49

MOLECULAR AND POPULATION GENETICS OF SCHIZOPHRENIA

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For much of this century, we have believed that genes play a role in the etiology of schizophrenia, but we have been frustrated in the search for specific mutations by diagnostic dilemmas and technologic shortcomings. Slowly, however, we have made substantive strides in the diagnosis and treatment of schizophrenia, and considerable progress toward an explication of its underlying neurobiology. In this chapter, we focus on the epidemiologic and genetic work that underlies much of the current clinical progress and the promises, perhaps to be fulfilled in the not-too-distant future, to facilitate the treatment, and even prevention, of this devastating disorder. We begin with updates on the epidemiology of schizophrenia and related disorders, followed by a review of its molecular genetic bases. We then consider research strategies that promise to explicate the genetic etiology of schizophrenia further in the next few years.

EPIDEMIOLOGY OF SCHIZOPHRENIA

Psychiatric epidemiology studies the distribution of disorders in well-defined populations. Its methodology emphasizes the use of representative samples with reliable and valid diagnoses, and a specified time of onset (1,2), to identify risk factors that explain why some populations are more vulnerable to psychiatric illness than others. Epidemiologic methods are also critical to an understanding of how frequently a disorder occurs, a concept often expressed in terms of prevalence, incidence, and lifetime risk. We consider the risk for schizophrenia by focusing on these measures.

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Prevalence Rate

The prevalence rate of a disorder is the number of people in whom the disorder is diagnosed divided by the total number of persons examined in the population under study. The computed rate depends on several factors: the definition of the disorder, the total number of individuals examined in the population, and the procedure used to choose whom to examine. Ideally, the sample used to compute prevalence should be representative of the population as a whole. The prevalence rate is usually expressed as the number of cases per thousand people surveyed within a year, which is called the *1-year prevalence per thousand.* Studies of schizophrenia from around the world usually report these rates to range from a low of 0.6/1,000 to a high of 17/1,000 (3–6). Lifetime prevalence rates usually range from 0.9/1,000 to 3.8/1,000 (7–10), depending on the diagnostic criteria and methods of ascertainment.

The prevalence rates for schizophrenia are relatively constant between countries. Whether we consider East versus West, developed countries versus less developed countries, or other classifications, the 1-year prevalence of schizophrenia is approximately 0.5% and the lifetime prevalence is approximately 1.0%. In other words, schizophrenia is found in approximately one-half of one percent of the population at any point in time.

Incidence Rate

Another way of reporting the rate of schizophrenia in a population is to estimate the number of *new* cases that appear in the population during a specified period of time; this is called the *incidence rate.* Prevalence rates (as discussed above) include both new and old cases because once schizophrenia has emerged, it usually demonstrates a chronic, unremitting course. In other words, once patients are classified as schizophrenic, they usually remain schizophrenic. Like prevalence rates, incidence rates vary according to a number of variables, including the standards of diagnosis. In addition, incidence studies of schizophrenia entail the difficulty

of how to define onset. Unlike the onset of some disorders (e.g., stroke or head injury), that of schizophrenia is often insidious and confused easily with other problems. For example, early symptoms like social withdrawal or unusual thinking may be ignored or mistaken for indications of depression or substance abuse. Because the time of onset is difficult to determine, incidence rates are usually based on a patient's first visit to psychiatric services for schizophrenic symptoms. Thus, misdiagnosed and untreated cases can affect the accuracy of incidence rates significantly.

The incidence rate is usually expressed as the number of new cases in a given period per 100,000 population. For schizophrenia, incidence rates range from a low of 0.10 to a high of 0.70 (11,12). As was the case with the prevalence figures, the incidence of schizophrenia is generally stable over time and across geographic areas (13).

Lifetime Risk

Most persons with schizophrenia first become ill between 20 and 39 years of age. We call this the *high-risk period* for schizophrenia. Men tend to be younger at the time of onset than women (14–16), although schizophrenia develops in men and women at approximately equal rates (2). Because of the variability of age at onset, prevalence and incidence rates vary according to the age and sex composition of the population studied. The age distribution is particularly important when the probability or risk that schizophrenia will develop in a person during his or her lifetime is estimated (i.e., the lifetime risk). To estimate lifetime risk, the age distribution of the population surveyed should be taken into account (17).

The lifetime risk for schizophrenia ranges from 0.3% to 3.7%, depending on the definition of schizophrenia and the method of survey used (11,12,18,19). The World Health Organization study shows a narrow range of lifetime risks in 10 countries around the world (0.5% to 1.7%) (13), although it is higher in some genetically isolated populations in Palau, Micronesia, and areas of Finland (20,21). Taken together, studies of the lifetime risk for schizophrenia in the general population suggest it is around 1%. In other words, a schizophrenic disorder will develop in approximately one in every hundred people at some time in their life.

Risk Factors

Schizophrenia occurs around the world and in all cultures. International differences in rates of the disorder are usually attributed to diagnostic differences rather than to differences in true rates of illness. The use of broad, ambiguous diagnostic criteria before the late 1970s was an important factor underlying artificial differences in rates of mental disorders recorded in different geographic locales. In the 1960s, for example, the hospital incidence of schizophrenia in the United States significantly exceeded that of Great Britain (28.0/100,000 vs. 17.9/100,000 population), whereas the incidence of mood disorders in British hospitals exceeded that in the United States (36/100,000 vs. 7/100,000). These differences disappeared, however, when identical methods of diagnosis and assessment were used (22). Similarly, in the World Health Organization study, differences in incidence among 10 countries diminished when narrow, standardized diagnostic criteria were utilized (13,23).

A variety of risk factors have received attention in schizophrenia. These include, among others, a family history of the disorder, low socioeconomic status, complications during pregnancy and childbirth, sex, and fetal viral infection (18,24–28). A family history of the disorder and a negative relationship to social class are especially strong and frequently replicated risk factors. The familial basis of schizophrenia is considered further in the section on the genetic epidemiology of schizophrenia. It should be emphasized that although the focus of this chapter is the genetics of schizophrenia, many of the risk factors for the illness cited above are environmental, a fact that underscores the combination of genetic and environmental factors underlying the disorder. This point is stressed further in the discussion of twin studies, below; given an identical twin with schizophrenia, the risk that the disorder will develop in the other twin (who has identical genes) is far less than 100%.

GENETIC EPIDEMIOLOGY OF SCHIZOPHRENIA

Family Studies

There is little question that schizophrenia (and related disorders) runs in families. In a review of 40 European studies, selected for similarities in diagnostic and ascertainment procedures and performed between 1920 and 1987, the lifetime risks for schizophrenia in relatives of schizophrenic patients were as follows: parents, 6.0%; siblings, 9.0%; offspring of one parent with schizophrenia, 13.0%; offspring of two parents with schizophrenia, 46%; and identical twin of a patient with schizophrenia, 46% (18,19). Note that the risk to offspring exceeds the risk to parents. Because the biological relationship is the same (i.e., first-degree relatives), the risk to offspring of patients should be identical to the risk to parents of patients. This would be true under any genetic model. The difference occurs because, by definition, parents have reproduced, and the presence of schizophrenia has an adverse affect on the probability of doing so. The risks to second-degree relatives ranged from 6.0% for half-siblings to 2.0% for uncles and aunts. First cousins, a type of third-degree relative, had an average risk of 2.0%. Consistent with a genetic etiology, these figures show that as the degree of biological/genetic relatedness to a schizophrenic patient increases, so does the risk for schizophrenia.

Although recent studies have used more rigorous research methods and narrower, criterion-based definitions of schiz-

ophrenia, they have essentially confirmed the familial basis of schizophrenia and provided evidence consistent with the genetic hypothesis. In their family study of the Roscommon area in Western Ireland, for example, Kendler et al. (29) reported that the risk to siblings of schizophrenic probands is 9.2%, consistent with the rates reported by Gottesman (18,19). The risk to parents of schizophrenic probands was 1.3%, somewhat lower than the 6.0% reported by Gottesman. In general, narrower definitions in modern studies result in somewhat lower risk figures than those reported previously. This point was demonstrated by Tsuang et al. (30), who reported the risk for schizophrenia in first-degree relatives of schizophrenics as 3.2%, compared with 0.6% for relatives of nonpsychiatric controls. Similarly, Guze et al. (31) reported rates of 3.6% and 0.56%, respectively. Despite the lower prevalence figures, both these studies reconfirmed that in comparison with the general population, relatives of people with schizophrenia are at significantly greater risk for development of the disorder. This in itself, however, is not sufficient to demonstrate a genetic basis. Additional strategies, such as twin and adoption paradigms, are necessary to parse out genetic and environmental determinants, and these are considered next.

Twin Studies

The two types of twins are monozygotic and dizygotic. Monozygotic twins (i.e., identical twins) share 100% of their genes, whereas dizygotic twins (i.e., ordinary brothers and sisters) share 50% of their genes. If both members of a twin pair have schizophrenia, they are considered *concordant* for the disorder; if one is schizophrenic and the other is not, they are considered *discordant.* If schizophrenia were caused by genetic factors alone, then concordance rates for monozygotic and dizygotic twins would be 100% and 50%, respectively. On the other hand, if it were caused essentially by environmental variables, then concordance rates for monozygotic and dizygotic twins reared in a common environment would be similar.

The empiric data lie between these extremes but provide clear evidence for a genetic component. For example, concordance rates from twin studies pooled by Kendler (32) were about 53% for monozygotic twin pairs and 15% for dizygotic twin pairs. A similar review by Gottesman (18, 19) demonstrated median concordance rates of 46% and 14% for monozygotic and dizygotic pairs, respectively. Interestingly, monozygotic twins reared apart have about the same concordance rates as do twins reared together (33). At the same time, monozygotic concordance rates lower than 100% demonstrate an important role for environmental factors. When they obtained quantitative estimates of the relative roles of genetic and environmental factors by translating genetic concordance rates into "heritabilities" (the proportion of the variance between individuals that is attributable to genetic factors), Kendler and Diehl (34) found that about 70% of the variance could be attributed to genetic factors in a series of twin studies. Several recent studies in which DSM-III, DSM-III-R, or DSM-IV diagnostic criteria were used provided even higher estimates of heritability, in the range of 80% to 86% (35–38).

Studies of twins discordant for schizophrenia have also shed light on the role of genetic and environmental factors. Gottesman and Bertelson (39), for example, followed the Danish schizophrenic twin sample of Fischer (40). They reasoned that if genetic liability is transmitted to the unaffected member of the twin pair, but not expressed because of environmental factors, then their offspring should demonstrate the same genetic liability. This hypothesis was supported when 24 children of unaffected co-twins showed a monozygotic concordance rate of 17%, a rate almost identical to that in the offspring of the twins who had schizophrenia. In contrast, although the risk for schizophrenia in the offspring of dizygotic twins with schizophrenia was similar to the risk in monozygotic twins, the risk in the offspring of unaffected dizygotic twins was only about 2%. What type of environmental factors might contribute to the risk for schizophrenia? A variety of possibilities exist, but adverse events occurring early in development (e.g., gestation, birth) have received the most attention recently, in part because the occurrence of such events during that period may have particularly far-reaching biological consequences. McNeil et al. (41) showed, for example, a relationship of smaller hippocampi and larger ventricles to complications of labor and delivery in the ill member of monozygotic twin pairs discordant for schizophrenia. Tsujita et al. (42) showed evidence of postzygotic genomic discordance in discordant twins, which could influence subsequent transcription in one or more genes. It is thus evident from twin studies with a variety of designs that both genetic *and* environmental factors underlie the expression of schizophrenia. Adoption studies, considered below, further support this conclusion.

Adoption Studies

Like twin studies, adoption studies can disentangle genetic and environmental causes of disease (43). An important series of studies was performed in Denmark, thanks in part to its system of national registers. Among these, Kety et al. (44) studied 5,500 children from the Greater Copenhagen area who were separated from their biological families and adopted between 1923 and 1947. Although schizophrenia or a related disorder developed in only 1.9% of the control adoptees, schizophrenia or a related illness was diagnosed in 8.7% of the adoptees who were separated from a schizophrenic parent. When the biological relatives of schizophrenic and control subjects were compared, 5.8% of the relatives of schizophrenic probands had (definite or probable) schizophrenia, in comparison with 0.9% of the control relatives. The rates of schizophrenia did not differ between the adoptive relatives of the schizophrenic and nonschizo-

phrenic adoptees. Moreover, children born to nonschizophrenic parents but raised by a schizophrenic parent did not show rates of schizophrenia above those predicted for the general population.

Limitations of adoption studies include the possibility of transmission by the mother during pregnancy or delivery of a liability for schizophrenia via nongenetic biological, or other type of environmental, factors, even if the child is adopted away soon after birth. However, Kety et al. (45) found that 13% percent of paternal half-siblings of schizophrenic adoptees had schizophrenia, in comparison with only 2% of paternal half-siblings of nonschizophrenic adoptees. Because paternal half-siblings have different mothers, these results cannot be attributed to *in utero* (environmental) effects. Many of Kety's findings were replicated recently for schizophrenia (45) and for certain schizophrenia spectrum conditions (46) in a sample drawn from the rest of Denmark.

Data from the Finnish adoption studies (47,48) provide additional support for both genetic and environmental influence in the transmission of schizophrenia. Together, family, twin, and adoption studies show consistently that the biological relatives of people with schizophrenia themselves show higher rates of schizophrenia and related disorders when compared with appropriate control groups, regardless of whether they are raised by biological or adoptive parents.

EPIDEMIOLOGY OF SPECTRUM DISORDERS

The term *related disorders* is used to describe schizophrenic illness of (generally) lesser severity. In fact, genetic studies provide evidence for a spectrum of disorders that are similar to schizophrenia and caused by the same genes. A disorder is considered to be in the schizophrenia spectrum if it occurs more frequently among the biological relatives of schizophrenic patients than it does among the relatives of people who do not have schizophrenia. Many of the behavioral genetic methodologies used to delineate genetic and environmental factors in the etiology of schizophrenia (e.g., family, twin, and adoption studies) have also provided evidence of a genetic etiology in schizophrenia spectrum conditions. Evidence for inclusion in the schizophrenic spectrum is considered next for several candidate disorders.

Psychotic Spectrum Disorders

In about 9% of the first-degree relatives of schizophrenic patients, a psychotic disorder develops that does not meet the criteria for either schizophrenia or a mood disorder (18, 49). Two prominent examples are schizoaffective disorder and psychosis not otherwise specified (NOS). As the name suggests, the term *schizoaffective disorder* describes patients with features of both schizophrenia and affective disorders (also known as *mood disorders*), although subgroups may exist in which either schizophrenia or affective symptoms predominate. *Psychosis NOS* is a residual diagnostic category for patients with psychotic symptoms who do not fit into a more narrowly defined category. In many cases, the NOS designation serves as a temporary diagnosis for patients with new onset of disease until the course of their symptoms reveals their true diagnosis.

Both schizoaffective disorder and psychosis NOS are more common among the relatives of schizophrenic patients than among the relatives of nonschizophrenic persons. For example, in a survey of family history and family, twin and adoption studies, Prescott and Gottesman (33) found that 13 of 15 studies demonstrated evidence of a familial/genetic component for schizoaffective disorder. Consistent with this finding, monozygotic twins show higher concordance rates for schizoaffective disorder than do dizygotic twins (36,50).

Nonpsychotic Spectrum Disorders

Personality Disorders

Milder forms of schizophrenic illness are characterized by nonpsychotic symptoms, such as poor social relationships, anxiety in social situations, and limited emotional responses. Less frequently, mild forms of thought disorder, suspiciousness, magical thinking, illusions, and perceptual aberrations are also present. These symptoms are observed most frequently in three personality disorders, including schizotypal, schizoid, and paranoid personality disorders. Several studies found that (DSM) cluster A personality disorder traits often precede the onset of psychosis in subjects in whom schizophrenia subsequently develops (51,52). Moreover, in the New York high-risk project (53), offspring of schizophrenic mothers demonstrated elevated rates of these personality disorders when they were considered together, although not separately.

Most studies of familial prevalence in the biological relatives of schizophrenic patients have related schizotypal personality disorder to the schizophrenia spectrum more strongly than they have either schizoid or paranoid personality disorder (54,55). Evidence in favor of including schizotypal personality disorder is consistent across family (56–58), adoption (45), and twin studies (59,60). Although not all studies have detected a higher rate of schizotypal personality disorder among relatives of schizophrenic probands (54), most investigations, and particularly those with large samples, show higher rates of schizotypal personality disorder among the relatives of index cases with schizophrenia than among the relatives of control subjects (61). The incidence of the disorder in schizophrenic families has been estimated at between 4.2% and 14.6% (57,58,62,63). In contrast, results for schizoid and paranoid personality disorders have been somewhat more controversial and contradictory, with positive findings sometimes occurring in combined paranoid–schizotypal or schizoid–schizotypal

samples (55). Thus, although some symptoms may overlap between schizotypal, schizoid, and paranoid personality disorders, schizotypal personality disorder is currently the strongest candidate among this group for a nonpsychotic, relatively mild condition that is related genetically to schizophrenia.

Schizotaxia

Paul Meehl (64) first used the term *schizotaxia* in 1962 to describe a genetic vulnerability to schizophrenia. He suggested that a subtle but widespread neurointegrative defect results from this vulnerability that predisposes individuals to the development of either schizotypy or schizophrenia, depending on the protection or liability afforded by environmental circumstances. Later, Meehl reformulated the concept to allow for the possibility that some people with schizotaxia would not progress to either schizophrenia or schizotypal personality disorder, although most would (65). Eventually, the term *schizotypy* entered the psychiatric nomenclature in the form of schizotypal personality disorder. *Schizotaxia* did not, although the term was used in a general sense by researchers to describe the liability for schizophrenia. Now, almost 40 years after the concept was introduced, a broad literature shows that the liability for schizophrenia can be characterized clinically by deficits or abnormalities in psychiatric, neuropsychological, neurobiological, and psychosocial domains in nonpsychotic, first-degree relatives of people with schizophrenia.

Psychiatric features in such relatives frequently include negative symptoms (e.g., asociality and anhedonia) that are qualitatively similar to, but quantitatively milder than, those often seen in schizophrenia (66). Positive symptoms, however, are usually less evident in these relatives than they are in schizophrenia or schizotypal personality disorder. Neuropsychological impairments in biological relatives of people with schizophrenia are also similar to those in patients with schizophrenia, but are generally of lesser severity (67–71). The most extensively documented of these impairments involve working memory/attention, verbal memory, and concept formation/abstraction.

We recently suggested a reformulation of Meehl's concept of schizotaxia that focuses on these features of negative symptoms and neuropsychological deficits (67). In addition to specifying the clinical consequences of schizotaxia more specifically, our view of the concept differs from Meehl's in a few other respects. Among these is whether schizotaxia always or even usually progresses to schizotypal personality disorder or schizophrenia. Our empiric analyses suggest that the basic symptoms of schizotaxia occur in 20% to 50% of adult relatives of patients with schizophrenia (68,69). This rate is considerably higher than the rates of schizophrenia or schizotypal personality disorder likely to develop in first-degree relatives ($\leq$ 10% for each condition), which suggests that schizotaxia does not lead inevitably to schizophrenia or schizotypal personality disorder. Our view of schizotaxia also differs somewhat from Meehl's formulation in that we include both genetic and nongenetic, adverse biological alterations occurring early in development (e.g., pregnancy and birth complications) in our conception of the syndrome.

Tsuang et al. (72) recently described a set of specific criteria for schizotaxia, but the clinical characterization is still evolving and the syndrome requires independent validation and further research before it can be used clinically (73). These criteria underlie a conception of schizotaxia as a neurodevelopmental condition resulting from genetic and adverse environmental (e.g., pregnancy or delivery complications) factors. Current criteria in adult, nonpsychotic, first-degree relatives of schizophrenic patients include moderate or greater levels of negative symptoms and neuropsychological deficits (as described above). The concept of schizotaxia demonstrates considerable utility because it accounts for clinical deficits in a sizable proportion of relatives who may not otherwise meet the criteria for a schizophrenia-related disorder. Moreover, because schizotaxia may be considered as a risk factor for schizophrenia, as well as a clinically meaningful syndrome in its own right, its recognition may eventually facilitate the development of early intervention and prevention strategies.

MODE OF INHERITANCE

Given the evidence outlined above for a substantial genetic contribution to the etiology of schizophrenia, methods such as complex segregation analysis (74) can be used to identify the most likely mode of inheritance. Commonly, a mixed model (75) comprising both major gene and polygenic effects is compared with the submodels of a single major locus and polygenic inheritance. However, large sample sizes are required to distinguish between models, especially the polygenic and mixed models, so that the practical usefulness of this approach has been limited to date.

Based on complex segregation analysis and related approaches, the pattern of risks in family and twin studies has been found to be incompatible with a single locus accounting for all the genetic liability to schizophrenia (76–78). However, it has not been possible to distinguish between a polygenic and a mixed model (77). The pattern of risks in family studies, in which the risk decreases rapidly as the degree of genetic relatedness decreases, is also compatible with a model of multiple loci with epistasis (interaction between genes) (79). However, the number of susceptibility loci, the disease risk conferred by each locus, and the degree of interaction between loci all remain unknown. The contribution of individual genes to the familiality of a disorder can be expressed in terms of λ_s (i.e., the relative risk to siblings resulting from possession of the disease allele (79). Risch (79) has calculated that the data for recurrence risks

in the relatives of probands with schizophrenia are incompatible with the existence of a single locus having a value of *(s* greater than 3. Unless extreme epistasis (interaction between loci) exists, models with two or three loci having values of *(s* of 2 or less are more plausible. It should be emphasized that these calculations are based on the assumption that the effects of genes are distributed equally across the whole population. It is quite possible that genes of larger effect are operating in a subset of patients—for example, those from families with a high density of illness.

These quantitative genetic investigations provide strong evidence that genetic factors increase the risk for schizophrenia. However, although it is possible to state that, as a group, siblings of individuals with schizophrenia, for example, have a roughly 10-fold increased risk in comparison with the general population, it is not currently possible to translate this figure to the level of risk for a particular sibling in a particular family. Similarly, heritability estimates refer to variations in liability to schizophrenia in the general population and have no simple meaning for an individual. Another important point is that risk to related individuals does not directly equate with genetic risk because some relatives carry one or more susceptibility alleles for schizophrenia but remain unaffected throughout their lives. In other words, the accumulation of susceptibility alleles, environmental risk factors, and complex interactions between risk factors probably all play a role in determining who becomes ill. Therefore, to quantify genetic risk, it is necessary to identify the susceptibility loci themselves at the molecular level.

MOLECULAR GENETICS: LINKAGE STUDIES

The first generation of systematic molecular genetic studies of schizophrenia effectively ignored the evidence for genetic complexity and targeted large, multiply affected pedigrees for analysis. This was done in the hope that such families, or at least a proportion of them, were segregating genes of sufficiently large effect that they could be detected unequivocally in this way. This approach has been successful in other complex disorders—Alzheimer disease, for example, in which mutations in three genes, *APP, PS1,* and *PS2,* are now known to cause rare forms of the disorder. In such cases, the disease is of unusually early onset and is transmitted through multiplex pedigrees in an autosomal dominant fashion (80–82).

Unfortunately, it has not proved possible to identify a phenotypic trait analogous to age at onset in Alzheimer disease by which to classify multiplex families segregating schizophrenia. Studies of such large families also initially produced positive findings in schizophrenia (83), but unfortunately these could not be replicated. The reasons for this have become clear as data from systematic genome scans have accumulated; highly penetrant mutations causing schizophrenia are at best extremely rare and quite possibly nonexistent (84,85). The false-positives were largely the consequence of a combination of multiple testing and the use of statistical methodology and significance levels derived from work on single-gene disorders.

Despite the failure to identify regions of unambiguous linkage in multiply affected families, modest evidence for several regions has been reported in more than one data set. Areas implicated for which supportive data have also been obtained from international collaborative studies include chromosomes 6p24-22, 8p22-21, and 22q11-12 (86,87; see refs. 84 and 88 for review). A number of other promising areas of putative linkage are also currently under investigation by international consortia. These include 13q14.1-32 (89–91), 5q21-31 (92,93), 18p22-21 (94), 10p15-11 (95–97), 6q (98,99), and 1q21-22 (100). However, in each case, both negative and positive findings have been obtained, and in only two cases, those of chromosomes 13q14.1-32 and1q21-22, did any single study achieve genome-wide significance at values of p of .05 (90,100).

These positive findings contrast with those from a large systematic search for linkage in which a sample of 196 affected sibling pairs, drawn typically from small nuclear families rather than extended pedigrees, was used (101). The results of simulation studies suggest that the power of this study is greater than 0.95 to detect a susceptibility locus of $(s = 3$ with a genome-wide significance of 0.05, but only 0.70 to detect a locus of $(s = 2$ with the conservative assumption that a locus lies midway between two adjacent markers. This study yielded evidence at the level of the definition of Lander and Kruglyak (102) of "suggestive" linkage to chromosomes 4p, 18p, and Xcen. However, none of the findings approached a genome-wide significance of 0.05, corresponding to Lander and Kruglyak's definition of "significant" linkage.

The findings from linkage studies of schizophrenia to date demonstrates several features that are to be expected in the search for genes for complex traits (103–106). First, no finding is replicated in all data sets. Second, levels of statistical significance are unconvincing and estimated effect sizes are usually modest. Third, chromosomal regions of interest are typically broad [often > 20 to 30 centimorgan (cM)].

At the present time, therefore, the linkage literature supports the predictions made by Risch (79); it is highly unlikely that a commonly occurring locus of effect size [$(s$] greater than 3 exists, but suggestive evidence implicates a number of regions, consistent with the existence of some susceptibility alleles of moderate effect [$(s = 1.5$ to 3]. Moreover, encouraging results in several chromosomal regions suggest that rarer alleles of larger effect may be segregating in some large, multiply affected families.

Linkage methods in sample sizes that are realistically achievable can detect smaller genetic effects than those in the studies to date. For example, it is possible to detect alleles with values of *(s* of 1.5 to 3 in a sample of 600 to

800 affected sibling pairs (107,108). We therefore suggest that priority should now be given to collecting such samples with a robust clinical methodology that is comparable across all interested research groups. However, if liability to schizophrenia is entirely a consequence of the operation of many genes of small effect, then even these large-scale studies will be unsuccessful.

CANDIDATE GENE ASSOCIATION STUDIES

Once genes of smaller effect than $(s = 1.5$ are sought, the number of affected family members required becomes prohibitively large (107–109). For this reason, many researchers have tried to take advantage of the potential of candidate gene association studies to identify such loci (109,110). Although a potentially powerful means of identifying genes of small effect, association studies are not without their problems. First, for a complex and poorly understood disorder such as schizophrenia, the choice of candidate genes is limited largely by the imagination and resources of the researcher. This places a stringent burden of statistical proof on positive results because of low prior probability and multiple testing (111). Second, case–control association studies have the potential to generate false-positives because of population stratification. This problem can be addressed by using family-based association methods (112), but because of stigma, adult age at onset, and the disruptive effects of mental illness on family relationships, family-based samples may be unrepresentative in addition to limited in size. Consequently, family-based studies may introduce more spurious results than do case–control studies (113). It would seem unwise, therefore, to discard the case–control study design, which has served epidemiology so well through the years. A third problem common to all molecular genetic studies in complex diseases is that they are prone to type 2 errors simply because they are often underpowered, and therefore to draw satisfactory conclusions from negative studies, larger sample sizes are required than have typically been used to date in psychiatric genetics (111). Fourth, even with larger samples, it is by no means certain that a given replication study will be sufficiently powered to replicate a particular effect. This is because variations may be noted in the contribution of a given susceptibility allele in different patient populations as a result of different allele frequencies at the locus of interest or at interacting loci. Further potential for heterogeneity occurs if the association with the marker is a result of tight linkage with the true susceptibility allele, or if different subtypes of the disease exist. Given that all the above factors may influence power, and that none of the above is known in advance, it is difficult to obtain an accurate measure of the power of a replication study. Because we cannot specify accurately the prior probability of a candidate gene, nor know the true power for replication, it is difficult to draw definitive conclusions from conflicting findings. However, the purpose of experiment is to reject a null hypothesis, and in the face of uncertainty, the burden of proof remains with the proponents of a particular candidate gene.

Most candidate gene studies have been based on neuropharmacologic studies, which suggests that abnormalities in monoamine neurotransmission, in particular dopaminergic and serotoninergic systems, play a role in the etiology of schizophrenia. Overall, the results in this extensive literature are disappointing, but it should be noted that the sample sizes in many of the older studies would now generally be regarded as inadequate, particularly in view of the fact that the polymorphic markers in question did not in themselves represent functional variants and that few genes have been systematically screened even for common functional variants. However, more promising reports of candidate gene associations have recently appeared, three of which are considered here.

Serotonin 5-HT_{2A}-Receptor Gene

Many novel antipsychotic drugs affect the serotoninergic system. The first genetic evidence that serotoninergic receptors may play a role in schizophrenia came from a Japanese group reporting an association between a T-to-C polymorphism at nucleotide 102 in the 5-HT_{2A}-receptor gene in a small sample (114). A large European consortium comprising seven centers and involving 571 patients and 639 controls then replicated this finding (115), which was further replicated with use of a family-based design (116). Although many other studies followed with mixed results, a recent metaanalysis of all available data from more than 3,000 subjects supports the original finding ($p = .0009$), and this does not appear to be a consequence of publication bias (117).

Since this metaanalysis was undertaken, a few further negative reports have followed, but none has approached the sample sizes required. If we assume homogeneity and if the association is true, the putative odds ratio (OR) for the C allele can be expected to be around 1.2 in any replication sample. Sample sizes of 1,000 subjects are then be required for 80% power to detect an effect of this size, even at a relaxed criterion of $p = .05$. Thus, the negative studies are effectively meaningless, but it is also true that the evidence for association, even in the metaanalysis ($p = .0009$), is not definitive if genome-wide significance levels are required (109). At present, all we can conclude is that the evidence favors association between the T102C 5-HT_{2A} polymorphism and schizophrenia, but the most stringent burden of proof has not yet been met.

If the association is real, it is unlikely that the T102C polymorphism is the susceptibility variant because this nucleotide change does not alter the predicted amino acid sequence of the receptor protein, nor is it in a region of obvious significance for regulating gene expression. T102C is

in complete linkage disequilibrium with a polymorphism in the promoter region of this gene, but no evidence has as yet been found that this has a functional effect either (116). Recent evidence of polymorphic monoallelic expression of the 5-HT_{2A} gene points to the possible existence of sequence variation elsewhere that influences gene expression (118), and this may be the true susceptibility variant.

D3 Dopamine-Receptor Gene

Association has been reported between schizophrenia and homozygosity for a Ser9Gly polymorphism in exon 1 of the D3 dopamine-receptor gene (*DRD3*) (119). As with the 5-HT_{2A} association, the results have now been confirmed in several independent samples, including one family-based study (120), but several negative studies have also been reported. Metaanalysis of data from more than 5,000 individuals has revealed a small (OR = 1.23) but significant ($p = .0002$) association between homozygosity at Ser9Gly and schizophrenia (120). Again, this cannot easily be ascribed to selective publication (120). Because it is a fairly uncommon genotype, we cannot be certain that homozygosity for the 9Gly allele alone is not associated with an increased risk for schizophrenia, and therefore it is not certain that the findings at D3 are an example of heterosis. However, a plausible biological explanation for D3 heterosis has been put forward in that possession of two different molecular forms of the receptor may allow the dopaminergic neuron to respond more flexibly (119).

At present, then, the status of the D3 dopamine-receptor gene is similar to that of the 5-HT_{2A}-receptor gene—that is, the balance of evidence at present favors association, but the null hypothesis still cannot be confidently rejected. Those wishing to replicate or reject these findings should bear in mind that to obtain power greater than 0.80 to detect an effect of this size at a criterion of $p = .05$, a sample of 1,500 cases and 1,500 controls is required. So far, no other polymorphisms have been found that might explain the putative D3 association, but several new polymorphisms have been identified in previously unknown exons 5′ to the exon referred to above as *exon 1* (121). These are currently being tested to establish whether variants in this region in linkage disequilibrium with the Ser9Gly polymorphism provide a more functionally plausible explanation of the association with schizophrenia.

ANTICIPATION AND TRINUCLEOTIDE REPEATS

The term *anticipation,* the phenomenon by which the age at onset of disease becomes earlier from one generation to the next, was first described in connection with severe mental disorder (122). A series of recent studies applying modern diagnostic criteria have now confirmed that the inheritance of schizophrenia is at least consistent with the presence of anticipation, although ascertainment biases offer an alternative explanation (123). Because pathogenic expanded trinucleotide repeats are the only known genetic mechanisms for anticipation, these findings have been taken as suggesting that such mutations may account for at least some of the complexity in the pattern of inheritance in this disorder (124). This hypothesis was supported by two groups who observed that the maximum length of the most common known pathogenic trinucleotide repeat, CAG/CTG, was greater in patients with schizophrenia than in unaffected controls (125,126). These findings were later replicated in a European multicenter study (127). Unfortunately, the early repeat expansion detection (RED) studies were followed by a series of unsuccessful attempts to identify the relevant repeat-containing loci by a variety of methods, and by several failures to replicate the RED findings (128–130), thus casting doubt over the CAG/CTG repeat hypothesis.

The trinucleotide repeat hypothesis was rejuvenated, however, with the report of an association between schizophrenia and alleles of a member of the family of calcium-activated potassium channel genes, *KCa3* (*hKCa3*/*KCCN3*) (131). For several reasons, *KCa3* seemed a remarkable candidate gene for schizophrenia. First, the gene contained two CAG repeats, one of which is highly polymorphic. Second, the family of genes to which it belongs is thought to play an important role in regulating neuronal activity, and it was therefore considered a functional candidate gene. Third, the gene was also thought to be a positional candidate, as it was believed to map to chromosome 22q11. Ironically, *KCa3* maps not to 22q11 but to 1q21 (132), which is also a region implicated by linkage studies as possibly containing a susceptibility locus for schizophrenia (100). Two further case–control studies have subsequently supported the findings of Chandy and colleagues (132,133).

However, although evidence from three case–control studies lends support to the hypothesis of *KCa3* as a susceptibility gene for schizophrenia, in other respects the case for this gene as a candidate is less certain. First, the RED data cited above lend no support to *KCa3* as a candidate because the polymorphic trinucleotide repeat in this gene is far too short to account for the RED associations (133). Second, a series of case–control and family studies have failed to replicate the findings (134–140). Thus, we are back to our familiar position in candidate gene analysis; although the data are insufficient to draw firm conclusions, we believe at present that the case for *KCa3* remains firmly with the null hypothesis.

What then is the status of the original associations between large CAG/CTG repeats and schizophrenia? It has been reported that large CAG/CTG RED products (repeat size > 40) are explained by repeat size at two autosomal loci, one at 18q21.1 and the other at 17q21.3 (141,142). If the explanation is correct, then it follows that one or both of these loci should be associated with schizophrenia.

Unfortunately, data from Vincent and colleagues (128) and unpublished data from Cardiff unequivocally show that expansions at these loci are not responsible for the RED associations. However, in both samples, only around 50% of large CAG/CTG repeats detected by RED could be explained by polymorphisms at these two loci, which suggests that at least one further locus is responsible for the RED data, a possibility supported by two recent studies on protein extracts from schizophrenic tissues (143).

HIGH RATES OF SCHIZOPHRENIA IN ADULTS WITH VELOCARDIOFACIAL SYNDROME

Velocardiofacial syndrome (VCFS), also known as *DiGeorge* or *Shprintzen syndrome,* is associated with small interstitial deletions of chromosome 22q11 in 80% to 85% of cases (144). First described by Shprintzen and colleagues (145), VCFS has an estimated prevalence of 1/4,000 births (146). Distinctive dysmorphology, congenital heart disease, and learning disabilities characterize the syndrome, although considerable phenotypic variability occurs. As the first recognized cohort of children with VCFS was followed into adolescence and early adulthood, evidence began to accumulate for a high prevalence of major mental illness. Early reports suggested that psychiatric disorders had developed in more than 10% of the cohort, which mostly resembled chronic schizophrenia with paranoid delusions, although operational criteria were not used (147). In a follow-up study of teenagers (age 17 years) in which DSM-III-R criteria were used, Pulver et al. (148) reported that 11 (79%) of their sample of 14 patients had been given a psychiatric diagnosis: 29% had schizophrenia (22%) or schizoaffective disorder (7%), 29% had simple or social phobia, 21% had depression, and 14% had obsessive-compulsive disorder. More recently, Papolos et al. (146) reported that of their sample of 15 children and 10 adults, four (16%) had psychotic symptoms and 16 (64%) met DSM-III-R criteria for a spectrum of bipolar affective disorders. Although none had schizophrenia, the two oldest members of their patient cohort (ages 29 and 34 years) both had schizoaffective disorder.

To try to gain a more precise determination of the prevalence and nature of psychopathology in adults with VCFS, rather than rely on clinical diagnosis, Murphy and colleagues (149) recently evaluated 50 cases with a structured clinical interview to establish a DSM-IV diagnosis. Fifteen patients with VCFS (30%) had a psychotic disorder, with 24% ($n = 12$) fulfilling DSM-IV criteria for schizophrenia. In addition, six (12%) had major depression without psychotic features. They were unable to replicate the findings of Papolos and colleagues (146) of a high prevalence of bipolar spectrum disorders in VCFS. However, these workers studied a small sample that included few adults, and in view of the fact that their oldest cases satisfied criteria for schizoaffective disorder, it is possible that the psychotic phenotype in VCFS varies with age. Prospective studies are now required to test this hypothesis.

The current balance of evidence favors the view that the high prevalence of psychosis results from hemizygosity for a gene or genes at chromosome 22q11 rather than ascertainment bias or a nonspecific association with a low intelligence quotient (IQ) (149). In particular, the prevalence of psychosis and schizotypy in VCFS appears to be much greater than that seen in most other congenital abnormalities affecting neural development, and appears not to be correlated with the degree of intellectual impairment (149). Many lines of evidence suggest that schizophrenia is a neurodevelopmental disorder (150). In VCFS, defective development and migration of mesencephalic and cardiac neural crest cells are believed to play a significant role in the pathogenesis of midfacial and cardiac abnormalities (151). Consequently, it has been postulated that a gene or genes causing disruption of neural cell migration may be a common neurodevelopmental mechanism for both VCFS and schizophrenia (152).

What then is the importance of 22q11 and VCFS in the etiology of schizophrenia as a whole? Karayiorgou et al. (153) reported that among 100 randomly ascertained patients with schizophrenia, two were found to have a 22q11 deletion. In contrast, when subjects with schizophrenia were selected for the presence of clinical features consistent with VCFS, 22q11 deletions were identified in 20% to 59% of cases (154,155). These findings suggest that a small proportion of cases of schizophrenia may result from deletions of 22q11, and that clinicians should be vigilant, especially when psychosis occurs in the presence of dysmorphology, mild learning disability, or a history of cleft palate or congenital heart disease (156). The question of whether genetic variation in 22q11 confers susceptibility to schizophrenia in cases without a deletion is more difficult to answer. As we have seen, the results of some linkage studies suggest the presence of a schizophrenia susceptibility locus on 22q. However, the linkage findings tend to point telomeric to the VCFS region (86,157). Nevertheless, modest evidence for linkage to the VCFS region has also been claimed (90, 158,159), and as we have noted above, linkage mapping in complex diseases is somewhat imprecise. It remains possible that the relationship between VCFS and "typical" schizophrenia is less direct, with little common ground between the genetic and neurodevelopmental mechanisms involved but with convergence on identical or at least similar psychopathologic syndromes.

Another important question concerns the factors that determine whether schizophrenia will develop in a person with VCFS. When adults with VCFS were tested with a quantitative measure of schizotypy, the patients with psychosis had the highest scores (149). However, perhaps of greater interest, those without psychosis had intermediate

scores in comparison with controls (149). If schizotypy is a trait marker for increased liability to psychosis, this suggests that the majority if not all of those with 22q11 deletions are at increased risk for psychosis, but that other genetic or environmental factors are required for this risk to be expressed. The genetic loci involved may reside elsewhere in the genome and include those involved more widely in psychosis, or lie within 22q11. The occurrence of psychosis does not appear to be related to the size of the deletion (153). However, it is possible that susceptibility to psychosis reflects allelic variation of a hemizygous gene or genes within the deletion. The gene encoding catechol-*O*-methyltransferase (COMT), an enzyme involved in the catabolism of catecholamine neurotransmitters, maps to the VCFS region and is therefore an obvious candidate for influencing the expression of psychosis in VCFS probands. This gene exists in two allelic forms encoding high- and low-activity isoforms of the enzyme, and it has been suggested that possession of the allele for low-activity COMT may be associated with the occurrence of schizophrenia in VCFS (160). However, Murphy and colleagues (149) found no evidence for an association between the allele for low-activity COMT and either schizophrenia or schizotypy in patients with VCFS.

FUTURE DIRECTIONS

Refining the Phenotype for Molecular Genetic Studies

The effectiveness of molecular genetic studies depends on the genetic validity of the phenotypes studied. Perhaps if we were better at defining phenotypes, we would be better at finding genes. It is worth reiterating at this point that the commonly used diagnostic criteria define phenotypes with high heritability. In principle, therefore, it should be possible to identify the genes predisposing to schizophrenia, as defined by current diagnostic criteria, if sufficiently large samples are studied. However, perhaps genetic validity could be improved by focusing on aspects of clinical variation, such as age at onset or symptom profiles, or by identifying biological markers that predict degree of genetic risk or define more homogeneous subgroups. Unfortunately, despite much work, it has not been possible to identify genetically distinct subtypes of schizophrenia. Instead, clinical variation is likely to reflect at least in part a combination of quantitative variation in genetic risk for the disorder and the effect of modifying genes that influence illness expression rather than the risk for illness *per se.* Examples of this phenomenon are probably age at onset and symptom pattern in schizophrenia (161,162).

The search for trait markers aims to move genetic studies beyond the clinical syndrome by identifying indices of genetic risk that can be measured in asymptomatic persons or by identifying markers of pathophysiologic processes that are closer to the primary effects of susceptibility genes than are clinical symptoms—so-called intermediate phenotypes or endophenotypes. Work in this area is developing fast; for example, candidate trait markers for schizophrenia include schizotypal personality traits, measures of cognitive processing, brain evoked potentials, and abnormalities in eye movements (163). It is also hoped that advances in brain imaging will lead to the identification of genetically valid trait markers. However, it seems unlikely that these phenotypes will provide a rapid solution to the problem. First, we will need to ensure that the measures used are stable and determine the extent to which they are affected by state. Second, to be of use in gene mapping, such measures will have to be practicably applied to a sufficient number of families or unrelated patients. Third, we will need to ensure that the traits identified are highly heritable, which will itself require a return to classic genetic epidemiology and model fitting. Finally, we cannot assume that the genetic architecture of such intermediate phenotypes will be simple.

Efforts to improve the selection of phenotypes are also concerned with enhancing the traditional categoric approach to defining psychiatric disorders by identifying genetically valid phenotypes that can be measured quantitatively. These can be used in quantitative trait locus approaches to gene mapping. Work in this area has begun but still faces problems, particularly those relating to the confounding influence of state-related effects. Perhaps the best hope of taking account of the complexity and heterogeneity of the schizophrenia phenotype comes from new methods of analysis in which aspects of the phenotype can be entered as covariates in linkage analyses (164).

Genome-wide Association Studies

In recent years, interest has increased in the possibility of systematic, genome-wide association studies (109,165). These have the potential of allowing systematic searches for genes of small effect in polygenic disorders. Optimism has been fueled by the fact that the most abundant form of genetic variation, the single-nucleotide polymorphism, is usually bi-allelic and potentially amenable to binary, high-throughput genotyping assays such as microarrays (so-called DNA chips). Moreover, as sequence data accumulate, it has become possible to contemplate the construction and application of very dense maps of hundreds of thousands of single-nucleotide polymorphisms (165).

Essentially two types of genome-wide association study have been proposed: direct and indirect. In the former, association is sought between a disease and a comprehensive catalogue of every variant that can alter the structure, function, or expression of every single gene. In contrast, indirect studies seek associations between markers and disease that are caused by linkage disequilibrium between the markers and susceptibility variants. The hope is that if sufficiently dense marker maps can be applied, it will be possible to screen the whole genome systematically for evidence of link-

age disequilibrium without actually having to screen every functional single-nucleotide polymorphism in the genome. However, a number of uncertainties and difficulties remain. These include, in particular, the difficulty of identifying functional single-nucleotide polymorphisms in regulatory rather than coding regions of the genome, uncertainty about the distances over which linkage disequilibrium is maintained, and the lack at the present time of a rapid, accurate, and cheap method for single-nucleotide genotyping (165).

Given these considerations, it seems clear that the era of genome-wide association studies, direct or indirect, is not yet at hand. Instead, studies in the next few years should probably focus mainly on the direct approach utilizing single-nucleotide polymorphisms from the coding sequence that actually alter protein structure in a wide range of functional and positional candidate genes. Preferably, complete functional systems should be dissected by the application of sensitive methods for mutation detection, followed by association studies in appropriately sized samples. We should also use our knowledge of functional pathways to make predictions about likely epistasis. However, given our ignorance of pathophysiology, the expectation should be that most reported associations will be false and resolved only by replication in large, well-characterized samples. At present, although the indirect approach is not widely applicable at a genome-wide level, smaller-scale studies focusing on specific regions indicated by the results of linkage studies may allow us to map loci. Additionally, such studies will generate the sort of data concerning patterns of linkage disequilibrium in typical "association samples" that will be required to determine whether genome-wide studies are likely to be feasible and what density of map will be required.

If the results are encouraging—that is, if linkage disequilibrium exists across useful distances in the genome—then until genotyping technology has sufficient capacity to permit mass genotyping at low cost, linkage disequilibrium analyses at the genome-wide level are most likely to be based on DNA pooling technologies (166–169).

Identifying Modifying Genes

It is sometimes assumed that variation in the clinical features of a disease or treatment response simply reflects pleiotropic effects of etiologic risk factors. However, it is becoming increasingly apparent that specific genes probably exist that influence clinical features and treatment response independent of those affecting liability. Thus, another potentially fruitful line of inquiry may be to design studies aimed at seeking modifying rather than causative genes, as these genes may in themselves allow novel drug targets to be identified. Evidence has already been found that variations in age at onset and symptom pattern in schizophrenia are probably caused at least in part by modifying genes (161,162). Furthermore, interest is increasing in pharmacogenetics in psychiatry (170), although with some trait markers, we need to exercise caution in the absence of genetic epidemiologic evidence that variations in drug response are under genetic control. We should also not forget that there is no *a priori* reason why responses to behavioral and psychological treatments should be less influenced by genetic factors than by pharmacologic treatments.

Animal Models

Another important challenge will be the development of suitable animal models to allow functional studies of putative disease loci (165). Disorders that predominantly involve higher cognitive function, such as schizophrenia, are likely to prove difficult to model in animals. However, certain features of the human phenotype, such as subtle abnormalities of cell migration, enlarged cerebral ventricles, and abnormalities of information processing, including defects in prepulse inhibition, can be detected in animals (171). In fact, a possible approach to producing a mouse model for at least some of the schizophrenia phenotype is suggested by the finding of an increased prevalence of schizophrenia in VCFS. Currently, attempts are under way to produce transgenic mice in which the syntenic region of mouse chromosome 16 is deleted (172). These animals should be investigated closely for neuroanatomic and behavioral phenotypes of possible relevance to schizophrenia, and such studies are already yielding encouraging findings (173).

Functional Studies

The most important and most obvious implication of identifying genetic risk factors for schizophrenia is that it will inspire a new wave of neurobiological studies from which, it is hoped, new and more effective therapies will emerge. However, although the unequivocal identification of associated genetic variants will represent a great advance, many years of work will be required before this is likely to translate to routine clinical practice. An early problem will be to determine exactly which genetic variant among several in linkage disequilibrium within a given gene is actually responsible for the functional variation. Even when a specific variant within a gene can be identified as the one of functional importance, functional analysis, in terms of effect at the level of the organism, is likely to be particularly difficult for behavioral phenotypes in the absence of animal models. An extra level of complexity is that we will need to be able to produce model systems, both *in vivo* and *in vitro,* that allow gene–gene and gene–environment interaction to be studied.

Genetic Nosology

Although the development of new therapies will take time, it is likely that the identification of susceptibility genes will

have an earlier effect on psychiatric nosology. If genetic risk factors are correlated with clinical symptoms and syndromes, it should be possible to study heterogeneity and comorbidity to improve the diagnosis and classification of psychosis. The prospects will also be enhanced for identifying clinically useful biological markers as an aid to diagnosis, so that we can move beyond the current situation of making diagnoses based entirely on clinical signs and symptoms. Improvements in diagnostic validity will clearly facilitate all avenues of research into these disorders. However, in the present context, we should point out that improvements in diagnosis and classification should enhance our ability to detect further genetic and environmental risk factors, so that a positive feedback between nosology, epidemiology, and molecular genetics can be envisaged.

Molecular Epidemiology

The identification of genetic risk factors can be expected to provide a new impetus to epidemiologic studies of schizophrenia by allowing researchers to investigate the ways in which genes and environment interact. Studies of this kind will require large, epidemiologically based samples together with the collection of relevant environmental data. This work could start now with DNA being banked for future use, although in schizophrenia, the identification of plausible environmental measures might require clues from the nature of the genetic risk factors yet to be identified. A major theme in relation to this work will be the bringing together of methodologies from genetics and epidemiology, which have traditionally adopted somewhat differing analytic approaches (174). Treating susceptibility alleles as risk factors in an epidemiologic context will allow estimates of effect sizes within a population to be made. Accounting for specific genetic effects will also facilitate the search for independent environmental factors and the investigation of potential gene–environment interactions. Scientific validity is likely to be enhanced by ensuring as far as possible that control samples are drawn from the same base population as patients. In addition, the use of incident cases should guard against the risk of identifying loci related to confounds, such as chronicity of illness, rather than susceptibility. Phenotypic assessment is likely to benefit from a prospective element to studies, which counteracts the tendency of patients to forget historical details and the difficulty of making observed ratings retrospectively from case records. However, the price of improved scientific rigor is likely to be considerably more expensive studies because of the longer period and larger number of investigators that will be required to ascertain detailed data from thousands of subjects.

Genetic Testing

A further implication concerns genetic testing. This is a complex area that raises a number of ethical issues, which have been discussed elsewhere (175). However, the potential for predictive testing has probably been overstated, given that susceptibility to schizophrenia almost certainly depends on the combined effects of predisposing and protective alleles at a number of loci and their interaction with the environment. Consequently, until we have a comprehensive molecular understanding of the etiology of schizophrenia, the predictive value of genetic testing is likely to be low. This applies, for example, to apolipoprotein E testing for late-onset Alzheimer disease, and has led to a recommendation that such testing not be performed in asymptomatic persons (175). Indeed, even when all the susceptibility genes for schizophrenia have been identified, it will still not be possible to predict the development of disease with certainty until the relevant genetic and environmental risk and modifying factors have also been identified and the nature of the various interactions understood. Such interactions may be complex and unpredictable (165). However, other possible roles of genetic testing are likely to be of greater value to patients and clinicians. For example, it might be possible to optimize treatment choices by testing genes found to influence treatment responses in psychiatric disorders and so provide more individualized treatment.

CONCLUSIONS

Attempts to identify the genes that predispose to schizophrenia face formidable challenges arising from both genetic and phenotypic complexity. Research to date has largely excluded the possibility that genes of major effect exist even in a subset of families. Evidence has been obtained of the location of some genes of moderate effect, but none of these findings can be regarded as conclusive, and proof in each case will probably have to await identification of the susceptibility locus itself. The clearest molecular genetic risk factor for schizophrenia that has been identified to date is deletion of a gene or several genes on chromosome 22, which can markedly increase the risk for schizophrenia. However, fairly strong data suggest that allelic variation in genes encoding the 5-HT_{2A} and D3 dopamine receptors confer a small degree of susceptibility.

As in other common diseases, it is hoped that advances will come through the use of a new generation of genetic markers and new methods of genotyping and statistical analysis (165). However, successful application of these methods requires access to large, well-characterized patient samples, and the collection of such data is a priority at the present time. We need to focus research on the development and refinement of phenotypic measures and biological markers. Success will also depend on the traditional medical disciplines of clinical description and epidemiology, and on our ability to integrate these with genetic approaches.

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