of disease entities, leading to diagnostic systems based almost completely on presence or absence of particular symptoms/signs/syndromes. By contrast, experimental psychologists have largely been concerned with quantifiable variance of specific measurable behaviors and experiences. These latter measurements, which include indices of qualities such as cognition, personality, or impulsivity are also technically phenotypes. Energized by the newfound possibility to identify genes for both the discrete disease entities of modern psychiatry and for the quantitative traits of experimental psychology, neuroscientists have recently established a new dialogue, which has, in turn, led to a new concept—"endophenotype." In fact, an endophenotype is essentially a special category of phenotype—one that has a special relationship with a more complex phenotype.

Irving Gottesman and James Shields (11) coined the term "endophenotype" for use in psychiatric genetics. As used by Gottesman and Shields, the term refers to the use of measurable phenotypes, which are associated with major psychiatric disorders, and which may be easier to quantify (and identify genes for) than the disorders per se. Gottesman and others (12–15) have defined a number of characteristics, which would make a phenotype a useful "endophenotype" for psychiatric research. A primary consideration is that the proposed endophenotype be highly heritable, if possible more so than disorders themselves. A second consideration is that the endophenotype should be associated with the disease or disorder of interest. It is also helpful if endophenotypes are quantifiable, which may allow entire pedigrees to be utilized in genetic mapping studies (rather than a handful of affected subjects). Because one of the primary aims of using endophenotypes is to better understand the genetic architecture of more complex (or more obscure) disease phenotypes, it is also critical that endophenotypes are seen in unaffected relatives of those with a particular disease phenotype at a level in between controls and affected individuals. Finally, if the endophenotype really is related to its associated phenotype, they should be jointly controlled by overlapping groups of genes.

These are all valid and minimal requirements to allow a particular phenotype to be considered an "endophenotype" for a related, more genetically complex phenotype. However, caution should be exercised: even when a particular trait is found that meets these requirements, it may be as complex (genetically and environmentally) as the disease it is associated with. Although it is believed that

endophenotypes mediate between gene(s) and disease, one must keep in mind the possibility that a putative endophenotype and a phenotype may only be indirectly related. Ultimately, it will not be until specific genes are identified for the categorical disorders (diseases) and genes are identified for potential endophenotypes for these disorders that the exact relation between genes and phenotypes may be ultimately unraveled.

At the present time, for those engaged in research on putative endophenotypes for disorders such as schizophrenia and bipolar disorder, the hope is that these related measures may strengthen the ability to identify genes for the diseases through the use of extended pedigrees, or that identification of the genes underlying the endophenotypes will begin to shed some light on the complex genetics underlying the related diseases. In two of several examples from other branches of medicine, quantitative traits associated with the complex diseases of diabetes type II and Alzheimer's disease have been utilized to more definitively identify the genes which underlie both these traits and the associated diseases (16,17). Recently, methodologies to identify and study psychiatric endophenotypes have been greatly enriched, and currently include neuropsychological, neurophysiologic, neuroanatomical (imaging), and biochemical approaches (Fig. 1).

ALTERNATIVE CLINICAL PHENOTYPES

Family, twin, and adoption studies have provided evidence for the heritability of most mental illnesses. For instance, heritability for schizophrenia is estimated to be from 73% to 90% and heritability for bipolar disorder is estimated to be between 73% and 93% (18,19). Given these relatively high heritability rates, early genetic mapping studies of schizophrenia and bipolar disorder used categorical diagnoses, in multiplex pedigrees, to try to locate the underlying genes for these disorders. As ascertainment of pedigrees with large numbers of clearly defined cases of schizophrenia or bipolar disorder has always been difficult, investigators have utilized several strategies to maximize the chance of finding disease-related genes. Among these are broadening of the phenotype to be studied. For example, including schizotypal relatives as affecteds in linkage analyses of schizophrenia or including recurrent major depression as an extension of the bipolar phenotype (20,21). Other strategies include studying more homogeneous populations (22,23) or studying large numbers of families with narrowly defined bipolar disorder or schizophrenia (24,25). Thus far, these approaches seem to have been moderately successful in identifying gene loci and some candidate genes for schizophrenia (26), but much less successful for bipolar disorder (27). Indeed, the lack of success in identifying replicable gene loci involved in bipolar disorder has led to a recent recommendation of a task force of the National Institute of Mental Health to propose that the search for endophenotypes for mood disorders should be a primary aim of geneticists currently attempting to better understand the foundations of these illnesses (28).

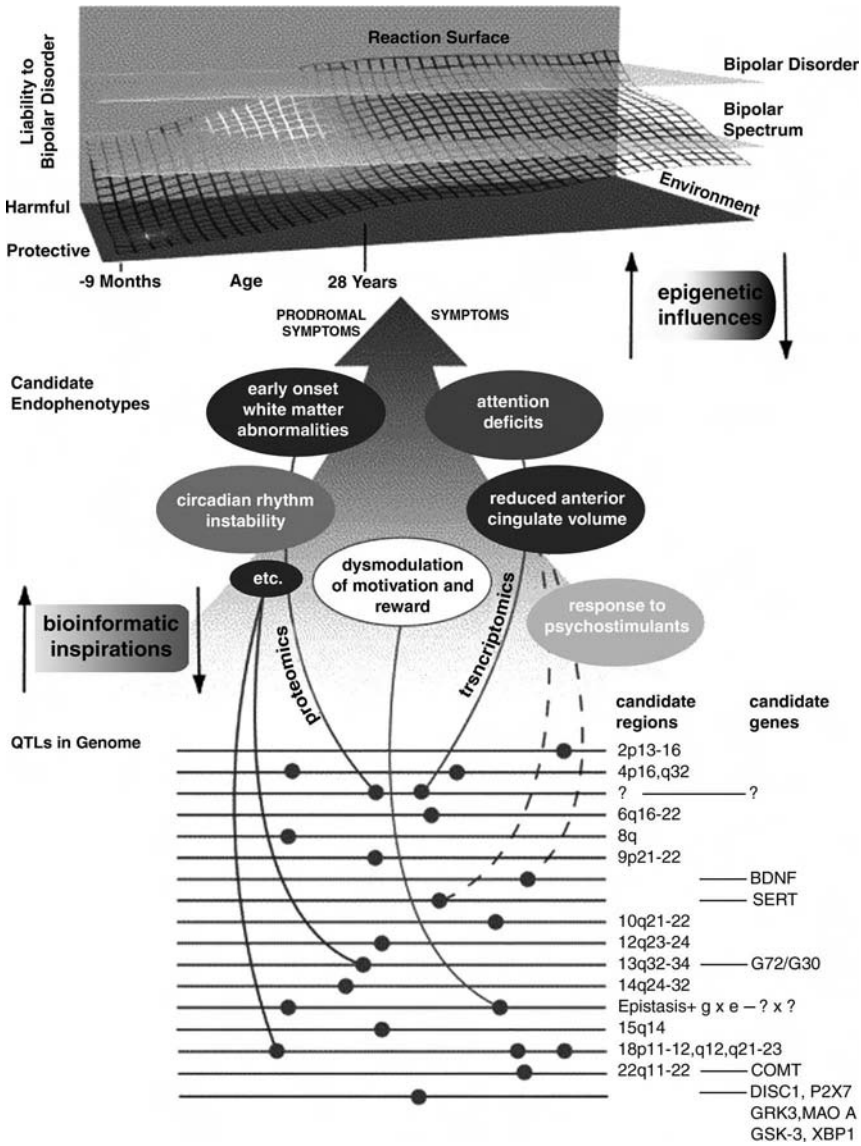

Figure 1 The relationship of potential genes, endophenotypes and phenotypes for bipolar disorder. *Source*: Copyright by Irv Gottesman; used with permission. From Ref. 127.

Although there have now been many published linkage screens for bipolar disorder and schizophrenia, there have been relatively few scans looking at alternative clinical phenotypes or quantifiable clinical phenotypes associated with these disorders. Alternative clinical phenotypes, especially if they are quantifiable

and well distributed in pedigree studies, could be potentially beneficial "endophenotypes" for these disorders. Brzustowicz et al. (29) found that ratings of positive symptoms of schizophrenia assessed in entire pedigrees were linked to chromosome 6. They reported that this quantitative phenotype yielded a stronger linkage score than the categorical diagnoses over the chromosome 6p region. At least three groups working in the field of schizophrenia have performed linkage studies utilizing the phenotype of psychosis (categorically defined) and found that this alternative phenotype yielded stronger linkage scores than the more classical phenotypes of schizophrenia and schizoaffective disorder (30,31,25). Walss-Bass et al. (32) recently reported on potentially different genomic regions being involved in manic versus nonmanic psychotic disorder, showing the potential utility of syndromes such as mania or depression as alternative phenotypes, which might link more directly to genes than the larger, complex disorders of schizophrenia, schizoaffective disorder, and bipolar disorder.

There is a paucity of reports utilizing alternative phenotypes in linkage or association studies in bipolar disorder. One approach that has proven promising is to subdivide bipolar families by the presence or absence of psychosis. In such studies (33), genetic linkage information for specific loci has been shown to be enhanced, in comparison to studies which combine all bipolar families in one analysis.

Quantifiable, dimensional rating systems for both bipolar disorder (34) and schizophrenia (35, 36) have existed for some time, yet they have rarely been -utilized in genetic mapping studies. In part, this may be due to the state dependence of most of these ratings. Alternative, lifetime dimensional scales, such as the Lifetime Dimensions of Psychosis Scale (37) and the Bipolar Affective Disorder Dimension Scale (38), have yet to be thoroughly employed in genetic mapping and association studies. Nevertheless, such scales may certainly prove to be of use, if they are applied to parse out genes which might underlie specific illness dimensions. There is significant evidence that both bipolar disorder and schizophrenia are composed of multiple, relatively independent dimensions, which can be measured at the level of clinical symptoms (39,40).

There are a large number of clinical anomalies that can be quantitatively measured, and which have been associated with schizophrenia and/or bipolar disorder, that could conceivably be used as endophenotypes for these disorders. For schizophrenia, these clinical anomalies/traits include electroencephelographic abnormalities (41), neurological examination abnormalities (42), eye tracking (43) and response to evoked potentials (44). For any of these measures, it will be necessary to test whether they meet the minimal additional requirements for being a potential endophenotype—namely, heritability, state independence, alteration in unaffected relatives, and cosegregation with illness in pedigrees. Some or all of these traits may also be of relevance to bipolar disorder (45–47).

NEUROCOGNITIVE ENDOPHENOTYPES

Neurocognitive traits, which have a long history of being studied in both the general population and in terms of their relationship to schizophrenia and mood disorders, present some of the more promising potential "endophenotypes." A fundamental requirement of any endophenotype is that it demonstrate heritability, and high rates of heritability have now been demonstrated for adult intelligence test performance (48–50) and, more recently, for the cognitive domains of attention, executive functioning, processing speed, and working and declarative memory (51–59). Thus, all of these cognitive domains are potential endophenotypes for mental disorders such as schizophrenia and bipolar disorder.

Patients with schizophrenia have profound impairments in a wide range of cognitive domains (60). These impairments are present prior to and during illness onset (61–64), and typically persist even after overt psychotic symptoms remit with treatment (65–67). High-risk and sibling paradigms have shown that first-degree relatives of schizophrenia probands perform better than patients but worse than healthy comparison subjects on a variety of neuropsychological tests (68–71). Thus, as a class, neuropsychological measures meet many of the criteria for endophenotypic markers.

To address the issue of which neuropsychological domains might be the best markers of genetic liability for schizophrenia, Cannon and colleagues (72) studied sets of twins and determined that neurocognitive tests of spatial working memory, divided attention, intrusions during recall of a word list [California Verbal Learning Test (CVLT) (73)], and choice reaction time (processing speed) best predicted genetic liability for schizophrenia in their sample. Interestingly, any one of these measures could not fully explain performance on the others, suggesting that each measure is independently sensitive to genetic loading for schizophrenia. Egan and colleagues (74) partially replicated the findings of Cannon and colleagues (72), showing significant relative risk for poor CVLT performance and Trails B [a visuo-spatial executive function test (75)]. In addition, relative risk for several other working (executive) and declarative memory measures reached trend level significance. In addition, Cornblatt and Malhotra (76) provide evidence that attention or vigilance dysfunction is sensitive to genetic liability for schizophrenia. Taken together, these findings suggest that several neurocognitive measures are potential candidate endophenotypic markers for schizophrenia.

Neurocognitive deficits in bipolar disorder have received significantly less attention than in schizophrenia, in part because the variable mood states found in bipolar disorder influence test performance. Fluctuating levels of cognitive performance observed in longitudinal studies of bipolar disorder (77,78) clarify that any study, which attempts to define cognitive endophenotypes for bipolar disorder must have a methodology to accurately assess the level of affective symptomatology present at the time of measurement. Although there is clear evidence that individuals with bipolar disorder exhibit widespread neurocognitive

dysfunction during acute episodes of mania (79) and depression (80), the discovery that these deficits endure in euthymic bipolar disorder patients raises the possibility that cognitive impairment may represent a trait rather than a state variable. Euthymic bipolar disorder patients exhibit limitations in several cognitive domains (81–85), including measures of executive function (45–86,88,78), declarative memory, and sustained attention.

Despite unresolved questions of residual mood effects, which may adversely affect performance on cognitive measures (89,90,91), a recent review concluded that the most consistent "trait" deficits in bipolar disorder appear to be verbal learning and memory, and sustained attention (92). Thus far, measures of these cognitive domains, in conjunction with tests of executive functioning, appear to be the most likely candidate neurocognitive endophenotypes for bipolar disorder (14).

There is growing evidence that first-degree relatives (e.g., siblings, parents) of bipolar disorder probands have mild executive impairments (93–95), particularly during tasks that require speeded judgments or sentence completion. Evidence for declarative memory deficits in unaffected siblings of bipolar disorder probands is less clear, with some groups reporting mild impairments (94,96,97), although others have not replicated this finding (95,98,99). However, currently examinations of unaffected relatives of bipolar disorder probands have been limited by sample size and inconsistencies of neuropsychological measures applied.

NEUROIMAGING ENDOPHENOTYPES

Twin studies of brain anatomy based on in vivo magnetic resonance imaging (MRI) images have found that brain volume and gray matter density is highly heritable (100). Baaré and colleagues (101) studied 112 twin pairs (54 MZ and 58 DZ), reporting that genetic factors accounted for most of the individual differences in whole brain (90%), gray (82%), and white (88%) matter volume. In contrast, individual differences in lateral ventricle volume were best explained by a model containing common (58%) and unique (42%) environmental factors.

Patients with schizophrenia have reduced brain volume in prefrontal, temporal/limbic, and parietal cortices, and increased ventricular size (102). Twin and family designs have shown that many of these abnormalities are associated with genetic predisposition for schizophrenia (103). Recently, reductions in gray matter density in schizophrenia probands and their unaffected MZ cotwins are focused in dorsolateral prefrontal cortex (DLPFC), superior temporal gyrus, and superior parietal lobule (104). Although ventricular enlargement is one of the more consistent neuroanatomical anomalies reported in schizophrenia (102), these abnormalities may be influenced by both genetic and environmental factors (105). Similarly, although reduced hippocampal volume seems to be associated with genetic liability for schizophrenia (106), the size of proband hippocampi is

significantly influenced by unique environmental influences (perinatal hypoxic events) (107).

In vivo volumetric MRI studies have reported subtle structural brain changes in bipolar patients in prefrontal, medial temporal, and limbic regions (108,109). Recently, Strakowski and colleagues (109) observed that although some abnormalities (e.g., subgenual prefrontal cortex, striatum, and amygdala) exist early in the course of bipolar illness, predating illness onset, other anatomic regions appear to degenerate with repeated affective episodes (e.g., cerebellar vermis, lateral ventricles, and inferior prefrontal regions) and may represent the effects of illness progression. Noga and colleagues (110) found caudate enlargement in both affected and unaffected monozygotic twins discordant for bipolar disorder, suggesting that striatal enlargement may be a heritable vulnerability factor for developing bipolar disorder.

McDonald and colleagues (111) used structural MRI to investigate the relationship between genetic risk for schizophrenia or bipolar disorder and neuroanatomical variation. They reported that although genetic risk for schizophrenia was associated with distributed gray matter volume deficits in the bilateral fronto-striato-thalamic and left lateral temporal regions, liability for bipolar disorder was associated with gray matter deficits only in the right anterior cingulate gyrus and ventral striatum (111). In addition, risk for both disorders was associated with white matter volume reduction in the left frontal and temporo-parietal regions, suggesting that these two disorders show both unique and overlapping patterns of brain structural pathology related to variable genetic risk. In a subsequent analysis of these same data, McDonald and colleagues did not find evidence for ventricular enlargement in either the patients with bipolar disorder or their unaffected relatives (112).

Although less studied, functional imaging may provide especially good endophenotypes for bipolar disorder and schizophrenia. Studies to determine whether fMRIs (functional magnetic resonance imaging) meet criteria to be useful endophenotypes have the challenge of being expensive. Nevertheless, the ability to study functional activation of particular areas of the brain during specific tasks make targeted fMRI studies potentially quite useful. Valdes et al. have recently found significant correlation between activation in the dorsal prefrontal cortex and scores on the Barrat Impulsivity Scale (113). Similarly, recent studies have shown correlation of fMRI signal abnormalities in the same brain region in the unaffected siblings of schizophrenic patients (114).

BEHAVIORAL ENDOPHENOTYPES

A wide variety of behaviors, many of which are altered in one form or another in persons affected with schizophrenia or bipolar disorder, may prove to be useful endophenotypes for these disorders. This is a largely unexplored and challenging area of behavioral genetics. Bipolar disorder type I is largely defined on episodes of mania occurring one or more times during a person's life. Driven for the most part by attempts to find pharmaceutical interventions for mania, scales are

available, which quantitatively measure key features of mania, such as decreased sleep, increases in grandiose ideation, pressured speech, and risky behaviors. Akiskal and colleagues have developed a rating scale (TEMPS-A) that measures various "dimensions" of bipolarity such as cyclothymic, hyperthymic, dysthmic, and anxious personality traits (115). This rating scale could define one or more behavioral endophenotypes for bipolar disorder. Similarly, actimetry (monitors, which measure activity levels) measures could be behavioral endophenotypes for bipolar disorder (116).

Other "pencil and paper" rating scales exist for other behavioral dimensions that are altered in many bipolar subjects; these include the Buss-Durkee scale for the measurement of hostility and the Barrat Impulsivity Scale for measurement of impulsivity (117,118). These scales were developed to measure stable personality traits in a general population, but have yet to be tested in terms of heritability and cosegregation with bipolar disorder in multiplex families. The Barrat Impulsivity Scale, which actually measures at least three domains of impulsivity, has been shown to correlate with related neurocognitive tests, such as the continuous performance task (119). As alterations in impulsivity are core features of not just bipolar disorder, but also of substance abuse disorders, attention deficit disorder, and borderline personality disorders, it is possible that similar or overlapping sets of genes contribute to all of these disorders.

The development of behavioral endophenotypes for schizophrenia may be even more challenging than in bipolar disorder, as many of the behaviors and experiences of schizophrenics seem quite discontinuous from the general population. In efforts to describe subclinical behavioral typologies that are potentially genetically related to schizophrenia, scales for measuring schizotypal disorder (120–122) and prodromal disorders (123) have been developed, which cover several of the key domains of behavior and experience that are disrupted in schizophrenia. Tsuang and colleagues have developed the concept of an extended phenotype for schizophrenia through the conceptualization of schizotaxia, and are currently testing whether this measurable combination of behavioral and cognitive impairment may be useful as an endophenotype for schizophrenia (124).

SUMMARY/FUTURE DIRECTIONS

The first decade of the 21st century is sure to see an increase in attention paid to alternative systems of classification and to the search for appropriate endophenotypes to help untangle the genetic underpinnings of the major psychiatric disorders. As reviewed in this chapter, the search for endophenotypes is currently driven largely by genetic researchers who have been frustrated at the relative lack of progress in identifying genes for psychiatric disorders. The challenge will first be to define what measurable traits satisfy the optimal criteria for being "endophenotypes" of the categorical psychiatric disorders, then to identify genes for those endophenotypes, and lastly, to redefine categories of psychiatric illness along the lines of the endophenotypes, rather than the current categorical diagnoses.

Although Gottesman and Shields initially wished that the field might one day find endophenotypes that mapped onto the current psychiatric disorders, it remains much more likely that endophenotypes will cut across our current categories of illness, revealing specific genetic factors, which cause spectrums of disorders (phenotypes).

For bipolar disorder and major depression, no quantitative measure or potential endophenotype has yet been studied in the depth necessary to define whether it is of use in mapping the genes underlying mood disorders. One can start with all measures that have shown association to these disorders, but the next step will be to perform studies, which test whether these measures are heritable, whether they segregate with disease in pedigrees, whether they are reliably measured, and whether they function as traits (rather than as state-dependent measures). Ideally, such studies might be performed simultaneously, in pedigree-based research or in studies, which analyze affecteds, first-degree unaffecteds, and controls. At the present time, some of the most intriguing potential endophenotypes for bipolar disorder are the structural brain differences in the right anterior cingulate and ventral striatum, the TEMPS-A personality scales, verbal learning and memory, sustained attention, and executive functioning. Several of these, however, especially the cognitive tasks, almost certainly are relevant to schizophrenia as well. For schizophrenia, several cognitive measures (of verbal learning, executive function, and attention), measures of schizotypy, and structural imaging of the prefrontal cortex are among the most promising potential endophenotypes. However, as for other psychiatric disorders, many potential endophenotypes have not yet been investigated.

For the psychiatrist in practice, who will most certainly continue to seek biological measures (markers) to help guide specific treatments, whether a biological alteration has been caused by genetic variation or environment may be of less interest than to define the abnormality and to treat it. In this regard, a byproduct of the search for endophenotypes may be that the psychiatric field may begin to focus more on the elements which underlie the complicated phenotypes currently classified by the DSM-IV. For instance, current trials are underway to determine if pharmacologic or alternative interventions, which enhance cognitive functioning (125,126) might be valuable in treating schizophrenic patients. Clinicians and psychiatric researchers will certainly also be interested in any studies which shed light on the biology of particular symptoms and "endophenotypes"—hence as genes are discovered, which influence specific endophenotypes, additional treatments (pharmacologic and otherwise) are certain to be developed to treat these specific deficits.

In closing, we remain optimistic that a large number of genes will be discovered during the next few decades, which contribute to both categorical phenotypes and to the more subtle clinical, behavioral, cognitive, structural, and physiological traits, which are distributed throughout the general population. If rigorous methods are utilized, which pay attention to not just genetic evidence, but also to the environmental, cultural, and philosophic issues relevant to the

phenomena of "mental illness," it is conceivable that our approach to classifying, diagnosing, and treating psychiatric disorders will soon undergo a major transformation. When all is said and done, the study of psychiatric "endophenotypes" may be one of the key new concepts which will help us to achieve the Aristotelian advice to understand how simple and complex elements of nature "come to be" what they are.

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Pharmacogenetics and Pharmacogenomics in Psychiatry: Clinical Applications

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INTRODUCTION

Pharmacogenetics and pharmacogenomics investigate the genetic factors that influence an individual's reaction to a drug. The difference between the two is a question of magnitude: pharmacogenetic studies investigate either single genes, or a limited number of genes, whereas pharmacogenomic research includes studies from several genes up to entire genome investigations. As the distinction between the two is not precisely defined, we will use the term *pharmacogenetics* unless specifically referring to genome-wide investigations.

The findings of the last decade, relating genetic variants to interindividual differences in drug metabolism, treatment side effects, and treatment response, created great expectations that pharmacogenetic research in psychiatry would yield clinical applications. However, although the development of high throughput genotyping techniques has greatly facilitated the laboratory work and increased the pace of genetic investigations, the interpretation of much of the data and translation to clinical utility is proving complex. Nevertheless, although much needs to be done before pharmacogenetic applications are commonplace in clinical settings; we can already speak of successes (e.g., genetic tests for determination of

patients' drug metabolic status and antipsychotic response prediction). In addition, in the coming years, the clinical implementation of more genetic tests for response and side effect prediction are likely. This chapter summarizes the most important findings in the field and their implications for clinical practice.

THE HERITABILITY OF DRUG RESPONSE

Epidemiological studies (twin, family, and adoption studies) are used to determine the heritability of genetic traits. However, the number of epidemiological studies investigating the heritability of response to psychotropic drugs is sparse. No large-scale systematic twin studies have been performed and only a relatively small study has been published, in which five monozygotic twin pairs showed higher concordance for antipsychotic-induced weight gain than seven same sex siblings(1). A number of reports of similar treatment response in twins, concordant for schizophrenia and treated with the same antipsychotic, have also been published (2–5). A similar trend in response to antidepressants was observed amongst family members (6). These studies cannot be taken as definitive proof of the heritability of antipsychotic and antidepressant response, but provide some evidence supporting the hypothesis of a genetic contribution to variability in response to psychotropic drugs. However, the exact magnitude of the contribution of genes to response variability cannot be quantified due to the lack of large systematic twin or family studies, and so assumptions have to be made in order to calculate the statistical power of experiments.

INVESTIGATING GENETIC INFLUENCE ON TREATMENT RESPONSE

Estimates of the efficacy of pharmacotherapy in mental illness vary: broadly speaking, 30% to 50% of treated patients do not respond adequately to antipsychotic treatment, and only 47% of patients treated with antidepressants show full remission, although a higher proportion show some improvement (7,8). This variability in overall response to certain drugs resembles a complex trait in which a number of contributing factors play a role. Not all aspects of pharmacotherapy, however, are explained by a multifactorial model, and specific aspects, such as rate of metabolism, appear to be controlled by a single gene. As an example of a single gene effect, there are several well-described functional mutations in cytochrome P450 2D6 (CYP2D6), a metabolic enzyme responsible for the oxidation of a number of antipsychotic and antidepressant drugs, that may cause an alteration in the metabolic rate of substrate drugs, especially compounds with no alternative metabolic pathway. The presence of these mutations in individuals is directly related to the development of side effects. Nevertheless, these genetically determined metabolic alterations are not the sole cause of adverse reactions, nor are they entirely responsible for the treatment outcome. A multigenic model where several genes contribute to clinical outcome would better explain the

variability in response. This would concur with the hypothesis that there is an important genetic influence determining treatment response, but with further influence from clinical (e.g., severity of illness, age of onset) and environmental (e.g., smoking, diet) factors.

The identification of the factors determining treatment outcome has obvious potential benefits for the management of psychiatric patients. In particular, the identification of the genetic determinants associated with good clinical response could facilitate the selection of the treatment most likely to help the patient without causing adverse reactions. Two major strategies are used for the identification of genes involved in complex traits: case-control genetic association studies and linkage studies.

Standard case-control genetic association studies require DNA from patients (unrelated individuals) receiving a particular medication. Traditionally, the selection of candidate genes is then undertaken based on existing biological knowledge (e.g., pharmacological profile of the drug, suspected target area or metabolic pathway) or from areas highlighted by linkage studies as likely to contain genes related to the investigated trait. Significant variation in the distribution of polymorphisms [DNA variants including single nucleotide polymorphisms (SNPs), variable number of tandem repeats (VNTRs), and other microsatellites] between cases and controls would indicate the involvement of the given candidate gene on the investigated trait. This strategy is particularly successful for the detection of genes of relatively minor or moderate effects, but is hindered by the propensity to produce false positive results if the case-control (or treatment responder and nonresponder) samples are stratified (come from different population groups) or not adequately balanced in terms of the numbers of cases and controls. Candidate genes have been selected with varied success for the investigation of metabolic enzymes and drug targets. Now, modern high throughput genotyping techniques have facilitated genome-wide association studies that do not require candidate gene selection. However, large-scale samples ($N > 1000$) are required to minimize spurious findings, and complex statistical analyses are needed to interpret the large yield of results. Nevertheless, large-scale genotyping studies are becoming the strategy of choice for the identification of novel candidate genes and for the better understanding of the mechanism of action of drugs.

Linkage studies investigate the cosegregation of markers with the disease or investigated trait in families including some affected members. If a marker segregates with the disease along generations, it would indicate that the investigated marker, or another closely linked genetic variant, may contribute to the development of the disease/trait. This strategy is particularly useful for the identification of genes with major effects, although it may fail to detect genetic factors of minor effect, which is highly likely to be the case in treatment response where the interplay of multiple genes of small effect is suspected. Because of the additional difficulty of obtaining appropriate samples for linkage studies (i.e., groups of relatives undergoing treatment with the same or similar antipsychotics or

antidepressants), association studies are the preferred strategy for pharmacogenetic investigations.

WHAT HAS PHARMACOGENETICS DONE FOR US?

Critics would say that little has been obtained from the application of the above strategies during the last two decades. However, the research findings of the last decades are leading to major breakthroughs (e.g., genetic determination of patients' metabolic status is possible) and with translation of these research findings into tests for routine clinical practice, these developments will produce clear health benefits (e.g., less adverse reactions, better treatment compliance). The following sections will summarize the pharmacogenetic findings in psychiatric research and their current and/or future clinical applications.

PHARMACOGENETIC FINDINGS

Historically, pharmacokinetic and pharmacodynamic processes have been independently explored. As work in the two fields has progressed, it has become clear that both processes contribute to the success and failure of pharmacotherapy. The independent findings of the two fields are summarized in the next two sections, but, in practice, the genetic determinants of response can be combined to obtain the full picture of variability.

Pharmacokinetic Investigation

CYP Enzymes: Determinants of Metabolic Rate

Since the early 1950s, it has been known that functional mutations in CYP metabolic enzymes may play an important role in drug metabolic rates (9). Of these CYP1A2, CYP2D6, CYP2C19, and CYP3A4 are the most important for the metabolism of psychotropic drugs. CYP2D6 is the main oxidative pathway of many antidepressants and several classical (first generation, typical) antipsychotics (10), and was the first enzyme to be investigated in relation to psychiatric drugs. The gene coding for this enzyme is known to contain functional polymorphisms (CYP2D6*3, *4, *5, and *6) that may severely disrupt the metabolic activity of the enzyme. Individuals possessing two copies of these variants are poor metabolizers (PM). Individuals with one copy of a deficient CYP2D6 and one normal copy show intermediate metabolism (IM), and normal or extensive metabolizer phenotypes (EM) are observed in individuals with two normal CYP2D6 variants. Additionally, duplications of the number of copies of the CYP2D6 gene can lead to ultrarapid metabolism (UM). Interestingly, the distribution of these functional polymorphisms shows clear ethnic variation: 4% to 10% of Caucasians present PM phenotype, whereas only 1% to 2% of Asians are poor metabolizers (11). To date, more than 90 variants in the CYP2D6 gene have been described, some of

Table 1 List of the Common Functional Polymorphisms of Cytochrome Genes

Enzyme	Mutation	Effect
CYP2D6	CYP2D6*3	PM
	CYP2D6*4	PM
	CYP2D6*5	PM
	CYP2D6*6	PM
	CYP2D6*1XN	UM
CYP2C19	CYP2C19*2B	PM
	CYP2C19*3	PM
CYP1A2	CYP1A2*1C	UM?
	CYP1A2*1F	PM
CYP2C9	CYP2C9*2	PM
	CYP2C9*3	PM

Abbreviations: CYP1A2, cytochrome P450 1A2; PM, poor metabolizer; UM, ultrarapid metabolizer.

them with important functional effects (12). Other relatively common functional polymorphisms that influence the rate of drug metabolism have been described in CYP1A2, CYP2C9, and CYP2C19, enzymes directly involved in the metabolism of antipsychotics and antidepressants (see Table 1). Polymorphic variants with decreased (*17) or increased (*18A) activity have also been described in the gene coding for CYP3A4 (13), an enzyme that plays an important role in the phase I metabolism of several antipsychotics including clozapine, risperidone, and haloperidol (14–16). A specific web page is dedicated to the collation of all mutations and polymorphisms described in CYP genes and their known functional influence (http://www.cypalleles.ki.se/).

Genetic association studies investigating the influence of these polymorphisms have shown that metabolic polymorphisms have important implications for therapeutic dosage (17,18) and may be related to toxic accumulations inducing side effects (19–27). Poor metabolizers may require lower therapeutic doses to avoid toxic accumulations, whereas ultrarapid metabolizers will require higher doses to obtain therapeutic response (18). However, non-genetic factors may also contribute to enzymatic rate alterations (see later section). Concomitant treatment with drugs competing for the same enzyme can lead to the saturation of metabolic pathways and toxic accumulations. Additionally, certain classical antipsychotics, antidepressants, and environmental factors, such as smoking and diet, can induce or inhibit metabolic enzymes in a less predictable manner (28). Without underestimating the importance of these environmental factors, genetic information has a predictive value on its own. Pretreatment genetic determination of metabolic status may guide and improve psychiatric treatment, reducing side effects by 10% to 20% and increasing treatment efficacy by 15% to 25% (29).

Not surprisingly, these pharmacogenetic findings have already been translated into clinical applications, which will be discussed in a later section.

Although much research has been performed on CYP enzymes, further research into metabolic variability is required. Phase II conjugation enzymes (e.g., *N*-acetyltransferases, thiopurine *S*-methyltransferases, UDP-glucuronosyltransferases, and glutathione *S*-transferases) may be important in determining the biotransformation of medication. Surprisingly, few studies have attempted to discern genetically determined variability in these enzymes. Additionally, transporter enzymes that regulate the bioavailability of psychotropic drugs on their target areas may influence treatment efficacy. This is illustrated by a recent study showing association between antidepressant response and polymorphisms in the gene coding for the P-glycoprotein (ABCB1), an enzyme that transports substrates through the brain barrier (30).

Pharmacodynamic Investigations

Genetic Variation in Neurotransmitter Systems: Influence on Clinical Outcome

The main pharmacological targets of antipsychotics, antidepressants, and medications used to treat attention deficit hyperactivity disorder (ADHD) and Alzheimer's disease (AD) have been the subject of investigation into genetically determined pharmacodynamic factors. These targets include neurotransmitter receptors and transporters, and the enzymes involved in neurotransmitter synthesis. In particular, dopamine (D) and serotonin genetic variants have been the subject of numerous association studies.

Dopamine receptors: Targeting of dopaminergic receptors is a common characteristic of drugs with an antipsychotic effect. With varying intensity, classical and modern antipsychotics display affinity for D receptors including D2, D3, and D4. Partial antagonism of D receptors has been suggested as a mechanism of action for certain antipsychotic drugs (31,32), such as clozapine and aripiprazole, which display moderate affinities for D2 and D3 receptors (32). Pharmacogenetic studies have provided further evidence of the importance of dopaminergic involvement. Several independent studies have related D2 receptor genetic variants with response to classical and atypical antipsychotics (33–37). D3 genetic variants have also been associated with general response to atypical antipsychotics and with improvement in positive symptoms in European and Chinese populations (38–40). Additionally, D3 variants have been related to antipsychotic-induced tardive dyskinesia (TD) and movement disorders (41,42). In particular, individuals possessing the D3 Gly9 variant have a higher risk of developing TD. However, a recent meta-analysis of D3/TD studies (43) has shown that the odds ratios of this association are relatively low (1.1–1.4), suggesting that the finding has no predictive value unless combined with other TD genes [serotonin type 2A (5-HT2A) and 5-HT2C receptor variants and CYP polymorphisms have also been suggested to contribute

to this adverse reaction]. D4 and D5 variants were associated with dose determination and treatment response to methylphenidate in children suffering from ADHD (44,45). No significant associations have been reported between dopaminergic variants and response to antidepressant medications, reflecting the different target area of these medications.

In summary, genetic association studies confirm the involvement of the dopaminergic system in the mechanism of action of antipsychotics. However, the reported associations are of moderate magnitude, and thus of limited clinical value. Clearer associations observed when investigating specific symptoms (e.g., improvement in positive symptoms) are still of limited predictive value when taken alone, indicating that other factors contribute and should be taken into account when predicting outcome.

Serotonin receptors: Genetic variants in serotonergic (5-HT) receptors have been repeatedly associated with variability in response to both antidepressant and antipsychotic medications. Several studies have reported associations between variants of the 5-HT2A receptor gene and clozapine and risperidone response, in Caucasian and Chinese populations respectively (46,47). Two studies have also related 5-HT2A variants with antidepressant response in European and Japanese patients (48,49). A recent study (the STAR*D) involving a large cohort of U.S. patients (N = 1,953) treated with the antidepressant citalopram, a selective serotonin reuptake inhibitor (SSRI), confirmed association of 5-HT2A variants with improvement and remission (7), although the study contradicted previous reports of association between response and serotonin transporter gene (SLC6A4) variants. 5-HT2A and 5-HT2C variants have been reported to contribute to movement disorders associated with prolonged antipsychotic treatment (50,51). 5-HT2A polymorphisms may also contribute to antidepressant adverse reactions (26). Associations have also been reported between 5-HT2C genetic polymorphisms and antipsychotic induced weight gain, response, and improvement in negative symptoms (50;52–54).

These pharmacogenetic studies confirm that the serotonergic system mediates, at least partially, the therapeutic efficacy of psychotropic drugs. However, as in the case of dopaminergic variants, individual serotonin variants cannot be used as clinical biomarkers. The moderate strength of the observed serotonin associations (rarely exceeding odds ratios of 2) indicates a contribution to response, rather than a major role in determining clinical outcome.

Other neurotransmitter receptors: Glutamate and adrenergic receptors are also obvious candidate genes for pharmacogenetic studies because of their involvement in the etiology of mental disorders and the affinity that psychotropic drugs display for these receptors. However, pharmacogenetic studies have neither confirmed nor rejected this hypothesis. Only one unconfirmed study of association between genetic variants of the *N*-methyl-D-aspartate (NMDAR1) receptor and clozapine response has been reported (55). Adrenergic variants have not been found to contribute to treatment response to psychotropic drugs (56).

Investigation of histaminic receptors revealed a marginal association between a genetic variant in the histamine 2 (H2) promoter region of the gene, a finding that requires confirmation (57). The lack of genetic association reports may reflect a dearth of studies performed on these systems or their limited role in psychotropic drug activity.

Neurotransmitter transporters: Serotonin and dopamine transporters (5-HTT and DAT, respectively) are blocked by antidepressants (in the SSRI group) and an ADHD treatment (methylphenidate). Important work has shown that sequence variants in these transporter genes may influence therapeutic outcome of these drugs.

Serotonin transporter. Several studies performed in Caucasians have related the short allele of a serotonin transporter (5-HTT) LPR polymorphism with poorer response to selactive serotonin re-uptake inhibitor (SSRIs) [for reviews of these studies, see (30,58,59)]. This allele, located in the promoter region of the gene, is associated with a lower expression of the 5-HTT protein, and therefore leads to lower levels of serotonin reuptake (60). Interestingly, the opposite is observed in Korean patients, where the short allele is associated with better response and higher levels of expression of the 5-HTT protein (61). This may suggest that the 5-HTT LPR polymorphism is linked with a genetic variant along the 5-HTT or on a nearby gene, and that the status of this linkage in inverted in Asian populations. Unexpectedly, the large STAR*D study (7) failed to find any association between 5-HTT polymorphisms and response to the antidepressant citalopram. This contradiction to the previously replicated findings of a number of independent studies may be the result of differences in treatments, study populations, or the symptomatology studied, and warrants further investigation. 5-HTT polymorphisms have also been reported to contribute to antipsychotic response prediction (62), although the strength of this association is weak in comparison with that observed in antidepressants.

Dopamine transporter. Methylphenidate, the most prescribed treatment for ADHD, acts by blocking the DAT, which results in an increase in the level of synaptic catecholamines. Several studies have linked a polymorphic VNTR in the gene coding for the DAT protein (DAT1) with methylphenidate response (45). The 10-repeat allele of this polymorphism has been reported to be associated with both poor and good treatment response in different studies, suggesting geographical variation in linkage disequilibrium, with a putative variant causing the observed effect on response. Nevertheless, the repetition of this finding in different population groups and the observed odds ratios (ranging from 1.3 to 2.6) suggests that DAT1 genotyping may be of use to predict clinical response to methylphenidate.

Other related genes: In addition to targeted neurotransmitter receptors and transporters, several categories of neurotransmitter-related proteins complete the list of candidate genes for psychopharmacogenetic studies. Investigations involving catalyzing enzymes such as the monoamine oxidase A (MAOA), an

enzyme that catalyzes the oxidative deamination of amines, and the catechol-*O*-methyltransferase (COMT) enzyme that catalyzes the degradation of catecholamines (including dopamine, adrenaline, and noradrenaline) have produced inconclusive pharmacogenetic results, although these genes may be related to the etiology of mental disorders (63). More success was observed when investigating variants of the tryptophan hydoxylases (TPH1 and TPH2), enzymes involved in the synthesis of serotonin from tryptophan. TPH1 and TPH2 variants may influence response to the antidepressants fluvoxamine and paroxetine in Caucasians (64–66). Most neurotransmitter receptors targeted by psychiatric drugs belong to the G-protein coupled category. Not surprisingly, variants in the gene coding for the G protein β3 subunit have been repeatedly associated with variance in response to antidepressants and antipsychotics (59,67). Recent studies on the hypothalamic-pituitary adrenal (HPA) axis and glucocorticoid receptors, whose functionality is altered in depressed patients, have revealed associations between SNPs in glucocorticoid-related genes and response to antidepressants (68). Polymorphisms in the angiotensin I-converting enzyme (ACE) and the β1-adrenergic receptor (β1AR) genes have also been related to antidepressant response, although these findings await confirmation (18).

It is important to note that many studies have failed to replicate the findings cited here. This lack of replication may be the result of spurious findings, but also of differences in study characteristics (e.g., type of medication, duration of treatment, patient population group, symptomatology, response assessment). Nevertheless, it may also reflect the complexity of the factors determining treatment response. Table 2 provides a brief list of pharmacogenetic studies reporting significant associations of pharmacodynamic genes that have been replicated and/or are of relatively important significance. The listed genetic associations are of moderate effect and most would have limited clinical value if considered individually. Clinical applications of these findings may require the combination of information from several genes, without forgetting that environmental and clinical factors may also have an important role in determining the success of drug treatment. A brief description of such factors is given in the following section.

NON-GENETIC FACTORS

Several studies have correlated demographic and clinical variants with the likelihood of successful treatment. Certain characteristics, for example, male gender, early age of illness onset, and long duration of untreated psychosis are associated with poorer treatment outcome in schizophrenia (69–71). Similarly, certain symptom profiles and measures of disease severity have been related to treatment outcome in mood disorders (72.73). Some of these phenotypes may be mediated by genetic factors, and their value in predicting treatment response is limited.

Environmental factors such as smoking and diet have been reported to induce or saturate metabolic pathways, directly affecting plasma levels of drug

Table 2 Summary of Pharmacogenetic Studies Reporting Genetic Associations with Psychotropic Drugs

Gene	Reported association
CYP2D6	Poor metabolizer variants associated with antipsychotic-induced movement disorders (19,22–25,27)
	Metabolic status important for dose adjustment (17,18)
CYP1A2	Variants associated with tardive dyskinesia and movement disorders associated with antipsychotics (21)
D2	Several SNPs associated with antipsychotic response in Asians and European Caucasians (33–37)
	Associated with neuroleptic malignant syndrome in Asians (93)
D3	Polymorphisms associated with improvement in positive symptoms after antipsychotic treatment (38–40)
	Ser9Gly polymorphism associated with antipsychotic-induced movement disorders (41–43)
DAT1	10-repeat allele associated with variable methylphenidate response (ADHD treatment) in different population groups (45)
5-HT2A	Two SNPs (102-T/C and His452Tys) associated with poor clozapine response (62)
	102-T/C associated with risperidone response (47) and antipsychotic-induced movement disorders (51)
	Several SNPs associated with response to antidepressants (7,26)
5-HT2C	Various SNPs associated with antipsychotic response and improvement in negative symptoms after treatment with risperidone (39,50)
	Promoter region polymorphisms associated with antipsychotic-induced weight gain (52–54)
5-HTT LPR	Several reports of association with variability in response to antidepressant medications (SSRIs) (30,58,59,61)
TPH1	Associated with fluvoxamine and paroxetine response (64–66)
GNB3	Gene variants associated with response to antipsychotics and antidepressants (59,67)

Abbreviations: ADHD, attention deficit hyperactivity disorder; CYP2D6, cytochrome P450 2D6; CYP1A2, cytochrome P450 1A2; D2, dopamine receptor; D3, dopamine receptor; DAT1, dopamine transporter 1; GNB3, guanine nucleotide-binding protein B3; HT2A, serotonin type 2A; SSR, selective serotonin reuptake inhibitor; SNP, single nucleotide polymorphism; TPH1, tryptophan hydroxylase 1.

metabolites. Caffeine intake inhibits CYP1A2 metabolic activity, whereas smoking induces this enzyme (28). Diet and smoking habits have different impact in different areas, as both are subject to significant geographical variation. Additionally, antidepressant and classical antipsychotic drugs are known to alter (induce or inhibit) metabolic rates, which may become problematic when more than one drug is prescribed (74). Concomitant treatment with two or more drugs competing for the same metabolic enzyme may also result in reduced biotransformation rates in individuals with normal enzyme variants. Although generally of limited predictive value, these factors may be important for the adjustment of

therapeutic doses of drugs metabolized by the affected enzymes, and may be considered in conjunction with other genetic determinants for response prediction.

CLINICAL APPLICATIONS: CURRENT AND FUTURE

Few of the pharmacogenetic findings reported here have, to date, found a clinical application. The low predictive values of individual findings have marred the translation of pharmacogenetic research into clinical practice. The combination of information from a number of different genes can improve the predictive values, but the accuracy of the prediction may vary, depending on the ethnicity of the population group and the clinical characteristics. Undoubtedly, the impact of pharmacogenetics on clinical practice would be greatly facilitated if a test could be applied to different clinical settings without significant adaptation to local population. If the functional mutation causing a response trait was known, a prediction test identifying this key gene variant would be of greater value as it could be applied to different patient groups. Unfortunately, this is not the case for most aspects of the treatment response spectrum. Aside from metabolic status (which may also be affected by environmental factors), treatment response and side effect liability are the result of multigenic interactions with some environmental contribution. Despite these limitations, several pharmacogenetic findings have been translated for clinical utility as prescribing aids. Currently, therapeutic dose adjustment and antipsychotic selection can be guided by pre-treatment genotyping of key genes, and pharmacogenetic selection of population groups likely to benefit from treatment is conducted during clinical trials. In the near future, genetic prediction tests for a variety of antipsychotics, antidepressants and adverse reactions are likely to be commercialized. The following sections review the current status and prospects for pharmacogenetic applications.

Adjustment of Therapeutic Dose

To date, this is the most important clinical application of pharmacogenetics. Pre-treatment determination of patients' drug metabolic status can help to adjust drug doses accordingly. As previously discussed, several functional polymorphisms in the genes coding for CYP2D6, CYP2C19, CYP1A2, and CYP3A4 are known. Recent studies by Kirchheiner and collaborators (17,18,58) have shown that genetically determined alterations in CYP2D6 and CYP2C19 enzymes can be counteracted by adjusting drug dosage. This may have important benefits for psychiatric treatment through the potential to reduce adverse reactions caused by toxic substrate accumulation (in poor metabolizers) and to increase efficacy by helping to reach therapeutic metabolic levels (in fast metabolizers). For example, CYP2D6 metabolic status is very important for psychotropic drugs with a narrow therapeutic window (e.g., tricyclic antidepressants) (30), although it has less influence on drugs with wider therapeutic ranges or a variety of metabolic pathways (e.g., SSRIs, antipsychotics). This is reflected in the dose adjustment recommendations according to patient's CYP2D6 and CYP2C19 status (18), as summarized in Tables 3 and 4.

Table 3 Recommended Adjustments to Therapeutic Doses According to Patient's Genetically Determined CYP2D6 Metabolic Status

Drug	UM (%)	EM (%)	IM (%)	PM (%)
Antidepressants				
Amitriptyline		120	80	70
Clomipramine		120	90	60
Desipramine	250	130	80	20
Fluoxetine		110	90	70
Flavoxamine		120	90	60
Imipramine		110	100	60
Mianserin	300	110	90	70
Nortriptyline	230	140	70	50
Paroxetine		130	70	20
Antipsychotics				
Aripripazole		113	92	70
Clozapine		94	104	113
Haloperidol		117	89	61

Abbreviations: CYP2D6, cytochrome P450 2D6; EM, extensive metabolism; IM, intermediate metabolism; PM, poor metabolism; UM, ultrarapid metabolism.
Source: Adapted from Ref. 18.

Table 4 Recommended Adjustments to Therapeutic Doses According to Patient's Genetically Determined CYP2C19 Metabolic Status

Drug	EM (%)	IM (%)	PM (%)
Antidepressants			
Amitriptyline	104	94	59
Clomipramine	110	79	62
Doxepin	105	91	48
Imipramine	105	91	77
Trimipramine	114	73	31
Citalopram	108	84	61
Fluoxetine	113	72	39
Fluvoxamine	101	97	93
Sertraline	105	90	75
Antipsychotics			
Clozapine	104	91	78
Zotepine	104	93	82

Abbreviations: CYP2C19, cytochrome P450 2C19; EM, extensive metabolism; IM, intermediate metabolism; PM, poor metabolism.
Source: Adapted from Ref. 18.

These tables exemplify the importance of pretreatment determination of an individual's metabolic status, especially for treatment with CYP2D6 or CYP2C19 substrates. Combining similar information for other enzymes (i.e., CYP1A2 and CYP3A4) will further increase the clinical value. It has been hypothesized that pretreatment determination of CYP metabolic status may lead to a 15% to 25% improvement in clinical efficacy and a 10% to 20% decrease in adverse reactions (29).

Prediction of Treatment Response: Are We There Yet?

Research into treatment selection based upon an individual's genetic profile (personalized medicine) has advanced to different levels in different disorders. Whereas research into antipsychotic and antidepressant genetic prediction methods is relatively advanced, pharmacogenetic prediction of response to other treatments is still in the early stages.

Prediction of Antipsychotic Response

Dopaminergic and serotonergic polymorphisms have been shown to clearly contribute to variability in response to treatment with antipsychotics, with likely contributions from other neurotransmitter systems. However, metabolic polymorphisms do not seem to have a major impact on response to most antipsychotics, with the exception of haloperidol and, to a lesser extent, aripripazole (18). This is likely to be the result of the number of different metabolic pathways involved in the metabolism of most antipsychotic drugs. The individual strength of the dopaminergic and serotonergic associations does not offer a high predictive value. However, in a previous study we have shown that a combination of information from a number of key genes may result in the prediction of response to clozapine with a relatively high accuracy ($>$70%) (62). A more developed version of this test is being marketed as a prescription aid by a clinical diagnostics company (LGC, London, U.K.). Several studies have already indicated that genetic prediction of response to olanzapine and risperidone may be feasible (38–40,47,75). Prediction tests for antipsychotic response are likely to be translated to clinical application in the near future.

Prediction of Response to Antidepressants

When compared to antipsychotic drugs, antidepressant medications have fewer defined targets, metabolic pathways, and supposed modes of action. Theoretically, this should facilitate the identification of genetic determinants of response. As mentioned previously, the vast majority of the pharmacogenetic studies on antidepressants have focused on metabolic CYP enzyme genes and the serotonin transporter, particulary when investigating SSRIs. CYP genotyping has been shown to be of use for antidepressants with narrow therapeutic ranges (see previous sections). Several studies have confirmed the contribution of genetic variability in the 5-HTT gene (59) and response to SSRI medication. When

considered separately, genetically determined pharmacokinetic and pharmocodynamic factors have only a moderate predictive value for response. At present, no study has attempted the combination of information in both areas. It is highly likely that such combinations will produce greater prediction levels, with clinical value, and that genetic antidepressant response prediction tests will be available in the near future.

Other Psychotropic Treatments

Less research has been performed on other psychotropic drugs, and with varied success. An interesting investigation by Cacabelos and collaborators reported a combination of genes associated with treatment response to a combination of drugs in AD (76). This was followed by a paper reporting a response prediction methodology using genotypic and clinical information (77). Although some advances have been made in identifying genes related to ADHD treatment response (45), no use of genetic tests to guide prescription choices has been reported. A number of studies have investigated the genetic contribution to variability in lithium response, without clearly significant results (59,78,79). With the exception of the anti-Alzheimer's treatments, it is unlikely that pharmacogenetic tests will be available for other pharmacotherapies in the near future.

Propensity to Develop Side Effects to Antipsychotics

It could be argued that the prediction of side effects, the main reason for treatment discontinuation and poor compliance, is at least as important as the prediction of treatment response. Numerous investigations have studied the genetic components of TD and drug-induced weight gain, particularly disturbing side effects of certain antipsychotics, and important advances have been made.

D3 polymorphisms were first associated with development of TD in a study by Steen and collaborators (41), and the association of the D3 Ser9Gly polymorphism was confirmed in later studies (42,80). The magnitude of the D3 association is moderate (odds ratios 1.1–1.4), indicating the involvement of other additional factors. CYPs, 5-HT2A, and 5-HT2C genetic variants have also been implicated (20,51,81–83), and, together, these polymorphisms account for an important proportion of TD cases. Despite this, their individual predictive value is not clinically valuable and additional information, genetic or phenotypic, is required before TD prediction is implemented in clinical settings.

Drug-induced weight gain has been significantly associated with variants in the 5-HT2C gene in several studies (52,53,84,85). The strength of this association varies in different population groups, although the initial reported odds ratios (OR=6) (52,53) suggest that this finding could be used as a predictor of weight gain. Additional polymorphisms in leptin and other genes have also been suggested to contribute (54,86). In view of these findings, it is likely that a prescription test for genetically determined propensity to gain weight after antipsychotic treatment can be developed in coming years.

PHARMACOGENOMICS: THE FUTURE IS HERE

The previous sections have summarized pharmacogenetic findings and their application in determining drug response. These findings have been based on current knowledge (e.g., known metabolic pathways or suspected drug targets). What pharmacogenetic studies have failed to do is to clearly ascertain the mechanisms of action of psychotropic drugs. The rapid development of high throughput genotyping techniques (87,88) has led to much more ambitious goals, including a better understanding of drug action. In contrast to studies investigating a limited number of carefully selected candidate genes (pharmacogenetics), high-throughput technologies have facilitated the simultaneous investigation of a large number of genes (pharmacogenomics). These rapid genotyping techniques allow the investigation of thousands of SNPs distributed through the entire genome. Previous knowledge-based hypotheses are no longer required and novel targets and response related genes can be discovered. This DNA chip technology, including information for the entire genome, has enabled the investigation of differential gene expression induced by antipsychotic and antidepressant treatments. Studies using DNA and RNA chips have identified novel genes involved in lipid metabolism, synaptic function, and regulation that are altered by treatment with antipsychotics (88,89). Additionally, genome array studies have reported alterations in the expression of dopaminergic and serotonergic genes (90), thus confirming the importance of these systems in antipsychotic activity already suggested by pharmacogenetic findings

Clinical Trials: Finding Genetic Determinants at Early Stages of Drug Development

Pharmacogenomics has also found applications in the new drug development. Pharmaceutical companies have embraced the idea of improving response levels in regulatory clinical trials by selecting subjects likely to benefit from treatment without experiencing side effects(91). Additionally, pharmacogenomic investigations for the identification of genes influencing response to the trial drug can be carried out without previous hypotheses or candidate genes. This type of strategy requires large sample sizes ($N > 1000$) to minimize the occurrence of false positive findings and, is appropriate for phase III clinical trials. The FDA and other regulatory authorities accept and encourage the creation of pharmacogenomic information during drug development as a complement to clinical information (92). Thus, future psychotropic drugs may incorporate pre-treatment genetic tests for patient selection. Undoubtedly, this approach will improve the treatment efficacy and reduce side effects.

CONCLUSIONS

Two decades of pharmacogenetic research into psychiatric drugs have produced a several tests of current clinical utility (e.g., metabolic status for dose adjustment, general response to certain antipsychotics) and other prediction tests likely

to be implemented in the near future (e.g., general response to antidepressants). A variety of methods (DNA chips, hybridization strips, light-cyclers, thermocyclers, and other devices that allow for the rapid genotyping of key genes) are already available to introduce these tests into different clinical settings (large-scale genotyping during clinical trials, individual characterization of patients in clinical labs). Further advances are required to improve our knowledge on the mechanism of action of psychotropic drugs. Pharmacogenomics promises to advance research in the field to a stage where more findings are translated into applications of routine clinical utility.

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10

Translational Research in Psychiatric Diseases

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TRANSLATIONAL RESEARCH: WHAT IS IT ANYWAY?

The first problem that one is faced with in addressing what might be considered a relatively new concept is that there is often a lack of clarity as to what it really is. Translational research, a term that has been popularized with the "Roadmap" initiative at the National Institutes of Health (NIH) is new in the sense of being in the forefront of announcements and emphasis panels focused on the relationship between health care delivery and the discovery process behind the understanding of pathophysiology of disease. In order to benefit mankind, discoveries in the world of basic biological science need to be *translated* into practical applications. The term "translational research" conveys a certain immediacy to the process, suggesting that very recent findings from the laboratory are carried over to the clinical research endeavors with the anticipation that a measurable effect in patients can be determined, and the results tabulated and taken back to the laboratory for refinement, if necessary. It implies that the work is "cutting edge" and on the forefront of the discovery process. It is in recognition that findings in the basic sciences were often of pure academic pursuits and may take many years before they could be useful in the clinic. There is a political force behind helping mankind "now."

The discovery of a basic illness process that leads to a novel medication that treats or cures the disease is the holy grail of "translational research." This is,

of course, not a novel process; all of medicine is, by and large, predicated on at least some understanding of basic biology or physiology and associated pathology or pathophysiology. Nor is it a novel process in that questions generated in the clinics lead to an iterative process between the clinic and a basic science laboratory (1). There are many classic examples; perhaps one of the best known is the cholesterol and cardiovascular disease story (2), wherein iterations between basic and clinical sciences led to an understanding of the increased risk for heart disease and the pathology of arterial plaques.

The latter part of the 20th century brought several promising molecular genetic findings that led many of us to believe that radical changes in the management of the associated diseases were at hand. Disease genes for cystic fibrosis (3), Fragile X (4), and Huntington's disease (5), to name a few, were described. The human genome was decoded. Although the understanding of the pathology of these disorders has undoubtedly been advanced, treatment options based on this new-found genetic understanding have not increased. It is in recognition of these gaps between the "bench" and the "bedside" that the NIH and the Roadmap initiative is attempting to accelerate the process of "translation" between these two entities. Translational research is a dynamic, "two-way" process; basic scientists will provide clinicians with new tools for the clinical operation and the clinicians will make observations at the clinical level that may be vital in developing hypotheses at the level of basic research. This will be the theme of this chapter; translational research is the deliberate and facilitated interaction between the basic science researcher and the clinic-based investigator. It is wildly optimistic to expect that the findings of basic science can be ported, without refinement, directly into clinical practice.

Although there is a multitude of promises, and indeed expectations, in psychiatry and neuroscience that the approach of translational research will be productive, it has to be acknowledged that we are still somewhat short on practical applications and that it is rather premature to attempt to make inferences at the individual patient level (the bedside) from findings at the bench. This chapter will provide some examples of promising translational research and will highlight the potential for future applications. The reader must remain skeptical, but, at the same time, appreciate the iterative processes of translational research; stronger ties and calculated iterations between bedside and lab will provide insights on the usefulness of a genetic test or a new medication. Translational research simply means that clinicians and basic science researchers are interacting more frequently and intensely. It does not mean that practical applications in the clinic are at hand.

WHAT IS THE DOMAIN OF TRANSLATIONAL RESEARCH?

Translational research in psychiatric disorders integrates the spectrum of psychological, social, cognitive, developmental, and biological phenomena. The activity of the central and peripheral nervous systems as it relates to the origins, expression,

regulation, and modulation of psychiatric disorders are important objects of study. Also included may be investigations or measures of overt behaviors (including substance abuse), personality and temperament assessment, and the environmental circumstances and experiences that shape psychiatric disorders. In essence, translational research could become a "catch all" for the integration of clinical and basic research. At the practical level, it is a forum or mechanism to ask direct questions between the bench and bedside: what is the effect of a specific genotype on the phenotype(s)? An emerging field of pharmacogenomics will begin to inform the field of therapeutics if an association between medication response and genotype can be consistently demonstrated.

TRANSLATIONAL RESEARCH: A NEW FOCUS, AN EMERGING FIELD

A potential advantage of the concept of "translational research" is that a specific focus has been emphasized at the NIH. It was recognized that as a concept, translational research is a powerful motivator toward developing and enhancing the necessary infrastructure to drive clinical and basic research aimed at solving human health related problems, rather than "research for the sake of research". It recognizes that there are frequent barriers between the bench and the lab, and that concentrated efforts were required to specifically address them. Translational research recognizes that very complex relationships exist between what ever it is that we measure and what we believe to be the disease state. The basic state of the individual is the genetic sequence, the genotype; genotype influences gene expression, physiology, and the biochemistry (biology). These basic states influence and accept feedback from neuropsychological states; the environment interacts with the neurophysiological states to ultimately produce the disease phenotype (Fig. 1). In the past couple of decades, we have been attempting to jump directly from the genotype to the phenotype, overlooking complex aspects of physiology, biochemistry, and neuropsychology.

TRANSLATIONAL PERSPECTIVES

The Genotype and Genomic Sequence

The core of any biological engine is the genomic sequence, from which the messages are decoded into genes, modifiers of genes, and, in all likelihood, functions that have yet to be discovered. In an attempt to associate regions or loci with human disease, scientists have used linkage and association analyses that rely on polymorphic markers near the putative susceptibility locus, the hypothesis being that variants identified will either be within the coding region of a gene and have a directly measurable affect on the gene and its products [e.g., catechol-*O*-methyl transferase COMT], or be near enough to serve as a marker. The past 20 years has produced over 21 genome-wide scans in bipolar disorder (6) and at least 25 in schizophrenia (7); no one single gene of major effect has emerged for either

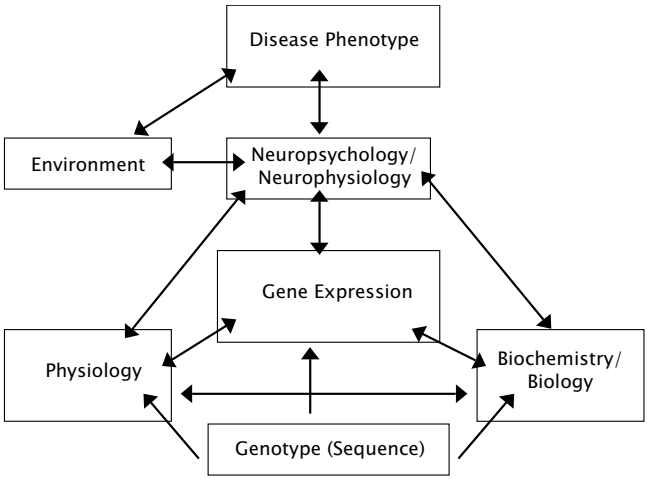

Figure 1 The relationship between genotype and phenotype represents a complex biological interaction involving biochemistry and physiology. Environment and psychological vectors interact with and modulate biological process to produce the observable phenotype.

disorder. In studies of schizophrenia, the genes dystrobrevin-binding protein (DTNBP1) and neuregulin (NRG1) have emerged as the strongest candidates. The complexity of the problem in the DTNBP1 findings is highlighted by the fact that the associated haplotype varies across studies, suggesting either that many haplotypes influence the gene product downstream or that there may be a variation that is common to the different associated backgrounds (7). In bipolar disorder, there have been at least three meta-analyses of the extant data, none of which have converged on specific loci. Nevertheless, there are a number of loci that are emerging. In the most recent and comprehensive meta-analyses, chromosome 6q and 8q24 stood out as being the strongest findings. There have been a number of "candidate" genes identified and proposed and they include D-amino acid oxidase activator (DAOA) (G72)/G30 (8,9), brain-derived neurotrophic factor (BDNF) (10) and G-protein receptor kinase 3 (GRK3) (11). Similar to the problem in the genetic findings in schizophrenia, the association studies in many of the candidate genes show association with different single nucleotide polymorphisms (SNPs) or haplotypes; the questions have been raised as to whether it is the actual diagnostic category that is associated with the SNP genotype or if it is a subtype of the phenotype that drives the association. Genetic linkage studies in other psychiatric disorders have identified susceptibility loci for other psychiatric disorders, such as obsessive-compulsive disorder (OCD) (12,13). Stratification strategies have suggested that there may be genes that underlie certain features related to phenotype, for example, it has been suggested that there may be genes

that drive the presence of psychosis per se in both bipolar disorder and schizophrenia (14,15); other loci may be driving the mood components (16) or specific characteristics such as age of onset (17,18) or polarity of onset (19). Although of tremendous interest, it is highly likely that many of the above features are modified through a variety of mechanisms at the level of physiology, biochemistry, and determinants of gene expression and regulation; environment and neuropsychology are likely to be a filter prior to the expression of the final disease phenotype seen in the clinical setting. Translational research will assist the basic science research by bringing data from the clinical analyses into the lab, to study the subtypes or possibly intermediate phenotypes known as endophenotypes (See Chapter 8).

Physiology, Biochemistry, and Psychiatric Genetics

There exists a wealth of research dating back to the 1950s on the biology of mood disorders and schizophrenia, beginning with the bioamine hypothesis of depression and dopamine hypothesis of psychosis (20). This led to a rather simplistic view of the pathophysiology of these diseases that unfortunately persists in many realms (mechanisms of medications are often referred to in terms of their "receptor profile"). Increased brain dopamine and pathology of dopamine receptors were considered to underlie schizophrenia (21). Disorders of mood were either the result of functional deficits of bioamines (depression) or excess (mania); medications had specific effects on these systems and, therefore, supported the hypothesis. There are several obvious problems in these hypotheses that have emerged over the past couple of decades. First, it is very clear that this approach is a dramatic and naïve simplification of a complex problem. Although these substances may play a role in the pathophysiology of psychiatric disorders, it is highly likely that the role will be one that is integrated into a complex pathway, influenced by the genetic variability of the gene itself [e.g., COMT has two known active forms that influence the activity of the gene (22)] along with genetic modifiers as well as the physiology and biochemistry of the given brain state in the individual subject. The "candidate gene" approach to psychiatry implied that one specific gene is tied to the disorder has been all but given up.

Stress and Inflammatory Mediators

A rather rich area of potentially relevant translational research is to be found in the arena of inflammatory responses and depression. Are there findings in this arena or research that can be translated into meaningful interventions at the level of clinical care? Stress and related environmental triggers have been consistently associated with depression and other psychiatric disorders (23). Losses and life events are associated with mood difficulties (24); high expressed emotion is associated with increased episode frequencies in several psychiatric disorders (25). Research into stress and inflammatory responses are perhaps best exemplified in the field of depression. Depressed patients generally have elevated levels of

proinflammatory cytokines, acute phase proteins, chemokines, and cellular adhesion molecules (26). There is evidence that these cytokines interact with many of the mechanisms associated with depression, such as the neurotransmitter system and its metabolism (26), neuroendocrine function (23), and synaptic plasticity (27). Stress, in addition to precipitating depression, promotes inflammatory responses through the autonomic nervous system (28). Suggestions of a stress hypothesis have also been made in relation to schizophrenia (29). This leads to the intriguing question of the relationship between inflammation, immunology, and depression or schizophrenia. Should we be rethinking the pathophysiological approach and questions? Would management and treatment of inflammatory mediators lead to enhanced treatment of depressed states? More specifically—are there depressed states that would be better managed by incorporating strategies to manage (i.e., reduce) the levels of inflammatory mediators? Which of the inflammatory mediators should be targeted? This is precisely the nature of questions and research that can be addressed using a translational approach—monitoring patients in the clinic with a facilitated relationship with the laboratory. It is, of course, important for the reader to appreciate that the applications at the level of patient care are premature, but that this is a highly promising avenue of investigation.

Inquiries involving BDNF and COMT are excellent examples of the translational research paradigm. BDNF research in mood disorders exemplifies the extension of investigation beyond the monoamine hypothesis that has preoccupied scientists and psychiatry for the past 40 years. Chronic stress and depression leads to neuronal atrophy and cell loss in the limbic system (28). Effective treatment with antidepressants leads to upregulation of BDNF (30). Stress itself decreases expression of BDNF (31,32,33); expression of BDNF has been found to be consistently lower in suicide victims (31). The upregulation of BDNF in the treatment of depression with antidepressants has been a relatively consistent finding from several investigators and independent laboratories (30,34,35), and has been found in selective serotonin reuptake inhibitors (SSRI) (36), and other classes of antidepressants such as the monoamine oxidase inhibitors (MAOIs) (36). BDNF is one of several growth factors that have been implicated in the pathophysiology of depression; fibroblast growth factor (FGF) (37) has been shown to have lower expression in depressed subjects, and in subjects treated for depression, the FGF expression level was similar to controls, suggesting that a threshold level of expression is necessary for euthymic mood.

The primary limitation of work focusing on neurotropic growth factors is the difficulty in *translation* of the findings to a level of clinical care. This is after all the primary purpose of research in psychiatric disease—to increase our understanding of disease and improve the quality of care. It is difficult to assess the significance of BDNF in serum, as it is outside of the blood brain barrier; there are reports of decreased serum levels of BDNF in depressed patients (38,39) and possibly other psychiatric disorders such as eating disorders (40). Preliminary evidence suggests that treatment with antidepressants reverses this effect, that is,

increases the serum BDNF (35,41,39). The changes in BDNF appear to be specific to mood and related disorders; there was no difference in serum levels when comparing schizophrenics and controls (42).

Catechol-*O*-Methyltransferase

The recent research on the COMT gene exemplifies the direction that translational research is likely to take, focusing not specifically on the disease in the broader concept, rather, parsing out specific symptoms or phenomenology that can be targeted for specific interventions.

At first glance, COMT might appear to be the quintessential molecule for translational research; its activity is measurable in peripheral red blood cells and the activity is largely determined by a G-to-A substitution resulting in the functional variability of the enzyme; the met variant results in a heat-labile protein with a four-fold reduction in enzymatic activity (43). There is a trimodal distribution of COMT in humans consistent with a codominant inheritance pattern (44). There have been clinical reports of elevated COMT enzyme activity in blood of schizophrenic patients (45). This led to extensive genetic analyses in the recent "genomics era," with the hypothesis that a functional variant of the COMT gene would be associated with schizophrenia. In fact, there have been conflicting results in the standard association studies; some have reported a modest association with the val allele, others with the met allele, and others with neither [reviewed by Fan et al (22)]. Additional findings have emerged to indicate that other polymorphisms affect the functioning of the gene, for example, a polymorphism near the 3′ untranslated region was associated with differential expression (46), and a further polymorphism at the 5′ regulatory domain affects COMT activity both in brain and peripheral cells (47). All of these polymorphisms appear to affect the cognitive functioning and executive functioning of the prefrontal cortex [reviewed by Tunbridge et al (48)].

The examples of BDNF and COMT in the quest for meaningful translational research highlight many of the problems that are encountered in attempting to "translate" between basic neuroscience and the day-to-day clinical care of our patients. How can clinical phenomena and their assessments be correlated to their relevant basic science underpinnings? Psychiatry and neuroscience investigation is complicated by the lack of accessibility to the primary tissue (brain) and subsequently compromised by the use of proxy measures, either with biochemistry, assaying inflammatory markers, imaging, or genetic polymorphisms.

Expression Profiling

Gene expression profiling technology using DNA microarrays has radically changed the environment of expression assessment, essentially the whole transciptome or gene expression profile can be assessed in a single experiment. The array contains fragments or targets representing the genes to be assayed; the sample is derived from the tissue of interest and RNA from the sample is used to

generate labeled probes that are hybridized with the array. The intensity of the hybridization reflects the RNA from the sample that is translated into a value representing the gene expression from the tissue. The most common gene expression experiments involving psychiatric disorder have focused on postmortem brain tissue (49), however attempts are being made to use peripheral sources such as lymphocytes (50).

Design of the experiment for profiling gene expression in postmortem samples must pay attention to the diversity in agonal factors including postmortem interval, age, comorbid features (substance abuse and other medical disease), and the specific anatomy of the tissue source. The presence of a normative control group against which to compare the experimental sample is crucial. Despite what might seem to be a rather daunting task, there have been initial findings in this emerging field that need to be interpreted with caution until replications are established. Among the most promising results have been those in major depression wherein neuropeptide Y (NPY) and thyrotropin-releasing hormone (TRH) were shown to have increased expression in three independent studies (51,52,53). These findings are of interest because intraperitoneal injection of TRH improves performance on the forced swim test (consistent with antidepressant affect) (54) and NPY is shown to be decreased in depression and increases in response to antidepressant medication (55). Expression studies focused on schizophrenia (postmortem brains) have identified expression motifs that have shown remarkable consistency across studies. Mitochondrial related genes were underexpressed in at least two studies (56,57). Genes involved with myelination and oligodendrocyte metabolism have also shown to be downregulated in most studies (58,59,60).

Expression studies in non-neuronal tissues have the advantage of ready accessibility over postmortem brain tissue, potentially increasing power and offering a cellular system that can be readily accessed and studied over time and under variable conditions of disease state and medication intervention. Although encouraging as an approach (61), the obvious problem is, of course, whether the gene expression in peripheral tissue, in any way, reflects the expression patterns that are altered centrally. The findings of Tsuang et al. (62) of eight putative markers that discriminated between schizophrenia, bipolar, and controls [catalytic polypeptide like apolipoprotein B mRNA edlting enzyme 3B (APOBEC3B), adenylosuccinate synthetase (ADDD), ataxia-telengiectasia mutated (ATM), Charcot-Leyden crystal protein (CLC), C-terminal binding protein 1 (CTBP1), death-associated transcription factor 1 (DATF1), chemokine C-X-C motif ligand 1 (CXCL1), and s100 calcium binding protein A9 (S100AS9)] have not been replicated. The array approach appears to be valid; in the findings, the level of microarray expression have been validated using an independent method. Vawter et al. (63), in a schizophrenia study, identified differential expression in the NPY1R and GNAO1 genes that were validated by quantitative PCR; however for other genes, there were some discrepancies with the brain expression data base, for example, MDH1 was overexpressed in the peripheral lymphocytes; yet there are data indicating that it is underexpressed in the central nervous system (CNS) (56).

Can gene expression profiling help the patient in the clinic? It must be appreciated that this technology is not much more than five years "out of the gate" and that we wait for either a convergence or divergence of findings. With predictions of a $1000 per person sequencing of their entire genome and relatively rapid assessment of gene expression profiles in select and accessible peripheral tissues, one can be relatively sure that there will be a wealth of data to be analyzed in parallel with the clinical outcome data. Questions are raised as to whether gene expression is related to the genotype of the actual gene itself or if it is related to a host of other factors, that is, the genotypes of other regulatory genes. Mirnics et al. (49) suggests a convergent hub hypothesis wherein expression levels of specific genes such as RGS4, GAD67, and NRG1 (all of which have been found to show altered expression in several studies) are the "hubs" whose expression may be influenced by converging inputs from elsewhere.

Neuropsychology, Environment, and Disease Phenotypes

For the purposes of this discussion, I view neuropsychology as a gateway between the phenotype and basic molecular biology (including genotypes and gene expression), physiology, and biochemistry. Neuropsychology represents the waking state that provides the expression of the individuals personality and, ultimately, their phenotype. Environmental stressors affect the physiology and biochemistry of stress through neuropsychological pathways. Implicit to neuropsychological perspectives are "endophenotypes" (Chapter 8), or middle states that themselves are heritable states, independent and measurable (or at least consistently observable) phenomena that are associated with the phenotype, and generally not seen "by the naked eye". Neuropsychology also includes the phenomenology of disease, the mood states of major depression and bipolar disorder, and psychotic experiences of the schizophrenic. The manifestation of symptoms and perceived severity are somewhat influenced by the environment. The endophenotypes are usually measurable entities, such as the attention and memory deficits measured in standard psychological testing or impairments in executive function measured using the Stroop test. As such, endophenotypes lend themselves to become conduits of translational research, allowing for integration of basic science research into the line of inquiry in endophenotypes; such has been the case for the COMT gene and functional cognitive assessments (48). In order for translational research to be successful, measurable phenotypic phenomena that can be correlated with basic science findings will help tremendously.

PLEA FOR DIMENSIONS IN PSYCHIATRY: CAN THEY EXIST WITHIN DIAGNOSTIC CATEGORIES?

So far in our discussions of translational research, it has been clear that the parameters of the basic sciences are measurable—the genotype, the level of gene expression, and physiological and biochemical measures. Endophenotypes are,

likewise, usually defined as a stable and measurable trait. It is, therefore, frustrating to the basic scientist who interacts with the clinic to learn that the phenotypes are categories based on tradition, expert consensus, and clinical utility (64). Although it is unlikely that dimensions will displace the categorical diagnoses, major depression, bipolar disorder, and schizophrenia are firmly entrenched in our clinical approach; it should be possible to add dimensions whenever possible to assess symptom severity, using the standardized scales (65,66) and to measure personality along the five-factor model (67).

Translational research implies the relative immediacy from the laboratory to the clinic and back to the laboratory. Relying on categories is unlikely to help in assessing the effect of environmental stress on expression of genes within specific tissue systems or the affect of altering expression patterns of specific genes on sleep or energy levels. This will require detailed measures and ongoing monitoring of symptoms and phenomenology; the measure need not be overly sophisticated or complex but, rather, focused on the target symptom. The STAR*D projects emphasized the importance of measurement-based care (68), or using relatively simple but standardized assessments of symptom severity in guiding the treatment of depression (69). It is a humbling point to make that most psychiatric care is based on the clinician's "impression" of how a patient is doing rather following a simple metric. Although not entirely fair, it might be compared to attempting to treat hypertension without regular measure of blood pressure, relying solely on clinical impressions!

Translational research will need dimensional measures of disease severity; major depression, bipolar disorder, and schizophrenia all are diseases of tremendous variability, yet we lump together each into their respective categories and refer to the fact that they meet the Diagnostic and Statistical Manual of Mental Disorders IV (DSMIV) criteria for the respective disease. I do wish to emphasize that I am not advocating replacing DSMIV with a purely dimensional schema; rather, that there needs to be measures within the categories that further characterize the disease and its severity. An example of such a dimensional characterization within a category can be found in the Bipolar Affective Disorder Dimensions scale (70); a dimensional scale useful in assessment of patients with bipolar disorder, it measures four dimensions: mania, depression, psychosis, and incongruence of psychosis. Using this instrument, Williams et al. (71) found that variants at the DAOA locus were more associated with the specific mood symptoms of bipolar disorder and schizophrenia and that elevated scores on mood incongruent psychosis were associated with variants at the NRG1 gene (72), generally considered to be a schizophrenia gene. Such a dimensional approach challenges the categorical approach, but is more likely to complement rather than replace the categories.

Dimensional measures imposed upon the DSM diagnostic categories are likely to be integrated into the next version of DSM (73) and the measure of personality and pathology lends itself well to this approach. Personality, like IQ, height, and blood pressure are dimensional categories; everyone has a measurable personality and a blood pressure. Translational research is all about

translating between the bedside and bench; in order to correlate between these two entities, there must be reliable measures on each side, descriptive categories have limited utility.

THE PROMISE OF TRANSLATIONAL RESEARCH

Translational research as an approach has its greatest value in the interactive and iterative processes between the clinical and basic science investigator. There are, as yet, few, if any, practical applications from genetic research in the current environment and understanding of genetic mechanisms and risk factors. It will be important for the clinician to be able to evaluate the meaning of new results and in the context of risk and the amount of variance explained by a specific finding, such as an association of a genetic variant with psychiatric disease or phenomena. Concepts of probability and likelihood must be understood, a topic that the average clinician found to be abstract and of questionable relevance during their training. The general public has, understandably, little appreciation of this, and are often persuaded to undergo testing procedures that are of little, if any, relevance to them. As far as the actual science goes, there is usually evidence for an effect, say, of variants at the serotonin transporter associated with an effect (74), followed by evidence against (75).

Such discrepancies should function to stimulate and not stifle "translational research" and accelerate interactions between clinics and labs in order to understand the phenomena, whether it is merely stochastic noise in the system or component to a meaningful pathophysiological process that will lead to an intervention in the clinic. However, the immediacy that the term translational implies is not the immediate that the public is expecting, and we must educate.

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