

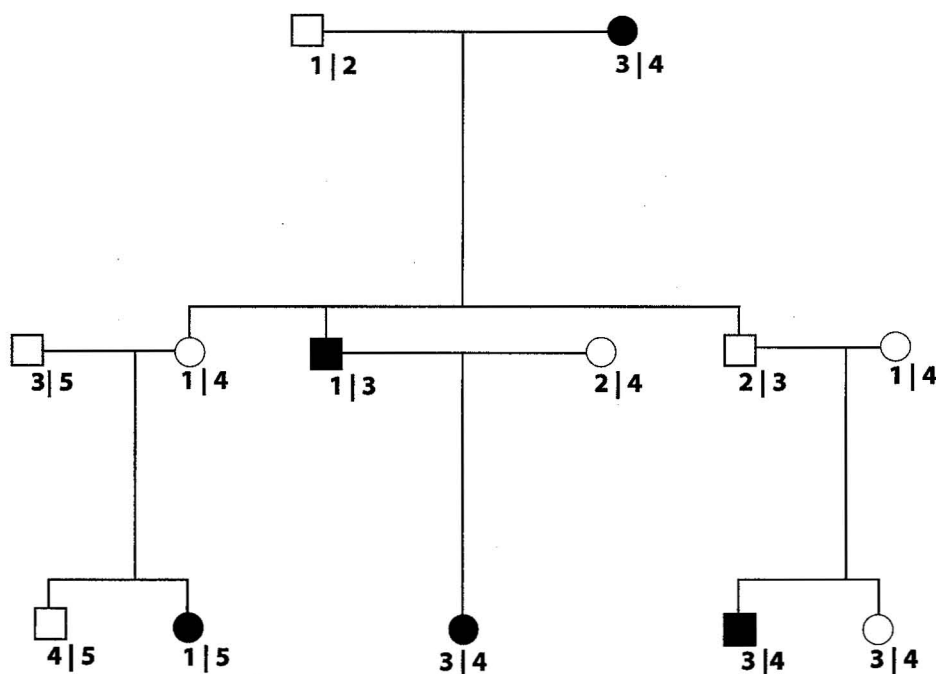
## Genetics of Childhood Disorders: V. Nonparametric Methods of Genetic Analysis

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Genes are responsible for many aspects of normal development. Mutations in genes are thought to underlie the etiology and pathophysiology of a number of psychiatric disorders. For decades, scientists have been trying to identify these genes. As reviewed in the last column, linkage analyses have been used to determine the approximate chromosomal location of genes involved in many different disorders. The inheritance pattern of a disorder is often studied in large pedigrees and is compared with the inheritance pattern for other genes or markers whose locations in the human genome are already known. These genetic markers are chosen either because of previous information about the supposed location of the disease gene or as part of a random genome scan. If a tight linkage is estab-

lished between the inheritance of the disease and the genetic marker, then the disease gene must lie relatively nearby.

Linkage analysis has been a powerful method for studying genetic disorders with a mendelian mode of inheritance, such as cystic fibrosis or Huntington disease. The analysis, however, depends on a set of assumptions. First, the investigator must establish whether the mode of inheritance is autosomal dominant, recessive, or another mode. In addition, it is essential to define who has the disorder and who does not. The penetrance—the chance that a subject who inherits the disease gene will express the disorder—must also be estimated. The gene frequency in the population must be determined, as well as whether there is genetic heterogeneity or not. The latter



**Fig. 1** Allele-sharing method of analysis. The amount of sharing of alleles between affected individuals is compared with what is expected by random transmission. Four of the 5 affected individuals share the 3 allele. This frequency is higher than expected by chance and suggests that this allele is associated with expression of the disorder. In this kind of analysis there is no need for setting parameters regarding mode of inheritance, penetrance, genetic heterogeneity, or phenocopies. Schematic representation of the pedigree: squares = males; circles = females; black = affected; white = unaffected. Genotype of the marker under study is noted under the corresponding individual. Different forms, or alleles, of the gene are given numbers (1–5). The 2 alleles that each individual carries are separated by a vertical line.

refers to the probability that different genes can separately cause the disorder under study. Finally, the rate of phenocopies must be determined, which establishes whether a disorder is also caused by nongenetic factors. These parameters are not known for most psychiatric disorders, and they must be guessed at before traditional linkage analysis can be performed. The chance of missing positive linkage is increased if any of these assumptions are incorrect.

The search for the gene for Tourette's disorder provides an interesting example of some of these pitfalls. Tourette's disorder is a neurodevelopmental disorder characterized by motor and vocal tics that begin in childhood. The importance of genetic factors contributing to the expression of this disorder was appreciated long ago. The expectations were high that a gene would be localized for this disorder by linkage analysis. These hopes relied on the relatively easy diagnosis of the phenotype and the apparent autosomal dominant mode of inheritance. Nevertheless, after 10 years of international collaboration in which dozens of pedigrees have been studied with markers spanning more than 90% of the human genome, no linkage has been established. In retrospect, parameters were assumed that were probably incorrect. For example, investigators now question whether Tourette's disorder is an autosomal dominant disorder. Also, the assumption that this disorder is caused by a single major gene is probably not true, and several genes acting in conjunction are likely to be responsible for expression of tics in the vulnerable child. Finally, separate and distinct genes might contribute to the expression of the disorder in different pedigrees. Thus the parameters that were initially specified were inaccurate and linkage could not be established. In other psychiatric disorders such as schizophrenia and bipolar disorder, the initially positive linkage results that were obtained have not been replicated in later studies, and researchers are similarly questioning the parameters that were assumed for these disorders.

To overcome some of these problems, several nonparametric approaches were developed that do not rely on the a priori determination of these parameters. There are 2 main types of approaches: association and allele-sharing. In association studies, the distribution of different forms of a given gene (called alleles) is studied in the general population. One compares the frequency of the various alleles in a group of unrelated patients with the frequency of these alleles in a group of normal controls. A higher than normal frequency of a certain allele would suggest a role for this allele in the expression of the disorder. This is how ApoE4, a specific allele for a lipoprotein, was found to be related to Alzheimer disease. More recently, an association was found between certain alleles of the dopamine D4 receptor (D4DR) gene and the novelty-seeking trait, and between the serotonin transporter gene and anxiety-related traits.

The advantages of association studies are the simple statistics that are used and the relative ease of obtaining a subject

group as there is no need to locate and interview relatives of the probands. On the other hand, as linkage usually cannot be detected by association studies, genome scans cannot be performed and alleles are chosen on the basis of some knowledge about their function and presumed involvement in the disorder. Thus the chance of finding the location of the gene for the disease is decreased. Moreover, genes that are found to be associated with the disorder often have a minor role in its etiology. For example, the serotonin transporter gene explains as little as 4% of the variance of the anxiety-related traits that were found to be associated with it.

Another significant difficulty with association studies is that allelic differences can stem from a number of factors. For example, differences in ethnic origin between the affected and the control group are likely to cause false-positive results. This is because allele frequencies are now known to vary tremendously among different ethnic groups. This might be one of the reasons for the difficulty in replicating the D4DR and serotonin transporter findings that followed the initial results of a positive association. To protect from this, a well-matched control group must be used. This is often accomplished by analyzing the nonaffected relatives of the subjects who are therefore of the same ethnic background.

The second nonparametric technique is allele-sharing analysis. In this method, related individuals with a disorder are studied. The sharing of the same allele by all affected members of a pedigree is calculated and compared with what is expected by chance. This can be done either in families with 2 affected siblings, called the sib-pair analysis, or in larger multigenerational pedigrees, called affected pedigree analysis. Affected Pedigree Member (APM) and Genehunter are examples of computer programs that use allele-sharing methods that are currently used for the study of larger pedigrees. Once again, the investigator looks for increased sharing of a particular allele. Increased sharing of an allele argues for an association between the studied marker and the disorder under study. This could imply linkage and help localize the disease gene. One of the important advantages of allele-sharing techniques is that no control group is required (Fig. 1).

In contrast with association studies, allele-sharing methods can be used for genome scans to detect genes with either a minor or major etiological role. Allele-sharing methods are used in the study of many disorders with complex inheritance. In diabetes, for example, sib-pair analysis was useful in assessing the role played by genes within the HLA complex, as well as finding other genes that are involved in its etiology. In the study of schizophrenia, sib-pair analysis has proved useful in providing additional support for the positive parametric linkage results of loci on chromosomes 6p and 22q that were reported. Researchers were also able to show increased allele-sharing on the 6p chromosome in affected individuals from large pedigrees with specific inherited forms of dyslexia.

Recently, increased allele-sharing of markers on chromosome 7q was found in sib pairs with autism.

Although very appealing, allele-sharing methods have one serious limitation. They are much less powerful than parametric linkage analysis. Thus hundreds of sib pairs and dozens of large pedigrees are needed to detect significant findings. Researchers can overcome this problem by combining samples from different populations. By doing so, however, they run the risk of genetic heterogeneity interfering with the results, and thus they actually may decrease the chance of finding increased allele-sharing. For this reason, allele-sharing methods are often used as an adjunct to more traditional parametric linkage analyses.

In summary, newer methods of genetic analysis enhance the ability to investigate the complex genetics of human behavior and development. Yet much effort is still needed to find sufficient numbers of related subjects with a disorder of interest and to search the entire genome for evidence of increased allele-sharing. It remains a difficult task to localize genes for complex disorders. Identifying the genes that are involved in these disorders is the first step toward understanding the underlying molecular mechanism. One must also appreciate that several genes are likely to be found to contribute to a particular disorder. Understanding how these genes interact with each other as well as with environmental factors poses the next challenge to researchers.

## WEB SITES OF INTEREST

<http://www.genome.wi.mit.edu/>  
<http://bioinformatics.weizmann.ac.il/cards/>  
<http://watson.hgen.pitt.edu/>

## ADDITIONAL READINGS

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**Social, Emotional, and Behavioral Functioning of Children With Cancer.** Robert B. Noll, PhD, Maria A. Gartstein, PhD, Kathryn Vannatta, PhD, Judy Correll, RN, William M. Bukowski, PhD, W. Hobart Davies, PhD

**Objective:** It was hypothesized that children with cancer would have more social problems and difficulties with emotional well-being than case control, same race/gender, similarly aged classmates. **Study Design:** Using a case controlled design, children with any type of cancer requiring chemotherapy except brain tumors ( $n = 76$ ), currently receiving chemotherapy, ages 8 to 15, were compared with case control classroom peers ( $n = 76$ ). Peer relationships, emotional well-being, and behavior were evaluated based on peer, teacher, parent, and self-report, and were compared using analysis of variance and structural equation modeling. **Results:** Relative to case controls, children with cancer were perceived by teachers as being more sociable; by teachers and peers as being less aggressive; and by peers as having greater social acceptance. Measures of depression, anxiety, loneliness, and self-concept showed no significant differences, except children with cancer reported significantly lower satisfaction with current athletic competence. There were also no significant differences in mother or father perceptions of behavioral problems, emotional well-being, or social functioning. Scores on all standardized measures were in the normal range for both groups. Comparisons of the correlation matrices of children with cancer and to the correlation matrix of the comparison children using structural equation modeling suggested they were not significantly different. **Conclusions:** Children with cancer currently receiving chemotherapy were remarkably similar to case controls on measures of emotional well-being and better on several-dimensions of social functioning. These findings are not supportive of disability/stress models of childhood chronic illness and suggest considerable psychologic hardiness. *Pediatrics* 1999;103:71-78. Reproduced by permission of *Pediatrics*, copyright 1999.