Methodological Issues Facing the Interpretation of High-Risk Studies: Biological Heterogeneity

by David L. Garver

Abstract

High-risk studies provide a unique opportunity to examine the interaction of biological vulnerability and psychosocial factors in the development of the major psychiatric illnesses. To parcel out the effects of psychosocial variables, high-risk populations need to be separated into offspring who are and who are not biologically/genetically vulnerable, since psychosocial modifiers will be interactive only in the biologically vulnerable offspring. Criteria are suggested for the use of "biological markers" to detect subjects at genetic/biological risk within high-risk cohorts. Presently, only the d' statistic of the continuous performance test (CPT) appears to satisfy the criteria for identification of such biologically vulnerable offspring.

One of the most exciting areas of psychiatric research is the interaction between biological and psychosocial risk factors in the development of the major psychiatric illnesses. High-risk studies are positioned at this important interface: such studies use populations at high risk for the development of major psychiatric disorder. Most such studies have defined a high-risk population on the basis of major psychiatric illness in a parent. The offspring of such affected parents have a considerably higher frequency of disturbance than does the general population. Neurointegrative and psychosocial parameters have been closely monitored during development and into the period of risk for psychotic decompensation. Results from the first generation of high-risk studies are coming in: the high-risk populations are now entering the age of risk. One major methodological issue has not yet been squarely addressed, however, and failure to address it will result in a profound loss of discriminative power in the parceling out of both psychotogenic and protective risk factors in the high-risk populations. The problem is biological heterogeneity, which likely occurs in both parent and offspring samples. First, it is probable that there is ongoing genetic risk within such populations for several different psychotic disorders. Recent conceptualization of the "schizophrenic syndrome" suggests that schizophrenia is a heterogeneous disorder comprising several different biological processes, which share only a final "common pathway" of delusions, hallucinations, and disorders of cognition. If several different psychotic disorders make up the "group of the schizophrenias," each may behave differently in the context of other risk factors that are being observed and quantified in the present high-risk studies.

Second, it is quite possible that some high-risk study families contain psychotic subjects who do not have a genetic illness but who have been made vulnerable to psychosis by central nervous system (CNS) trauma, by viral infections of the CNS, and so on. Again, it would not be surprising if such psychotic illnesses behaved differently under the conditions of the other risk factors that are being studied in the present populations.

Third, the offspring—the focus of most high-risk studies—are heterogeneous at yet another level. Miosis of the germ-cell line separates DNA and distributes it differently to the...
offspring. The results are different genetic constitutions (different biological risks). In the context of a single-locus model, the risk of genetic/biological transmission is an all or nothing phenomenon, and there is a 50 percent chance of the offspri

The data from high-risk studies have the power to demonstrate that pathological development and outcome occur in the offspring of pathological parents and that pathological family interactions occur in families with pathological parents and are associated with poor outcome in offspring. More important, such data can define specific risk factors for such pathological development and outcome; alternatively, they can identify protective factors that will interfere with such pathological development and outcome in biologically predisposed offspring. But before we can use this potential to parcel out risk factors, we must define who is at risk (who is biologically predisposed for the psychosis) and what the risk is for (for what biological variety of psychosis).

Some of the critical issues can be brought into focus by an example. If a nonpsychotic sibling (of a psychotic proband) is not genetically/biologically predisposed to develop psychosis (does not have "schizophrenic DNA"), the pursuit of other protective factors that have prevented the development of psychosis will lead us astray, since the sibling is not at risk. And unless the absence of biological risk is recognized, such a case will contaminate the analysis with unwanted variance.

The critical point is that the first parceling out in high-risk studies has to be that of the presence or absence of genetic/biological risk in each individual subject. Without separating subjects who are at biological risk from those who are not, the precision and power of further risk analyses are lost.

As an aside, it is vital to study not only a single child within a high-risk family but siblings as well. It is in comparisons of siblings that the critical analysis of other risk factors for various types of psychopathology can take place. For example, pathological effects on offspring occasioned by pathological family interactions independent of biological risk in the probands can be separated from the pathological effects of pathological family interaction that interact with biological predisposition for psychosis.

The question is, how are the subjects with genetic/biological predispositions to be identified? Several of the articles in this issue of the Schizophrenia Bulletin have referred wistfully to the process of chromosomal identification of subjects (both parents and offspring) who are at biological risk for a psychotic disorder. Molecular geneticists may not be too many years away from the use of oligonucleotide probes through which abnormal DNA sequences can be detected—sequences that predispose and underlie one of several psychotic disorders. With such a DNA probe, the high-risk investigator can get an immediate answer to biological risk for a particular psychosis in each subject, and can then parcel out the other factors related to increased risk or prevention of psychosis. At present, such techniques only enrich the fantasy life of the investigator of high-risk populations, although they may be possible within the next 5–25 years. Until such tools are available, what alternatives might be used? What other "biological markers" might allow us to move ahead with analyses of data, permitting us to parcel out the risk contributions of genetic/biological versus social/interactional factors?

For a "marker" to be sufficient to the task, several criteria must be met (table 1). First, it is important that the distribution of the marker in psychotic populations differ from distribution in normal populations. Here it might not be unusual for the distribution of the marker to be unimodally distributed within normal populations and bimodally distributed in psychotic populations. A deviant mode of distribution in a subset of a psychotic population could be the first step toward identifying a marker relevant to detecting an abnormality in that genetic/biological subset of the psychotic population. Second, the deviant distribution of the marker in a sub-

<table>
<thead>
<tr>
<th>Table 1. Criteria for a &quot;marker&quot; to detect genetic biological risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Distributed differently in psychotic than in control populations.</td>
</tr>
<tr>
<td>2. Is a stable trait rather than state-associated deviation.</td>
</tr>
<tr>
<td>3. Deviation also occurs with higher frequency in family members of identified deviant psychotics than in the general population, and is associated with psychotic spectrum disorder in family members.</td>
</tr>
<tr>
<td>4. Deviation also occurs at higher frequency in offspring of deviant psychotics before development of psychotic spectrum disease clinically.</td>
</tr>
<tr>
<td>5. Deviation in the offspring is associated with later development of psychotic spectrum disease.</td>
</tr>
<tr>
<td>6. Marker is relatively noninvasive and can be used by a variety of investigators with high reliability.</td>
</tr>
</tbody>
</table>
group of the psychotic population needs to remain stable despite fluctuations in symptoms; the deviant distribution is not simply a state-related phenomenon, but has the characteristics of a persistent trait abnormality. Third, deviant distributions must occur with greater frequency in biological family members of psychotic patients than in the general population, and must be associated with a higher prevalence of schizophrenia spectrum disorder than in nondeviant relatives. Such evidence supports genetic transmission of the marker. Fourth, the deviant distribution must appear with greater frequency in the as-yet-nonpsychotic offspring of a psychotic parent who has a deviant biological marker; this confirms that the abnormality is passed on to at least some of the offspring before they enter the period in which psychosis appears. Fifth, if parents have both psychotic spectrum disorder and the marker, offspring who have the same deviant marker should be at particularly high risk to develop psychotic spectrum disorders. Neither the psychotic parent nor the offspring (both of whom may have psychotic spectrum disorder) may have the particular marker being studied; each may have the marker for another type of psychotic spectrum disorder. Sixth, a relevant biological risk marker for use in high-risk studies must be relatively noninvasive and easily standardized across centers for reliability.

An example may help clarify these issues. Several investigators have reported abnormalities on the continuous performance test (CPT) and on the span of apprehension test, as well as noting neurointegrative deficits in both parents and off-spring of some families manifesting disorders of the psychotic spectrum. While it needs to be verified that each of these three “markers” is tapping into the same attention-processing disorder, for the sake of illustration let us assume that the same disease is being detected by each. First, the distribution, as shown by the span of apprehension test, has been described as unimodally distributed in normals but bimodally distributed in a population of psychotics. Only a subgroup of psychotic patients are positive for the abnormality. Second, the CPT d’ has been reported to be deviant both when the patient is frankly psychotic and during periods of partial recovery. Third and fourth, the frequency of CPT d’ deviance or neurointegrative defects has been reported to be increased in the family members of patients with similar deviance, and especially in the offspring of such CPT d’-deviant psychotic spectrum parents. Fifth, offspring who have the CPT d’ deviance or neurointegrative defects and are now entering the period of risk are frequently presenting psychotic spectrum disorders; the high-risk special population is becoming phenotypically psychotic spectrum. Sixth, the CPTd’ has now been standardized and is available for widespread use with the Apple II Computer. It has been suggested that this will make the determination of CPT d’ deviance reliable across centers under varying conditions, reliable for detecting deviance of the distribution and for detecting subjects who are genetically/biologically at high risk for a particular type of psychotic spectrum.

The group of psychotic spectrum patients who have deviant CPT d’ should not be called “schizophrenic”; a more relevant diagnostic statement might be “CPT d’-deviant psychotic spectrum disorder.” Having thus segregated subjects as “positive” or “negative” for the CPT d’ deviance, the investigator can examine data for other risk factors. The psychotic-spectrum-disordered families who do not have members deviant on the CPT d’ cannot contribute data to this analysis because they represent a different type of psychotic spectrum disorder, which may have a different interactive pattern with psychosocial risk factors. Thus, the analysis is not an analysis of “schizophrenia” but of CPT d’ psychotic spectrum disorder.

In the current absence of techniques to do sequence analysis to convincingly identify DNA abnormalities associated with various psychotic spectrum disorders, we need to look at what other biological or neurochemical markers might be effective in initially determining the presence or absence of genetic/biological risk in each subject. There might be enzyme-activity deviance associated with psychotic processes, deviant constituents or proportions of constituents of biological membranes, or deviation of ion transport systems associated with psychotic spectrum diseases. Such “biological markers” of deviance may reflect underlying DNA abnormalities. For each potential marker, we need to know whether it would meet the criteria to detect genetic/biological risk subjects.

Unfortunately, no neurochemical, receptor, compositional, or other “marker” currently meets the criteria listed in table 1. In part, this may reflect the fact that the markers have not yet been explored adequately in family members of psychotic patient populations. How could potentially useful biological markers be explored quickly? The speed of exploration is critical, because with the passage of time, the present at-risk populations will enter the period of risk, become psychotic, and be on various drugs that may alter the markers. Moreover, the needed
markers on parents will become increasingly unavailable because of parents' deaths. Furthermore, data analysis of the wealth of information from the present high-risk cohorts requires knowledge of who is at risk and who is not. The biological samples need be collected now, before both parents and offspring are lost to further study.

What type of samples need to be collected? First, there are certain biological markers that show promise of beginning to meet the criteria—although they have not yet been demonstrated to be genetically transmitted to offspring. It may be that such potential markers can be explored on a small subset of high-risk patients and their parents to determine whether they meet the criteria for use in wider-scale investigations. Deviance of brain size (computed tomographic or magnetic resonance imaging), electroencephalographic topographic imaging, and positron emission tomography (PET) might be used in select samples to determine their wider applicability. Similarly, studies of cerebrospinal fluid and plasma-amine metabolites or neurophysiological studies such as galvanic skin response could be investigated for possible use. Some such procedures are more than mildly invasive, a consideration that needs to be weighed carefully in determining their usefulness.

Second, probably the most important samples that need to be taken, which can be done relatively noninvasively, are DNA samples to be preserved for future analyses. Samples of DNA from white blood cells from each of the high-risk subjects, their siblings, and biological parents can be preserved for analysis years from now. Then, as oligonucleotide probes become available, they can be used for identification of the various forms of psychotic spectrum disorders and for determination of which offspring are and are not at genetic risk for the development of which varieties of psychotic spectrum disorder.

Third, the confluence of interests of high-risk study groups and biological psychiatrists interested in biological markers for psychotic spectrum diseases is particularly opportune. As already noted, a variety of potentially useful biological markers have been found in psychotic-spectrum-disordered patients. Few, if any, have been explored for familial transmission, and none has been used prospectively to predict which high-risk subjects will develop psychotic spectrum disorders. Tissue samples from high-risk offspring, their siblings, and parents could be deposited in a tissue culture bank on which approved investigators with promising biological markers could draw. This would permit rapid investigations of biological markers with respect to genetic transmission and the presence or absence of clinically associated psychotic-spectrum illnesses. Such a culture bank would not only spur collaboration with neuroscientists but might quickly provide viable markers to the high-risk groups, permitting them to distinguish individuals with and without biological risk for the development of psychotic spectrum disorders, which is the first step in analyzing other risk factors.

My strongest recommendation is for the immediate establishment of a bank of parent and offspring DNA; equally important is systematic banking of the data base of the present series of high-risk studies. The availability within the foreseeable future of DNA identification of subjects at risk for psychotic spectrum disorder will permit reanalysis of the present data in the light of biological risk for one of several psychotic disorders.

Of next priority is the establishment of a tissue bank with continued cultures from both parents and offspring. Such a culture bank of fibroblasts and lymphocytes will serve two purposes: (1) It will permit neuroscientists to explore immediately possible genetic/biological markers found in psychotic populations. The rate of identification and confirmation of markers that meet the criteria for use in high-risk studies will be profoundly accelerated. (2) Discovery and confirmation of such biological markers (e.g., receptors, enzymes, and membrane composition) will permit them to be used in the first step of the analysis of the high-risk data (i.e., distinction of subjects with respect to presence or absence of genetic/biological risk factors).

Without waiting for confirmation of the usefulness of biological markers or DNA probes, it is important that the available tools for identifying genetic/biological disorders of attention-apprehension-neurointegrative deviance (especially CPT d') be used immediately across several high-risk populations (both parents and offspring). The instrument for the CPT d' is already available on disk, and presumably can be easily translated with high reliability across study centers. The use of this technique within the consortium can permit the first genetic/biological separation of risk/no risk for analysis of data of families in which a parent shows the neurointegrative or CPT d' abnormality.

In summary, clear interpretation of high-risk studies requires (1) that subjects who are at genetic/biological risk for psychotic spectrum disorders be distinguished from those who are not, and (2) that the specific
psychotic spectrum disorder for which they are at risk be identified. Tools or markers to aid in the determination of biological risk are underdeveloped. Only neurointegrative testing (e.g., with the CPT d') appears to meet the criteria for separating subjects who are and are not at risk for a particular type of psychotic spectrum disorder. Current collaboration with neurobiologists and human geneticists in the preservation of DNA samples and the acquisition of tissue cultures promises advancement of molecular-genetic and biological understanding of the psychotic spectrum disorders. It should also hasten the time when appropriate genetic/biological tools will be available for the analysis of high-risk study data.

The Author

David L. Garver, M.D., is Professor of Psychiatry, Pharmacology, and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH.

Schizophrenia: Questions and Answers

What is schizophrenia? What causes it? How is it treated? How can other people help? What is the outlook? These are the questions addressed in a booklet prepared by the Schizophrenia Research Branch of the National Institute of Mental Health.

Directed to readers who may have little or no professional training in schizophrenia-related disciplines, the booklet provides answers and explanations for many commonly asked questions of the complex issues about schizophrenia. It also conveys something of the sense of unreality, fears, and loneliness that a schizophrenic individual often experiences.

The booklet describes "The World of the Schizophrenic Patient" through the use of analogy. It briefly describes what is known about causes—the influence of genetics, environment, and biochemistry. It also discusses common treatment techniques. The booklet closes with a discussion of the prospects for understanding schizophrenia in the coming decade and the outlook for individuals who are now victims of this severe and often chronic mental disorder.

Single copies of Schizophrenia: Questions and Answers (DHHS Publication No. ADM 86–1457) are available from the Public Inquiries Branch, National Institute of Mental Health, Room 15C–05, 5600 Fishers Lane, Rockville, MD 20857.