Prospects for the Genetic Analysis of Schizophrenia

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Abstract

This issue of the Schizophrenia Bulletin provides a forum for the presentation of early results and speculative hypotheses based on the application of molecular genetic methods for linkage studies in schizophrenia. Contributors were given the freedom to explore the historical and theoretical perspectives on the genetics of schizophrenia. They were also asked to balance their enthusiasm and cautious skepticism at the new suggestions of linkage involving chromosome 5 (Sherrington et al. 1988). In this overview, the epidemiologic evidence for a genetic factor in schizophrenia and recent linkage studies are briefly discussed. In addition, the potential and limitations of different linkage strategies in schizophrenia are examined.

A number of different factors figure in the analysis of genetic linkage in schizophrenia. The diagnosis of schizophrenia and its relationship to a valid phenotype are clearly primary. This issue is discussed in light of the compelling epidemiologic evidence for the role of genetic factors in schizophrenia. The present enthusiasm for pursuing genetic linkage methods in schizophrenia reflects the potential significance of this new technology. The vocabulary used to describe many aspects of this work is new to most clinicians and to many researchers in the field. For this reason, a glossary of terms (table 1) is presented to be used as a starting point in understanding both the methods and the results of the studies being described and discussed in this issue. Finally, an overview of the different contributions to this issue summarizes both important recent findings and some of the theoretical issues currently being studied.

The diagnosis of schizophrenia is based on the presence of characteristic psychotic symptoms which have endured for 6 months (DSM-III-R; American Psychiatric Association 1987). Different diagnostic systems have provided both narrower and broader definitions of schizophrenia than DSM-III-R, but none necessarily defines a distinct homogeneous disorder. Schizophrenia is a clinical syndrome with no pathognomonic feature that allows the diagnosis to be made with 100 percent certainty, and the central feature of the illness—psychosis—can be present in a number of disorders. However, systematic and objective strategies for the diagnosis of schizophrenia have greatly improved the reliability of the diagnosis over the last 30 years. Agreement on the diagnosis between examiners, given the presence of specific symptoms, can be over 90 percent as exemplified by the Flexible System devised by Carpenter et al. (1973). In addition, the potential role of spectrum disorders in schizophrenia research has become more apparent (Siever and Gunderson 1979). Objective approaches to personality disorders have improved greatly in the last few years, thus enabling researchers to assess family members more reliably.

The ability to analyze and compare a number of different studies of schizophrenia depends largely on the ability to communicate objec-

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Table 1. Glossary

**Allele**—One of several alternative forms of a gene.

**Autosome**—A nonsex chromosome; there are 22 homologous pairs of autosomes in humans. In individuals, two sex chromosomes form the 23rd pair.

**Centimorgan**—The genetic distance in which the probability of a recombination occurring is 1 percent.

**Chromosome**—A rodlike structure present in the cell nucleus containing genes. The chromosome is made up of a long double helix of DNA and associated proteins.

**Cytogenetics**—The light microscopic study of the structure of chromosomes.

**Dominant**—A phenotype caused by one allele is said to be dominant with respect to a phenotype caused by a second allele if an individual carrying both alleles shows the former (not the latter) phenotype.

**Expressivity**—The extent to which a given phenotype is manifest in an individual.

**Gene**—An inherited Mendelian factor transmitted from parent to offspring.

**Genome**—A complete set of chromosomes considered as genes.

**Genotype**—An individual’s genetic composition.

**Karyotype**—A complete set of chromosomes considered from a cytogenetic point of view.

**Linkage**—The tendency of two alleles at different loci on the same chromosome to be inherited together. The greater the physical proximity, the smaller the probability of genetic recombination occurring between them and therefore the greater the probability they will be coinherited.

**Locus**—A position on a chromosome occupied by a gene or marker.

**Lod score**—The log to the base 10 of the probability that a given set of data about genetic recombination arises by virtue of two loci being linked at a specified recombination fraction divided by the probability that the data would arise by nonlinkage.

**Mode of inheritance**—The pattern of inheritance (e.g., dominant or recessive) of a particular allele.

**Penetrance**—The proportion of individuals with a given genotype that actually manifest a particular phenotype.

**Phenocopy**—An individual exhibiting a trait due to nongeneric factors.

**Phenotype**—An observable trait.

**Recessive**—Opposite of dominant.

**Recombination**—The process by which a pair of homologous chromosomes physically exchanges sections yielding a new combination of genes.

**RFLP**—Restriction fragment length polymorphism: When a restriction enzyme is used to digest DNA, chromosomal regions are cut into fragments of characteristic lengths. Some of these fragment lengths are variable in the population. Loci where this is the case are RFLPs; they provide convenient markers for linkage studies.

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tively the characteristics of the subjects and families being studied. Even when the diagnosis of schizophrenia follows systematic criteria, it may not completely describe a specific and unique phenotype. There is significant evidence for a range of possible phenotypes (clinical presentations) even within a single large family (Pato et al. 1989). This single pedigree contained at least 11 members with chronic psychosis; of these, the majority were diagnosed with chronic schizophrenia, but several were classified as having schizoaffective disorder according to the criteria in DSM–III–R. At least four family members were diagnosed with major depression without psychotic features, and one was diagnosed with manic-depressive illness. DeLisi et al. (1987) examined siblings for symptom concordance within pairs and were unable to demonstrate a significant correlation. The recent claim of linkage to chromosome 5 in seven Icelandic and English families (Sherrington et al. 1988) is also consistent with the theory of a range of phenotypes, since the systematic broadening of the diagnostic scheme for inclusion as an affected
member led to an increased lod score (log to the base 10 of the odds ratio), which strengthened the linkage finding in these families. These scores are by no means definitive evidence, but they suggest the possibility of variable expression leading to a range of phenotypes.

The Genetic Hypothesis

A genetic factor in the etiology of schizophrenia has long been suspected. Some of the studies that have supported a genetic association based on the strong family aggregation data for schizophrenia will be briefly summarized. For example, Heston (1966) studied children of chronic schizophrenic mothers who had been removed from their mothers within 3 days of birth and were brought up in foster homes. These children were compared to a matched control group whose parents had no history of psychosis or psychiatric illness and who had also been given up for adoption. The children of schizophrenic mothers were found to have an age-corrected risk of 16 percent compared to 0 percent for those from the control group. Kety et al. (1968) used an alternate strategy and identified adopted children who developed schizophrenia and studied the prevalence of schizophrenia in their biological and adoptive parents in an attempt to identify the relative contributions of genes and environment. Their results indicate as much as a threefold higher risk for schizophrenia in the biological relatives than in the adoptive relatives (Kety et al. 1968).

If there is no “genetic predisposition” to schizophrenia, the concordance rate in monozygotic twins (who share all their genes) should be no higher than the concordance rate in dizygotic twins (who share only half their genes). Gottesman and Shields (1982) reviewed the results of 11 studies of adult twins representing data on 1,300 pairs of same-sex twins. The concordance rate in monozygotic twins is approximately 50 percent compared with a rate of 12–14 percent in dizygotic twins (Gottesman and Shields 1982; McGue and Gottesman 1989). The 50 percent concordance rate in monozygotic twins supports the role of genetic factors, while the 50 percent rate of discordance in monozygotic twins shows the crucial importance of nongenetic factors—such as environment and development. Discordant monozygotic twins provide a special population. They may allow the study of environmental factors and their role in the etiology of schizophrenia. Torrey and coworkers are currently studying a population of discordant monozygotic twins focusing on the characterization of the twin pairs (Suddath et al. 1989; Torrey et al. 1989). Discordant twins also provide an opportunity to investigate genetic factors: an unaffected twin should transmit the same genetic risk of schizophrenia as his affected sib. Fischer (1971) and McGue and Gottesman (1989) explored this question and found the risk to the child of a non-schizophrenic twin to be equal to that of the children of the schizophrenic twin.

Gottesman and Shields (1982) summarized the results of a composite series from several different family studies. They cautioned the reader that diagnosis was rarely if ever done blindly, and that the investigators were usually aware of relatives already carrying the diagnosis. With this in mind, it still appears that the closer the genetic proximity to a schizophrenic relative, the higher the morbid risk. There is a large body of evidence consistent with a significant heritable liability to schizophrenia (Gottesman and Shields 1982; Kendler 1987). However, the mode of transmission of this liability remains unclear. The combination of new analytic methods (i.e., multipoint linkage—where one assesses the coinheritance of a group of markers that are linked to each other and a disease locus [Diehl and Kendler 1989], and a simultaneous search of multiple regions of the genome) and molecular genetic techniques may be sufficient to resolve the difficulties in defining the mode or modes of inheritance of schizophrenia and its specific biological characteristics (Gershon et al. 1987; Kendler 1987; Eaves et al. 1988).

Linkage Strategies

The advent of molecular genetic linkage techniques has provided a new strategy for studying the genetics of complex familial disorders like schizophrenia. In the past, few markers were available to study linkage; however, as first suggested by Botstein et al. (1980), common variations in DNA sequence provide a limitless supply of genetic markers for studying inheritance. Such variations in DNA sequence can be easily detected when they disrupt a recognition site for a restriction enzyme giving rise to restriction fragment length polymorphisms (RFLPs); over 3,000 such RFLPs have been identified to date. In the last few years many fields of medicine have embraced this new technology to try to understand the genetic contributions to illnesses such as
Huntington's disease (Gusella et al. 1983), muscular dystrophy (Hoffman et al. 1988). The efforts of clinical researchers have been complemented by the laboratory research that has steadily progressed in defining an increasingly detailed RFLP map of the human genome (White 1986; Donis-Keller et al. 1987). The potential for using these methods to understand schizophrenia is one of the most promising avenues open to us at this time.

Despite the great promise it holds, the program of searching for genetic linkage in schizophrenia is fraught with potential problems.

The most serious issue is defining a phenotype that corresponds to the actual underlying genetic entity. If too broad a definition of a phenotype is used (effectively lumping schizophrenia together with other conditions), then it will prove impossible to find a locus that cosegregates with the phenotype. If too narrow a definition is used (effectively excluding true cases), then it will prove much more difficult to locate a locus. The latter case is somewhat less serious than the former, because individuals who inherit the disease-predisposing form of the disease but fail to manifest the disease can be treated as cases of incomplete penetrance. Such cases will not absolutely prevent the finding of linkage, provided that linkage analysis allows for a substantial degree of incomplete penetrance. However, the failure to exploit information from such cases will greatly increase the difficulty of gathering enough evidence to draw a statistically significant conclusion.

The second serious problem is the likely genetic heterogeneity of schizophrenia. A disease is said to be genetically heterogeneous if it can be caused by mutations at any one of several loci. The obvious difficulty with mapping a heterogeneous disease is that it may show linkage to one locus in one family and to another locus in a different family. Thus, evidence for linkage in one family is offset by evidence against linkage at that point in another family. Possible strategies for overcoming genetic heterogeneity include: (1) studying large families with a sufficient number of affected individuals to reach a statistically significant conclusion with a single family (a good approach in principle but rarely practical in schizophrenia without resorting to an overly broad definition of the disease); (2) studying a collection of families in a genetically isolated population (with the hope that the population has a homogeneous genetic etiology); (3) applying analytical techniques, such as a simultaneous search of multiple regions of the genome, to find a collection of loci which together account for most of the inheritance of the disease; and (4), characterizing the patient population according to some biological phenotype (with the hope that the biological distinction corresponds to a genetic distinction and thereby produces subpopulations which are genetically more homogeneous).

The efforts to map schizophrenia are not premised on the notion that all, or most, cases have a common etiology. Indeed, mapping studies at this early stage are best directed at finding those situations, suspected to be rare, in which simple Mendelian single gene inheritance is most likely. Only after such loci are found does it become appropriate to assess their contribution to the overall etiology of schizophrenia.

In short, genetic linkage analysis of schizophrenia is an exciting challenge which the field has greeted with enthusiasm. It currently offers the best hope for elucidating the molecular basis for schizophrenia, and it therefore merits vigorous pursuit over the next decade, even if success is not certain.

Overview

This issue of the Schizophrenia Bulletin provides a forum for the presentation of early results and speculative hypotheses based on the application of molecular genetic methods for linkage studies in schizophrenia. Contributors have been asked to explore the historical and theoretical perspectives while sharing a mixture of enthusiasm and cautious skepticism at the new suggestions of linkage involving chromosome 5 (Sherrington et al. 1988).

Bassett identified an oriental man and his nephew, both with the diagnosis of schizophrenia, as well as some facial dysmorphism and other abnormalities. Such a combination of features is suggestive of a chromosomal abnormality (Bassett et al. 1988; Bassett 1989). High resolution karyotyping identified a partial trisomy of the q11–q13 region of chromosome 5 in both patients. This finding led workers to focus their linkage studies on this region. In this issue, Bassett describes these findings at some length and their influence on the direction of recent linkage studies. In this expansion of her earlier report, special attention is paid to the ethical issues involved in informed consent for these studies and the need to maintain con-
fidelity. This study is an example of how cytogenetic screening may prove useful in identifying other candidate regions for study. DeLisi et al. (1988) screened 20 patients with schizophrenia, despite finding no abnormalities in this small group. The ramifications of linkage findings to specific family members who might be carriers are also discussed (Bassett 1989).

Recently, Sherrington et al. reported genetic linkage of schizophrenia to two loci (D5S39 and D5S76) on the proximal long arm of chromosome 5 in studies of seven families (five Icelandic and two English) with multiple affected members diagnosed as schizophrenic (Sherrington et al. 1988; Gurling et al. 1989). These reports are the first to demonstrate a specific genetic linkage to schizophrenia in more than one family using molecular genetic techniques. An interesting feature of the Sherrington study is the linkage strategy employed. Starting with a strict definition of schizophrenia and sequentially broadening the diagnosis to include spectrum disorders (schizoid and schizotypal personality) and, finally, "fringe" disorders led to an increasing lod score to a maximum of over 7.0. This result is taken as an indication that the nonschizophrenic diagnoses are possibly the result of a variable expression of a schizophrenia susceptibility locus (Gurling et al. 1989). The statistical effect of performing multiple analyses of this type is not clear and needs to be further studied. It should be noted that there is no proof yet that the locus implicated by Sherrington et al. actually maps within the trisomic region identified by Bassett. Until this has been demonstrated, it remains possible that there is no connection between the two findings. If these two findings are unrelated, they would represent two independent forms of schizophrenia. However, if the same genetic liability is present in the families studied by Sherrington and coworkers and in the oriental family studied by Bassett, this susceptibility locus might be more generalizable to the rest of the population with schizophrenia.

Kennedy and colleagues have examined the same region of chromosome 5. Focusing on one large Swedish pedigree with 31 schizophrenic members, they have tested linkage to seven loci (D5S76, D5S56, D5S39, D5S78, D5S21, HEXB, DHFR). Their work excludes linkage in a 56 centimorgan region of chromosome 5 (Kennedy et al. 1988, 1989). Given the high prevalence of schizophrenia within this one family, one suspects a genetic etiology. Therefore, this suggests that more than one genetic factor can present as a schizophrenic phenotype. It should be noted that significant diagnostic differences may exist between the Swedish pedigree studied by Kennedy (focusing on identified family members suspected to have schizophrenia) and those studied by Sherrington (designed to characterize as many members of the pedigrees as possible for a variety of diagnoses).

Kaufmann et al. (1989) describe the rationale for using cytogenetic abnormalities, such as the trisomy discovered by Bassett, as potential regions of interest for linkage analysis. They also point out the need to define the actual physical location of a susceptibility locus for schizophrenia. They present data defining the physical map positions of several markers that are either physically within the trisomic area described by Bassett or in close proximity to it. Four American families with schizophrenia were examined for linkage to this same region. Linkage was excluded to a narrow area in the same region of chromosome 5, and no evidence for linkage to the rest of the region was found (Kaufmann et al. 1989). This work, along with the work by Kennedy et al. (1989), provides a more detailed linkage map of the region. It can significantly increase the informativeness of affected individuals within a pedigree, and facilitate the linkage analysis to this region.

DeLisi and Crow (1989) examine evidence implicating the sex chromosomes in the transmission of schizophrenia. A number of potentially associated regions of the X chromosome are suggested and contrasted to the alternative concept that psychotic illness is part of a continuum ranging from affective illness to schizophrenia and implicating a single genetic locus for both illnesses. These and other preliminary reports serve to underscore the possibility that schizophrenia is genetically heterogeneous.

Genetic heterogeneity is one possible explanation for a lifetime risk of schizophrenia as high as 1 percent in the general population. Mental retardation can serve as a model of a disorder with multiple genetic forms and many nongenetic forms. In just such an analysis, Gottesman and Shields (1982) estimate that the prevalence of mental retardation is approximately 3 percent of the population, and that as many as 300 rare genetic disorders can cause mental retardation. They point out that these 300 different genetic disorders actually account for only a small portion of the total
incidence of mental retardation. This example highlights the difficulty of testing genetic linkage in a heterogeneous disorder. Garver et al. (1989) discuss some of the approaches to probable heterogeneity, including admixture analyses and the use of external measures to define genetically informative subtypes of schizophrenia for linkage analysis. The work done by Gurling et al. (1989) demonstrates the potential increased power of including spectrum disorder cases as affected. However, the approaches to including these broader definitions in a linkage analysis are not fully tested (Diehl and Kendler 1989; Garver et al. 1989). No common strategy has been accepted. The Gurling model illustrates a strategy that starts with a narrow definition of schizophrenia and systematically broadens the diagnosis; an alternate model might start with a broad diagnosis and systematically narrow the definition of affected. The statistical validity of either of these strategies must be tested.

Approaches that would either broaden or subtype the syndrome must be used cautiously to generate new and testable hypotheses.

The potential for genetic mapping is discussed by Garver et al. (1989) using the example of work done by Kunkle and coworkers on Duchenne's and Becker's muscular dystrophy (Hoffman et al. 1988). These two clinical entities have very similar clinical presentations, with the exception of the severity and time course of the illness. They are easily diagnosed and have similar patterns of inheritance. Finding linkage to an Xp21 locus in both disorders led to the identification of the protein dystrophin, the product of the associated gene. The pathophysiology of these disorders is now much more readily understood. Each of these disorders arises from separate mutations in the same gene. This accounts for both the similarities and the differences that are clinically observed. Similarly, one may hope that such genetic distinctions may help define subtypes within the schizophrenia spectrum once enough affected pedigrees have been studied.

McGue and Gottesman (1989, p. 460) point out that despite sophisticated attempts using segregation analysis, "Nobody has ever been able to demonstrate statistically that a single major gene accounts for a large share of the overall risk for schizophrenia." In this issue, they discuss the limitations of a single major gene model for schizophrenia. In outlining the characteristics of a single gene model, it becomes clear that one needs to explain deviation from a Mendelian pattern of inheritance. If one accepts reduced penetrance and the existence of phenocopies, more complex patterns of inheritance are predicted (McGue and Gottesman 1989). These need not be unreasonable assumptions and are not the only potential contributions to the apparent deviations from a typical pattern of inheritance. Variable expression could lead to the failure to recognize certain forms of a phenotype and also obscure a relatively typical pattern of inheritance. However, the deviations from a Mendelian pattern could also be explained by a polygenic or a multifactorial model.

The multifactorial threshold model for schizophrenia (Gottesman and Shields 1967; McGue and Gottesman 1989) assumes polygenic factors combining with nongenetic factors until a certain threshold is reached. If this threshold is passed, the individual develops schizophrenia. The authors contend that this type of model fits the empirical data more closely than a single major gene model; their simulations of risk to relatives under a multifactorial threshold model result in an exponentially decreasing risk with increasing genetic distance from the affected relative. If this type of model is accurate, then current linkage studies will not be likely to find linkage in most families. Of course, this does not imply that a single major gene cannot account for a particular subtype of schizophrenia.

Diehl and Kendler (1989) review the potential benefits of using epidemiologic and molecular genetic strategies in parallel. In their own work, Diehl and Kendler start with a family study of all cases of schizophrenia in a specific population sample in Ireland. This study is being done in parallel with a study designed to identify high-density families through the county psychiatric hospitals. The authors hope that the concurrent case-controlled epidemiologic family study will help define the nature of the schizophrenia phenotype in this population. The use of segregation analysis to model the inheritance pattern present in the population-based study might clarify what definition of a schizophrenia phenotype (i.e., inclusion or exclusion of spectrum disorders) best fits a typical pattern of inheritance. This would facilitate linkage studies performed on the high-incidence families identified from the same population. One potential limitation to this method is that the different ascertainment methods in the high-incidence and the population-based studies may identify different phenotypes (Diehl and Kendler 1989).

Molecular genetics offers exciting new tools for research on the causes of schizophrenia. In this
issue, Gurling et al. (1989) provide a detailed review of the first reported linkage to schizophrenia implicating a susceptibility locus on chromosome 5. In contrast, several reports of failure to find linkage to chromosome 5 in other families with schizophrenia are also presented (Kaufmann et al. 1989; Kennedy et al. 1989). This issue brings together a number of major investigators in an attempt to provide some understanding of the implications of such findings. Different approaches are discussed and several potential problems are highlighted. Even if none of this work proves that a basic genetic cause is present in all, or most, cases of schizophrenia, there is clearly much to be excited about in the study of the genetics of schizophrenia. No other technique offers so much potential for defining the basic pathophysiology of schizophrenia. Even if schizophrenia is actually a heterogeneous disorder, these tools may provide some understanding of physiological defects or pathways that may be involved even in non-genetic cases of schizophrenia.

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