BIPOLAR DISORDERS: REVIEW OF MOLECULAR GENETIC LINKAGE STUDIES

WADE BERRETTINI

This chapter reviews evidence of the heritability of bipolar (BP) and recurrent unipolar (RUP) disorders. This evidence has accumulated through family, twin, adoption, linkage, and candidate gene studies. Both the twin and family studies suggest that unipolar and bipolar disorders share some fraction of genetic susceptibility. Data from the twin and family studies can be used for genetic counseling, and this will be discussed briefly from a clinical perspective.

Efforts to find susceptibility genes through linkage studies have yielded several confirmed regions of the genome where such genes will be found. These linkage studies will be discussed and summarized from methodologic perspectives.

Candidate gene approaches to BP and RUP disorders will be reviewed, with some suggestions for improving methods. A few of the most promising candidate genes will be noted.

Mechanisms on nonmendelian inheritance may be involved in the complex genetics of BP and RUP disorders. Imprinting, triplet repeat expansion, and mitochondrial inheritance are reviewed briefly as examples of nonmendelian mechanisms possibly involved in these disorders.

Finally, the implications of the Human Genome Project for future progress will be discussed.

GENETIC EPIDEMIOLOGY

Family Studies

Family studies can answer three critical questions concerning the inheritance of a human phenomenon:

1. Is the phenomenon found more frequently among the biological relatives of an affected individual compared to biological relatives of unaffected persons? Alterna-

- tively, are relatives of an affected subject at increased risk for the disorder compared to relatives of control subjects?
- 2. What other phenomena (possibly genetically related) are also found more frequently among relatives of an affected individual? Alternatively, what other disorders (or clinical characteristics) may share a common genetic vulnerability with the phenomenon in question?
- 3. Can a specific mode of inheritance be discerned?

Family studies are executed as follows. A proband that (most likely) has the phenomenon in question is examined to determine its presence. This person's biologic relatives are similarly examined for its presence. Simultaneously, relatives of unaffected probands are examined in the same fashion for its presence. The importance of simultaneous examination of control families cannot be overestimated, as the risk of psychiatric disorders in the general population varies according to the diagnostic criteria and according to the judgment of specific interviewers and diagnosers. Thus, it is rarely acceptable to rely on data collected by others to estimate risk for a control population. The risk of a particular disorder can then be calculated for the relatives of both affected probands and control probands. If the disorder in question is heritable, a family study should show that relatives of a proband with the disorder are at significantly higher risk to show the disorder themselves, compared to relatives of control probands. Often, the risk for a certain class of relatives of affected probands is expressed as a ratio. Shown below is an example for siblings:

Sibling relative risk =

[Risk for siblings of affected probands]

/[Risk for siblings of control probands]

A family study is not the same as a "family history" study, in which the relatives are not directly examined, but information from the proband or other persons is used to establish the affections status of relatives. The reliability of the family history method is not as high as the family study

Wade Berrettini: University of Pennsylvania School of Medicine, Center for Neurobiology and Behavior, Philadelphia, Pennsylvania.

method. The discrepancy in reliability is a function of the phenomenon under study, but for psychiatric diseases the discrepancy is often great enough to render the family history method undesirable.

As with any disorder with a variable age of onset, the prevalence of illness among relatives must be corrected for the fraction of the age of risk that each relative has yet to live through. There are several ways of performing this correction. Commonly, using age-at-onset data from the relevant population, the number of relatives in a particular age decade is multiplied by the fraction of affected people who became ill by that decade of life. This product is known as the *bezugziffer*. A bezugziffer is calculated for each decade of life and the sum of them represents the total number of relatives at risk. When the total number of ill relatives is divided by this sum of bezugziffers, a morbid risk value (risk of developing the illness at some point in life) is determined for the relatives.

Family studies of bipolar disorder (BPD) show that a spectrum of mood disorders is found among the first-degree relatives of BPD probands: BP I disorder, BP II disorder with major depression (hypomania and RUP illness in the same person), and schizoaffective (SA) disorders and RUP depression, as described by multiple investigators (1-10). Nearly all family studies of BPD probands reveal that their biologic relatives are at increased risk for BPD, SA, and RUP diagnoses. These results suggest that RUP and SA diagnoses, within a familial context of BPD disorders, may be alternative phenotypic expressions of the same genetic susceptibility. Thus, this spectrum of mood disorders represents a reasonable BPD affection status model, in that it can be expected that some genetic susceptibility to BPDs may partially explain genetic susceptibility to SA and RUP disorders.

The first-degree relatives of RUP probands are at increased risk for BPD and RUP disorder (1,2). If the general population risk for BPDs is \sim 1%, then the risk to first-degree relatives of BPD probands is \sim 10%. Similarly the first-degree relatives of BPD probands are at increased risk of RUP disorders: if the general population risk if \sim 8% (1, 2), the RUP risk to first-degree relatives of BPD probands is \sim 15% (1). Historically, SA disorder has been defined as a nosologic category that may describe psychotic affective disorders in some diagnostic systems. This makes estimates of risk of SA disorder among relatives of BPD probands difficult to compare across family studies.

Several family studies have also reported a higher risk of RUP illness among the first-degree relatives of RUP probands (2,4,11–14). The first-degree relatives of RUP probands also show significant increases in risk for BPD diagnoses (1,2). Thus, first-degree relatives of RUP and first-degree relatives of BPD probands are at increased risk for both RUP and BPD diagnoses. These data suggest that the two nosologic entities may share some genetic susceptibility and/or environmental risk. This tentative conclusion,

which must await molecular studies, is supported by twin studies (see below).

Thus, family studies of BPD suggest that the first-degree relatives are at increased risk of BPD, SA disorder, and RUP disorder. Similarly, a review of SZ family studies reveals that the first-degree relatives of schizophrenia (SZ) probands are at increased risk of SZ, SA, and RUP disorders (10,15). Kendler et al. (16) describe an increased risk of psychotic affective illness among relatives of SZ probands. Despite numerous carefully conducted investigations, no family study of SZ reports increased risk for BPD among firstdegree relatives of SZ probands. However, the first-degree relatives of SZ probands and the first-degree relatives of BP probands are at increased risk of SA and RUP disorders. The overlap in elevated risk of SA and RUP diagnoses is evident. Therefore, these family studies are consistent with partial overlap in familial susceptibility for BPD and SZ disorders. It will be instructive to determine whether putative BPD susceptibility loci are mapped to regions of the genome at which SZ susceptibility loci are thought to exist (see below).

Twin Studies

A phenomenon that is under genetic control should be more "concordant" (similar) in monozygoric (MZ) twins compared to dizygotic (DZ) twins. By comparing the concordance rate (how often the second member of a twin pair demonstrates the phenomenon in question when the first member has it) for MZ and DZ twin pairs, evidence of the genetic determination of a phenomenon can be obtained. There are two methods for calculating concordance rates in twin studies. "Pairwise concordance" involves a simple calculation of the percentage of twin pairs in which both members demonstrate the phenomenon in question. In "proband-wise concordance" every affected person is considered a proband (within a family, a proband is usually the first person who comes into contact with the investigators), independent of status within a twin pair, meaning that twin pairs in which both are affected are counted twice. Studies in which twin subjects are selected at random from a population most appropriately apply the pairwise method. However, in most twin studies of psychiatric diseases, the twins are not selected at random, but are selected because one member has the disease in question. Therefore, these studies most appropriately use the proband-wise concordance, although both types of analyses can yield similar results. The results of both types of analyses are often reported. Differences between the MZ and DZ concordances are usually larger when the proband-wise calculation is used.

If the variable being measured is genetically determined, then the concordance rate may be significantly higher in MZ twins compared to that for DZ twins. This suggests, but is by no means conclusive, that the variable in question may be heritable. The magnitude of the difference for the

MZ versus DZ concordance rates may provide an estimate of the heritability of the variable in question. Although there are several methods for calculating this heritability, one of the most simple is Holzinger's index:

Heritability =

When the MZ concordance rate is 100% and the DZ rate is 50%, the variable is a purely genetically determined phenomenon. More complex path modeling of heritability estimates from twin data is commonly used today (e.g., 16,17). MZ concordance rates lower than 100% suggest reduced penetrance (the gene is present but the disease is not expressed because of protective environmental events) or the presence of phenocopies (individuals appear to exhibit the variable in question but do not have the genetic diathesis for it).

Twin studies conducted over the past 70 years have indicated greater MZ twin concordance compared to DZ twin concordance for BPD and RUP disorders (for review see ref. 18). More recent twin studies (16,17,19), conducted with operationalized diagnostic criteria, validated semistructured interviews, and blinded assessments, confirm the earlier research, showing significantly greater MZ twin concordance. The MZ twin concordance rate (~65%) indicates decreased penetrance of inherited susceptibility or the presence of phenocopies (nongenetic cases). Among MZ twin pairs concordant for mood disorder, when one twin has a BPD diagnosis, RUP illness is present among 20% of the ill co-twins (20,21). This suggests that BPD and RUP syndromes share some common genetic susceptibility factors. This result is nicely concordant with the family study results reviewed above, which demonstrate that the first-degree relatives of BPD and the first-degree relatives of RUP probands are at increased risk for both BPD and RUP disorders. The twin and family study data suggest that BPD and RUP disorders share genetic susceptibility.

This has some clinical implications. For example, these genetic epidemiologic data suggest that RUP individuals who have lithium-responsive BPD relatives should be treated with lithium for prophylaxis of RUP, if maintenance treatment with an antidepressant is not successful.

Adoption Studies

Most adoption studies proceed through identification of affected probands that have been adopted early in life. Similarly, a control group of unaffected, adopted probands is identified. Risk for the disorder is compared in four groups of relatives: the adoptive and biological relatives of affected adoptees and the adoptive and biological relatives of unaffected adoptees. For partially genetic phenomena, there will be an increased risk among the biological relatives of affected

probands, compared to the other three groups of relatives. Alternatively, risk for illness in adopted-away children of ill parents can be compared with risk for illness in adopted-away children of well parents.

In the "cross-fostering" design, researchers ascertain two groups of adopted-away children—those of ill parents and those of unaffected parents—both of whom are adopted away early in life and are raised by well parents. Researchers also ascertain two additional groups of adopted-away children—those of ill parents and those of unaffected parents-both of whom are similarly adopted away early in life and raised by affected adults. If the presence of the illness in the family environment increases risk for development of the disease, then the risk for children raised by affected parents will be greater than the risk for children raised by unaffected parents. If only genetic factors are important in the pathogenesis, then children with ill biologic parents will be at increased risk for illness, independent of the presence of illness in the adoptive parents. These adoption studies cannot exclude intrauterine or perinatal events, which may yield results similar to those of genetic diseases.

Mendlewicz and Rainer (22) reported a controlled adoption study of BPD probands, including a control group of probands with poliomyelitis. The biological relatives of the BPD probands had a 31% risk for BPD or RUP disorders, compared to 2% risk in the relatives of the control probands. The risk of affective disorder in biological relatives of adopted BPD patients was similar to the risk in relatives of BPD patients who were not adopted away (26%). Adoptive relatives do not show increased risk compared to relatives of control probands.

Wender et al. (23) and Cadoret (24) studied RUP and BPD probands. Although evidence of genetic susceptibility was found, *adoptive* relatives of affective probands had a tendency to excess affective illness themselves, compared with the adoptive relatives of controls. Von Knorring et al. (25) did not find concordance in psychopathology between adoptees and biological relatives when examining the records of 56 adoptees with unipolar (UP) disorders. Sample size may have limited the conclusions of this study. It is also possible that heritable factors may be more prominent for BPD disorders, compared to RUP disorders.

Genetic Counseling

Frequently, patients with BPD or RUP disorder are aware of the genetic component to these illnesses, and, quite naturally, they are concerned about risks of illness to other members in the family, most often children. Clinician responses to these requests for genetic counseling properly use risk estimates derived from controlled family studies (e.g., 1,2). Family studies of BP and RUP illness have established the risk for offspring of affected parents. When one parent is BP (and the other parent is unaffected), the risk for a child of developing BP illness is ~9%, whereas the risk of RUP

disorder is \sim 18%. When one parent is UP (and the other parent is unaffected), there is \sim 16% risk of RUP illness and a \sim 3% risk of BPD. These risks are elevated compared to the general population risk of \sim 1% for BP and \sim 8% for RUP disorders. When both parents are BP or one parent is BP and the other RUP, risk of either BPD or RUP disorder is \sim 75%.

In the near future, when BP and RUP susceptibility genes are identified, and risks of the common variants (alleles) at those genes are estimated (through large-scale population studies), it may be possible to assay patient DNA samples to determine which of the common susceptibility gene alleles are present in an individual at risk. With this information, it may be possible to provide improved estimates of risk.

METHODOLOGIC CONSIDERATIONS IN LINKAGE

Linkage and association analyses are commonly employed methods to locate and define susceptibility genes for diseases. In the family shown in Fig. 71.1, a BPD mother has alleles X,Y at some anonymous DNA marker, while unaffected father has alleles U,V. Mother transmits allele Y to affected children and allele X to the unaffected children. The probability that a parent will transmit a specific allele to each child within a family is 50%. A logarithm of odds (LOD) score statistic assesses the probability that, within a family, co-segregation of illness and a marker allele has occurred randomly, versus the probability that the co-segregation of illness and a marker allele has occurred because the marker allele is located near a disease gene on the same chromosome, such that the two are transmitted together more often than expected by chance (=50%). In a single family, as shown, segregation of BPD illness with an allele at this marker locus could be a random event. However, if such a segregation was observed in >25% of 50 such BPD families, the probability that this is a random event would be remote. LOD scores for individual families can be

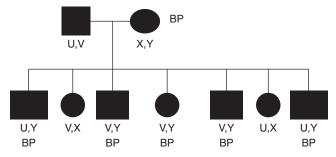


FIGURE 71.1. A family in which the bipolar disorder (BPD) mother has alleles X,Y at some anonymous DNA marker, whereas the unaffected father has alleles U,V.

summed to provide evidence that a region contains a susceptibility gene.

LOD score calculations require specification of the disease allele frequency in the population, the mode of inheritance (dominant or recessive or some intermediate model), and the penetrance. If the mode of inheritance is misspecified, then the LOD score may not detect linkage when it is present (26). For psychiatric diseases, none of these parameters is known. In practice, investigators usually calculate LOD scores under dominant and recessive models of inheritance with reduced penetrance.

A commonly employed analysis in complex trait studies is the affected sibling pair (ASP) statistic. Pairs of siblings will share 50% of their alleles randomly, and the expected distribution of this allele sharing is as follows:

No. of alleles shared: 0 1 2 Percent of all sibling pairs: 25% 50% 25%

Pairs of affected siblings will tend to share alleles to a greater extent when the DNA marker alleles are located near a disease gene that contributes to the illness in the affected siblings pairs. Consider the ten possible affected siblings in the pedigree diagram above. Whereas four affected sibling pairs share two alleles, six pairs share one allele, but none share no alleles. This skewing of the expected random distribution of allele sharing toward greater sharing is consistent with the hypothesis that the DNA marker is located near a BPD susceptibility gene (i.e., linkage is present). This statistical method has been extended to all types of affected relative pairs (27,28). Because this approach does not require specification of several parameters, including mode of inheritance, penetrance, and disease allele frequency (as is necessary for the LOD score method), these statistical methods are often described as nonparametric methods.

The validity of a linkage study is demonstrated by delineation of the functional DNA sequence variants that explain the linkage statistics, or through independent confirmation in another set of families. Statistical guidelines for judging validity of linkage reports in complex disorders have been described (29). These guidelines suggest thresholds for an initial report of "significant" linkage (LOD score = 3.6 or nominal p = .00002) and for confirmation (LOD score = 1.2 or p = .01). These guidelines should limit false positives to less than 5%. It should be remembered that these guidelines refer to analysis of a single phenotypic definition (e.g., BP I and BP II disorders). If multiple phenotypes are analyzed, some statistical adjustments for multiple hypothesis testing may be necessary.

In genetic linkage analysis of common complex disorders, failure of subsequent studies to confirm previously nominated susceptibility loci has become commonplace. This is as true for diabetes mellitus (30,31) as it is for SZ and bipolar disorders. This failure to confirm has multiple origins most probably the manifestations of multiple susceptibility loci, within an affected individual, which interact

with each other and the environment to produce these wellknown syndromes. For these disorders, no single susceptibility locus has a major effect on risk for illness in a majority of the ill population. Loci that increase risk by factors greater than 2 are unusual for common, complex disorders. Despite Herculean efforts in numerous disorders, only two loci that increase risk by a factor >2 in a large fraction of ill people have been detected: one is human leukocyte antigen (HLA) for insulin-dependent diabetes mellitus [increased risk = ~ 3 (32)], and the other is apolipoprotein E in late-onset Alzheimer's disease (33–35,79,80). Substantial sample sizes are required to detect such loci that increase risk by factors \leq 2. As Hauser et al. (36) have shown, \sim 400 affected sibling pairs are needed to have >90% power to detect (p < .0001 or LOD >3) loci, which increases risk by a factor of 2. No single linkage study of BPD or SZ disorders published in the 1990s has exceeded this sample size, although metaanalyses of multiple independent data sets have larger sample sizes.

A second major reason for lack of confirmation in linkage studies of common complex disorders has been delineated by Suarez et al. (37), who conducted simulation studies to evaluate the power to replicate linkage. They simulated linkage data for a complex disease caused in part by six equally frequent independent (unlinked) disease loci. They found that a larger sample size was required to confirm linkage of a previously detected locus, because independent pedigree samples might (through sampling variation) contain an overrepresentation of different susceptibility loci, rather than the locus initially detected. Given that investigators often draw their pedigrees from different ethnic backgrounds (in which prevalence of a particular susceptibility locus might vary), sampling variation is an important origin of confirmation failure. Thus, expectations of universal agreement (even when sample size is adequate) regarding susceptibility loci for common complex traits are unrealistic.

If three loci of equal effect size are used in an interactive, multiplicative model to explain the increased relative risk in BPD disorder (each locus increases relative risk by \sim 2), then these three hypothetical interactive loci explain most of the relative risk (2 × 2 × 2 = 8). Thus, loci that increase risk of BPD disorder will have minor to moderate effects. Substantial sample sizes are required to detect such loci of minor effect. As Hauser et al. (36) have shown, \sim 400 affected sibling pairs are needed to have >95% power to detect initially (LOD \geq 3.6, or $p \leq$.00002) loci that increase risk by a factor of 2, whereas 200 pairs are needed to have >95% power to provide confirmation ($p \leq$.01) of a previously detected locus.

Linkage Studies of Bipolar Disorder

To focus attention on the most promising linkage reports in BPD, this chapter limits consideration to those BPD linkages that meet criteria for validity (29), as noted above. Table 71.1 describes those BPD linkages that have at least one principal report with $p = \sim .00002$ and at least one independent confirmation at $p = \sim .001$. It is undoubtedly true that each of these confirmed linkages has been the subject of multiple negative reports. This is unavoidable when detecting loci of modest or minor effect, where the locus-specific relative risk is less than 2. Nearly all the negative reports are perhaps secondary to inadequate power to detect the initially described evidence of linkage. These negative reports will not be reviewed here.

Berrettini et al. (38,39) reported evidence of a BPD susceptibility locus on 18p11 using ASP and affected pedigree member (APM) methods ($p = 10^{-4}$ to 10^{-6}). Independent evidence of confirmation of this finding was reported

TABLE 71.1.	CONFIRMED	LINKAGES	IN BIPOL	AR DISORDER

Genomic Location	Principle Report	Independent Confirmations	Comments
18p11.2	Berrettini et al., 1994 (38) and 1997 (39)	Stine et al., 1995 (40); Nothen et al., 1999 (41); Turecki et al., 1999 (42)	Paternal parent-of-origin effect; see Schwab et al., 1998 (43)
21q22	Straub et al., 1994 (44)	Detera-Wadleigh et al., 1996 (45); Smyth et al., 1996 (46); Kwok et al., 1999 (47); Morissette et al., 1999 (48)	
22q11–13	Kelsoe et al., 2001 (49)	Detera-Wadleigh et al., 1997 (50) and 1999; (51)	Velocardiofacial syndrome region; possible overlap with a schizophrenia locus
18q22	Stine et al., 1995 (40)	McInnes et al., 1996 (52); McMahon et al., 1997 (53); De Bruyn et al., 1996 (54)	See Freimer et al., 1996 (55)
12q24	Morissette et al., 1999 (48)	Ewald et al., 1998 (56); Detera-Wadleigh et al., 1999 (51)	Principal report in a Canadian isolate
4p15	Blackwood et al., 1996 (57)	Ewald et al., 1998 (58); Nothen et al., 1997 (59); Detera-Wadleigh et al., 1999 (51)	See Ginns et al., 1998 (60)

by Stine et al. (40), Nothen et al. (41), and Turecki et al. (42). Evidence of linkage was found most often among those families with paternally transmitted illness (40,41,61). As part of Genetic Analysis Workshop no. 10, independent BPD chromosome 18 linkage data sets, including \sim 1,200 samples, were assembled for metaanalyses (62). An affected sibling pair (N=382 sibling pairs) metaanalysis yielded $p=2.8\times10^{-8}$ at marker D18S37 (63).

In light of the family studies suggesting partial overlap in susceptibility for BPD and SZ (see above), it is of interest to determine whether any of these confirmed BPD loci might overlap with confirmed SZ susceptibility loci. Schwab et al. (43) employed \sim 20 chromosome 18 markers in a linkage study of 59 multiplex German and Israeli SZ pedigrees, in which there were 24 affective disorder cases (two were BP). When these data were analyzed in two-point parametric methods, the maximum LOD score was 3.1 at D18S53. A multipoint nonparametric analysis using Genehunter (28) revealed p = .002 at D18S53.

One possible explanation for the results of Schwab et al. (43) is that their kindreds were misdiagnosed or unusual in some undetected characteristics. If the SZ kindreds of Schwab et al. (64) were nosologically unique (perhaps misclassified affective disorder kindreds), then one would not expect to find confirmations of other SZ loci in those kindreds. For example, these kindreds show linkage to chromosome 6p (65), as reported in other series of multiplex SZ kindreds (66,67). Similarly, Faraone et al. (68) and Straub et al. (69) report SZ linkage to 10p14, as did Schwab et al. (64). Nosologic misclassification does not explain the chromosome 18p11.2 linkage to SZ detected by Schwab et al. (43). Thus, one region of partial overlap in genetic susceptibility to BPD and SZ may be 18p11.2.

Straub et al. (44) initially described linkage of BPD disorder to 21q21 markers, in a study of American and Israeli BPD kindreds. One BPD pedigree with a LOD score of 3.41 was reported from a series of 57 BPD kindreds; further nonparametric analysis provided evidence of linkage (p < .0003 for the phosphofructokinase locus). An emendation of this original work has been published by Aita et al. (70). A confirmation has been described in a two-locus analysis of genotypic data from 21q21 and 11p15.5 (46). This 21q21 BPD susceptibility locus has been confirmed by Detera-Wadleigh et al. (45), who employed multipoint nonparametric analyses (p < .001). Kwok et al. (47) described confirmatory evidence for linkage to this region in nonparametric analyses. Kelsoe et al. (49) report a LOD >2 in this region. Morisette et al. (48) also report a confirmation for this locus in a population isolate of French an-

Lachman et al. (71), Edenberg et al. (72), Kelsoe et al. (49) described evidence of a BPD susceptibility locus on chromosome 22q11-13, near the velocardiofacial syndrome (VCFS) locus. Kelsoe et al. (49) report a LOD score of 3.8 at D22S278. Detera-Wadleigh et al. (51) report p=.008 for markers in this region. This VCFS has been associated

with microdeletions of the 22q region. These individuals have a psychosis in $\sim 30\%$ of cases. The syndromal form of the psychosis has been termed schizophrenia-like (73), whereas others have described it in terms of bipolar disorder (74,75).

Another region of the genome that harbors a BPD susceptibility locus is 18q22. McMahon et al. (53) initially reported linkage to this region in 28 American BPD kindreds (LOD is 3.51 for D18S41) and the ASP method (p = .00002 at D18S41). In an extension of this work, McMahon et al. (53) provided additional evidence for linkage to 18q21-2 in 30 new BPD kindreds. This locus may have been detected by Freimer et al. (55) and McInnes et al. (52) who studied Costa Rican BPD kindreds. McInnes et al. described evidence of increased allele sharing at some of the same markers identified by McMahon et al. For example, at D18S55, McMahon et al. (53) reported a nonparametric LOD score of 2.2, whereas McInnes et al. (52) at this same marker reports a maximum likelihood estimate of the LOD score as 1.67. Although the genetic map position of greatest significance for these two studies are not identical, there is sufficient map location overlap so that the simplest conclusion is that the two studies have detected the same locus.

Morissette et al. (48) reported evidence of a chromosome 12q24 BPD susceptibility locus, detected through the study of a population isolate (French ancestry) from the Saguenay River region of Quebec province. Detera-Wadleigh et al. (51) observed modest support for this locus in a study of 22 American kindreds of European origin.

An extended Scottish kindred showed linkage (LOD 4.1 at D4S394) to 4p16 DNA markers (57). Confirmation of the 4p locus has been reported in a paper by Nothen et al. (59), in which increased allele-sharing was noted at D4S394 (p = .0009). Another confirmation was described by Ewald et al. (58), who noted a LOD of 2.0 at D4S394. Ginns et al. (60) reported linkage to this region for a *mental health locus*, meaning absence of any psychiatric disorder. This requires additional investigation. Thus, the 4p16 region has a confirmed BPD susceptibility locus.

CANDIDATE GENE APPROACHES

The confusing array of disputed claims for association of candidate genes with psychiatric disorders becomes more comprehensible (and expected) if we recall two key issues. First, these candidate genes confer a small risk (if any), so that adequate power to confirm the originally described effect size is frequently absent in subsequent reports. Second, because the population genetics history of our species is unknown, associations detected in one ethnic group may not be detected so easily in another ethnic group. For example, the protective effect of aldehyde dehydrogenase deficiency on risk of alcoholism is easily demonstrated in

Chinese, Korean, and Japanese populations, because the deficiency allele has a frequency of $\sim 30\%$ (76,77). Much larger sample sizes are required to detect this influence in European populations, because the protective allele frequency is lower by an order of magnitude.

Candidate gene influences on risk of disease can be detected by demonstrating that certain candidate gene alleles are found more frequently among affected individuals compared to unaffected individuals. These studies are often termed "case-control association" investigations. This process is quite reliable when the effect size is robust. Candidate gene effect sizes can be considered as genotype relative risk (GRR), in which the risk associated with a particular genotype is compared to the general population risk. In general, there are four possible models of GRR: dominant, recessive, additive, and multiplicative. Let us consider each of these models for the general population risk, R, for a given disease, caused partially by a disease allele D, which triples the general population risk (the normal allele being d):

Model/genotype	DD	Dd	dd
Dominant	3R	3R	R
Recessive	3R	R	R
Additive	6R	3R	R
Multiplicative	9R	3R	R

An often-cited example of a multiplicative effect is apolipoprotein E4 in Alzheimer's disease (33,35,50–78), where one copy of the E4 allele increases risk for Alzheimer's disease by a factor of \sim 4 (at age 75), whereas homozygosity for E4 increases risk by a factor of \sim 14. Thus the influence of E4 on Alzheimer's disease risk may follow a multiplicative model.

Thus, one can genotype cases and unaffected individuals, comparing risk for disease across the three possible genotypes. However, in complex diseases such as BPD and RUP illness, the same disease allele may act in dominant or recessive mechanisms, depending on genetic background and environmental influence. Thus, a straightforward comparison of disease allele frequency in cases and controls can be recommended.

The major difficulty in comparing genotypes (or allele frequencies) in cases and controls is the risk of false positives because of subtle genetic differences between the case and control populations, differences that are independent of disease risk (81). Sometimes this is termed "population stratification." This danger can be illustrated by the following example. Suppose we are interested in testing the hypothesis that glucose-6-phosphate dehydrogenase (G6PD) is a disease gene in diabetes mellitus, using the case-control method. Let us also suppose that we are unaware that G6PD deficiency protects against malaria, and is found at increased frequency among individuals of Mediterranean origins. We select cases from a population enriched with individuals of Mediterranean origin, where G6PD deficiency is fairly common. Our controls also come from a population of individuals of European ancestry, but mostly northern Europe, where G6PD deficiency is relatively uncommon. We test our cases and controls, and find that the diabetics have increased frequencies of alleles that result in marked enzyme deficiency. We conclude falsely that these G6PD alleles are risk factors for diabetes mellitus.

One method to protect against such errors is known as a family-based association test (82-84). Such methods generally employ DNA samples from an affected individual and his/her parents. In one form, the transmission disequilibrium test (TDT) (84), the putative susceptibility allele is examined for excess transmission from heterozygous parents to affected children. Consider this nuclear family (Fig. 71.2), consisting of an affected child and two parents, whose affection status is unknown. Genotypes at a putative candidate gene are listed. Randomly, the affected child has a 50% probability of inheriting allele 1 from her heterozygous father. Let us hypothesize that allele 1 increases risk for the disorder present in the child. If this hypothesis is true, allele 1 should be inherited from heterozygous parents by affected offspring greater than 50% of the time. If DNA samples are collected from 500 parent-affected child trios, and those samples are genotyped at the candidate gene, the hypothesis can be tested. Note that this method does not require any diagnostic information from parents, only their DNA samples. Clearly, this method is not applicable to disease with onset late in life, such as Parkinson's disease or Alzheimer's disease. However, derivatives of this approach that use discordant siblings have been described (85-87).

The disadvantages to the family-based association methods include the greater difficulty in collecting parent-child trios, compared to unrelated cases and controls. Also, only those parents who are heterozygous are informative. Given that most variants in the human genome have only two alleles, parental homozygosity can be a significant problem. One compromise paradigm is to conduct a large case-control association study of candidate gene polymorphisms. Where one sees a positive result, it can be confirmed in a smaller family-based confirmation. If the family-based sample provides confirmation, one can have greater confidence that the original case-control positive result was not due to population stratification.

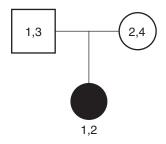


FIGURE 71.2. A nuclear family consisting of an affected child and two parents, whose affection status is unknown. Genotypes at a putative candidate gene are listed.

Although a multitude of candidate genes have been examined in populations of BPD and RUP patients, there are no candidate genes that have been unequivocally established. It is instructive to review one candidate gene intensively studied, monoamine oxidase A (*MAOA*), located on the short arm of the X chromosome, Xp11.23. These studies exemplify the difficulties outlined above, including possible population stratification, limited power, and different ethnic groups. Although no linkage studies have suggested Xp11 as a genomic location of a susceptibility gene for affective disorder, the role of *MAOA* in the deamination of serotonin and norepinephrine and the therapeutic efficacy of MAO inhibitors suggest that this gene should be evaluated as a potential risk factor for affective disorders.

There have been numerous independent association studies of BPD and RUP and an MAOA (CA)n repeat polymorphism in European (88–94) and Asian (95,96) studies. Those studies reporting a positive association (89,92,94,95) generally detect an overrepresentation of allele 5 or 6 of the MAOA (CA)n repeat among BPD patients, compared to controls, an observation that may be particularly evident among women. The effect size is small, the odds ratio being 1.49 (94), and the sample size required for adequate power to detect is larger than most of the negative studies (88,90, 91,93,96). There is also an MAOA promoter polymorphism (97). These studies involve multiple ethnic groups, casecontrol methods, and family-based designs, with some studies having limited power to detect a small effect size. Thus, it is understandable that conflicting studies are reported.

PARENT-OF-ORIGIN EFFECTS

Parent-of-origin effects refer to unequal rates of transmission of a disorder from fathers, compared to mothers. In BPD, McInnis et al. (98) first observed an excess of maternal transmission in multiplex kindreds selected for a linkage study. This observation was confirmed by Gershon et al. (15) in an independent series of multiplex BPD kindreds. Although this observation has not been confirmed by other investigators (99), it raises the possibility that the complex genetics of BPD may involve mitochondrial inheritance and/or imprinting.

Mitochondrial DNA is a nonnuclear circular 16,500 base pair molecule that is solely maternal in origin. It contains genes for oxidative phosphorylation and genes for transfer RNA (tRNA) molecules, among others. Defects in mitochondrial DNA sequence can contribute to genetic susceptibility for complex disorders, such a diabetes mellitus (100) and some forms of nonsyndromic deafness (101). If a fraction of all BPD included a mitochondrial susceptibility gene, then this would be consistent with the excess maternal transmission observed in BPD (15,98). However, variations in mitochondrial DNA have not been associated with BPD.

Another mechanism consistent with excess maternal

transmission is that of imprinting (see ref. 102 for review). Imprinting results in the transcriptional silencing of the allele of a sex-specific parent. For some genes, imprinting is paternal, meaning that the paternal allele is not transcribed into messenger RNA (mRNA). For other genes, maternal imprinting is present, and the maternal allele is transcriptionally silent. This results in a "functional hemizygosity," so that defects in the single active allele may have a greater impact on the phenotype. How might this mechanism give rise to an apparent excess of maternal transmission, as has been observed by McInnis et al. (98) and Gershon et al. (15)? If the putative BPD susceptibility gene is paternally imprinted, then the paternal allele is transcriptionally silent, and defects in the expressed maternal allele may be more often detected in the phenotypes of the offspring. In the embryonic gonads of each generation, the imprinting mark is reset, so that the alleles can be properly regulated in the next generation. Consider an example of paternal imprinting (Fig. 71.3). Note that individuals are heterozygous at the DNA level, but they are hemizygous at the mRNA level, expressing only the maternal allele. Note also that the imprinting mark is reset with each generation, so the woman who inherits (and expresses) allele 3 from her mother transmits allele 2 to her son, and this allele is transcriptionally active in the son, who does not express allele 5, which he inherits paternally. Thus if allele 2 is a BPD susceptibility gene, it will be expressed in the third generation, and influence the risk of disease, due to maternal imprinting. However, allele 2 is not expressed in the second generation, and will not influence risk of BPD.

Molecular mechanisms of imprinting are complex, but involve methylation of the promoter regions of the target genes (102), resulting in nontranscription. Imprinting defects give rise to human diseases, the classic examples of which are Prader-Willi and Angelman's syndromes. Imprinting can be tissue-specific, such as the serotonin receptor subtype 2A (5-HT_{2A}) gene (103), which is imprinted in specific brain regions. Imprinting has been described for

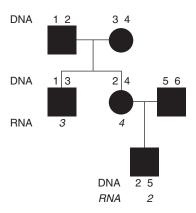


FIGURE 71.3. An example of paternal imprinting.

~50 human genes. It is likely that imprinting will explain some of the complex inheritance of BPD.

ANTICIPATION AND TRIPLET REPEATS

Anticipation is the term used to define an observation that a familial disorder occurs with earlier age at onset and/or increasing severity among younger generations, compared to older generations. Anticipation occurs in several neurodegenerative diseases, including Huntington's disease, fragile X, myotonic dystrophy, spinocerebellar ataxias, and others (see ref. 104 for review). The molecular explanation for anticipation in these disorders involves unstable intragenic trinucleotide repeats, which expand in subsequent generations, giving rise to increasing levels of gene disruption and thus to earlier age at onset and increasingly severe phenotype in younger generations.

Evidence of anticipation has been reported in several family studies of BPD illness (98,105-107), but some authorities suggest that there is intractable ascertainment bias (108,109). Individuals with earlier age-at-onset BPD disorder may have reduced capacity to reproduce, so parents with such early-onset disorders may be underrepresented in the general population. Individuals with familial BPD disorder may come to treatment earlier than those with sporadic disease, such that less severe mood disorder episodes are detected medically, and an earlier age at onset is defined. Such individuals (by virtue of their familiarity with mood disorder symptoms) may be more likely to report minor mood disturbance in terms of "diagnosable syndromes." Some evidence of anticipation in BPD comes from extensive studies of multiplex BPD families for linkage studies. These linkage studies select for earlier age-at-onset cases, because preference is given to densely affected kindreds. Among broader cultural factors possibly underlying the evidence of anticipation, if stigma concerning mood disorders is less among younger affected persons (compared to older individuals), then younger cohorts might describe their experiences more easily in terms of a diagnosable mood disorder, because denial (due to stigma) is less prevalent among the younger cohorts. These potential confounding factors make detection of anticipation in BPD disorder difficult.

The hypothesis that anticipation in BPD disorder reflects causative expanding trinucleotide CTG repeat sequences has generated genomic searches for such sequences (110–113), using the repeat expansion detection method (114). These three groups have noted increased lengths of CTG repeats in BPD disorders, especially among those with familial disease. However, not all studies have reported this difference (115), and no report shows transmission of an expanding repeat within BPD families, the definitive evidence. Furthermore, greater than 90% of the expanded CTG repeats detected by the method of Schalling et al. (114) are from two apparently nonpathogenic unstable

CTG repeats on 17q and 18q21 (116). The hypothesis that unstable trinucleotide repeats represent BPD susceptibility factors warrants continued study.

IMPLICATIONS OF THE HUMAN GENOME PROJECT

The Human Genome Project is nearing completion of one of its primary goals, the sequence of the human genome. However, the additional goal of defining all expressed sequences (genes) may require several additional years of work. Once this goal is achieved, the most important task will be the definition of function for each expressed sequence (functional genomics). Implied (but not included) in this goal is the function of each protein (functional proteomics), involving interactions between proteins. A third goal of the Human Genome Project is the definition of $\sim 300,000$ common single nucleotide polymorphisms (SNPs), including several within each gene.

The cloning of individual susceptibility genes for BPD and RUP disorder will be facilitated remarkably by the completion of these Human Genome Project milestones noted above. At present our knowledge base regarding central nervous system (CNS) function and the biochemical etiology of BPD and RUP disorder is so poor that too many brainexpressed genes may be considered candidates. This limitation is made more severe by the fact that linkage studies of all complex traits result in genomic regions of interest that typically span 20,000,000 base pairs of DNA. Because \sim 20 to 50 genes (most of which are unknown today) are usually found in every 1,000,000 base pairs of DNA, the task of discovering a single disease gene within such a region, implicated by linkage, is the proverbial equivalent of finding a needle in a haystack, with currently available DNA technologies. However, once all expressed sequences are known, and their functions are understood, it is possible to focus on the few best candidates. This reduces an intractable problem (from a DNA technology perspective) to a manageable size. Thus, finding susceptibility genes implicated by linkage results will become progressively less difficult as the Human Genome Project goals are approached.

SUMMARY

Despite the extensive data (from twin, family, and adoption studies) for genetic factors in BPD, gene identification through linkage studies has been elusive. There are multiple confirmed BPD linkage regions across the human genome, but the effect sizes are uniformly small at each locus. Cloning genes from these small effect size regions is a challenge for current molecular techniques. Part of the complexity of BPD genetics may be due to imprinting, mitochondrial

1036

inheritance, and trinucleotide repeat expansion. These nonmendelian influences require additional research.

ACKNOWLEDGMENTS

This paper was prepared with the support of National Institutes of Health (NIH) grant MH59553 and a National Alliance for Research on Schizophrenia and Depression Distinguished Investigator Award.

REFERENCES

- Gershon ES, Hamovit J, Guroff JJ, et al. A family study of schizoaffective, bipolar I, bipolar II, unipolar, and normal control probands. *Arch Gen Psychiatry* 1982;39:1157–1167.
- Weissman MM, Gershon ES, Kidd KK, et al. Psychiatric disorders in the relatives of probands with affective disorder. *Arch Gen Psychiatry* 1984;41:13–21.
- 3. Baron M, Gruen R, Anis L, et al. Schizoaffective illness, schizophrenia and affective disorders: morbidity risk and genetic transmission. *Acta Psychiatr Scand* 1983;65:253–262.
- Winokur G, Tsuang MT, Crowe RR. The Iowa 500: affective disorder in relatives of manic and depressed patients. Am J Psychiatry 1982;139:209–212.
- Winokur G, Coryell W, Keller M, et al. A family study of manic-depressive (bipolar I) disease. Is it a distinct illness separable from primary unipolar depression? *Arch Gen Psychiatry* 1995;52(5):367–373.
- Helzer JE, Winokur G. A family interview study of male manicdepressives. Arch Gen Psychiatry 1974;31:73–77.
- 7. James NM, Chapman ČJ. A genetic study of bipolar affective disorder. *Br J Psychiatry* 1975;126:449–456.
- Johnson GFS, Leeman MM. Analysis of familial factors in bipolar affective illness. Arch Gen Psychiatry 1977;34:1074–1083.
- 9. Angst J, Frey R, Lohmeyer R, et al. Bipolar manic depressive psychoses: results of a genetic investigation. *Hum Genet* 1980; 55:237–254.
- Maier W, Lichtermann D, Minges J, et al. Continuity and discontinuity of affective disorders and schizophrenia. Results of a controlled family study. *Arch Gen Psychiatry* 1993;50: 871–883.
- 11. Slater E. The inheritance of manic-depressive insanity. *Proc R Soc Med* 1936;29:981–990.
- 12. Mendlewicz J, Baron M. Morbidity risks in subtypes of unipolar depressive illness: differences between early and late onset forms. *Br J Psychiatry* 1981;139:463–466.
- 13. Bland RC, Newman SC, Orn H. Recurrent and nonrecurrent depression. *Arch Gen Psychiatry* 1986;43:1085–1089.
- 14. Kupfer DJ, Frank E, Carpenter LL, et al. Family history in recurrent depression. *J Affective Disord* 1989;17:113–119.
- Gershon ES, DeLisi LE, Hamovit J, et al. A controlled family study of chronic psychoses. Arch Gen Psychiatry 1988;45: 328–336.
- Kendler KS, McGuire M, Gruenberg AM, et al. The Roscommon family study. Arch Gen Psychiatry 1993;50:527–540.
- 17. Kendler KS, Neale MC, Kessler RC, et al. A population-based twin study of major depression in women: the impact of varying definitions of illness. *Arch Gen Psychiatry* 1992;49:257–266.
- 18. Nurnberger JL Jr, Berrettini WH. *Psychiatric genetics*. London: Chapman and Hall, 1998.
- 19. McGuffin P, Katz R, Watkins S, et al. A hospital-based twin register of the heritability of DSM-IV unipolar depression. *Arch Gen Psychiatry* 1996;53:129–136.

- Bertelsen A, Harvald B, Hauge M. A Danish twin study of manic-depressive disorders. Br J Psychiatry 1977;130:330–351.
- Allen MG, Cohen S, Pollin W, et al. Affective illness in veteran twins. A diagnostic review. Am J Psychiatry 1974;131: 1234–1239.
- 22. Mendlewicz J, Rainer JD. Adoption study supporting genetic transmission in manic-depressive illness. *Nature* 1977; 368(5618):327–329.
- 23. Wender H, Kety SS, Rosenthal D, et al. Psychiatric disorders in the biological and adoptive families of adopted individuals with affective disorders. *Arch Gen Psychiatry* 1986;43:923–929.
- Cadoret RJ. Evidence for genetic inheritance of primary affective disorder in adoptees. Am J Psychiatry 1978;135(4): 463–466.
- 25. Von Knorring AL, Cloninger CR, Bohman M, et al. An adoption study of depressive disorders and substance abuse. *Arch Gen Psychiatry* 1983;40:943–950.
- Clerget-Darpoux F, Bonaiti-Pellie C, Hochez J. Effects of misspecifying genetic parameters in LOD score analysis. *Biometrics* 1986;42:393–399.
- 27. Weeks DE, Lange K. The affected-pedigree-member method of linkage analysis. *Am J Hum Genet* 1988;42:315–326.
- Kruglyak L, Daly MJ, Reeve-Daly MP, et al. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 1996;58:1347–1363.
- 29. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genet* 1995;11:241–247.
- 30. Hanis CL, Boerwinkle E, Chakraborty R, et al. A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. *Nature Genet* 1996;13(2):161–166.
- 31. Concannon P, Gogolin-Ewens KJ, Hinds DA, et al. A secondgeneration screen of the human genome for susceptibility to insulin-dependent diabetes mellitus. *Nature Genet* 1998;19: 292–296.
- 32. Davies JL, Kawaguchi Y, Bennett ST, et al. A genome-wide search for human type 1 diabetes susceptibility genes. *Nature* 1994;371:130–136.
- Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993;261:921–923.
- Mayeux R, Stern Y, Ottman R, et al. The apolipoprotein E4 allele in patients with Alzheimer's disease. *Ann Neurol* 1993; 34:752–754.
- 35. Tsai M-S, Tangalos EG, Petersen RC, et al. Apolipoprotein E: risk factor for Alzheimer disease. *Am J Hum Genet* 1994;54: 643–649.
- 36. Hauser ER, Boehnke M, Guo S-W, et al. Affected sib pair interval mapping and exclusion for complex genetic traits. *Genet Epidemiol* 1996;13:117–137.
- 37. Suarez B, Harpe CL, Van Eerdewegh P. Problems of replicating linkage claims in psychiatry. In: Gershon ES, Cloninger CR, eds. *Genetic approaches to mental disorders*. Proceedings of the 82nd Annual Meeting of the American Psychopathological Association. Washington, DC: American Psychiatric Press, 1994: 23–46.
- Berrettini W, Ferraro T, Goldin L, et al. Chromosome 18 DNA markers and manic depressive illness: evidence for a susceptibility gene. *Proc Natl Acad Sci USA* 1994;91:5918–5921.
- 39. Berrettini W, Ferraro T, Choi H, et al. Linkage studies of bipolar illness. *Arch Gen Psychiatry* 1997;54:32–39.
- Stine OC, Xu J, Koskela R, et al. Evidence for linkage of bipolar disorder to chromosome 18 with a parent-of-origin effect. Am J Hum Genet 1995;57:1384–1394.
- 41. Nothen MM, Cichon S, Rohleder H, et al. Evaluation of linkage

- of bipolar affective disorder to chromosome 18 in a sample of 57 German families. *Mol Psychiatry* 1999;4:76.
- 42. Turecki G, Grof P, Cavazzoni P, et al. Lithium responsive bipolar disorder, unilineality and chromosome 18: a linkage study. *Am J Med Genet* 1999;88:411–415.
- Schwab SG, Hallmayer J, Lerer B, et al. Support for a chromosome 18p locus conferring susceptibility to functional psychoses in families with schizophrenia, by association and linkage analysis. Am J Hum Genet 1998;63:1139.
- 44. Straub RE, Lehner T, Luo Y, et al. A possible vulnerability locus for bipolar affective disorder on chromosome 21q22.3. *Nature Genet* 1994;8:291–296.
- Detera-Wadleigh SD, Badner JA, Goldin LR, et al. Affected sib-pair analyses reveal support of prior evidence for a susceptibility locus for bipolar disorder on 21q. Am J Hum Genet 1996; 58:1279–1285.
- 46. Smyth C, Kalsi G, Brynjolfsson J, et al. Further tests for linkage of bipolar affective disorder to the tyrosine hydroxylase gene locus on chromosome 11p15 in a new series of multiplex British affective disorder pedigrees. *Am J Psychiatry* 1996;153: 271–274
- Kwok JB, Adams LJ, Salmon JA, et al. Non-parametric simulation-based statistical analyses for bipolar affective disorder locus on chromosome 21q22.3. Am J Med Genet 1999;88:99–102.
- 48. Morissette J, Villeneuve A, Bordeleau L, et al. Genome-wide search for linkage of bipolar affective disorders in a very large pedigree derived from a homogeneous population in Quebec points to a locus of major effect on chromosome 12q23-q24. Am J Med Genet 1999;88:567–587.
- Kelsoe JR, Spence MA, Loetscher E, et al. A genome survey indicates a susceptibility locus for bipolar disorder on chromosome 22. Proc Natl Acad Sci USA 2001;98:585–590.
- Detera-Wadleigh SD, Hsieh W-T, Berrettini WH, et al. Genetic linkage mapping for a susceptibility locus to bipolar illness: Chromosomes 2, 3, 4, 7, 9, 10p, 11p, 22, and Xpter. Neuropsychiatr Genet 1997;74:206–218.
- Detera-Wadleigh SD, Badner JA, Berrettini WH, et al. Evidence for a bipolar susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. Proc Natl Acad Sci USA 1999;96:5604–5609.
- McInnes LA, Escamilla MA, Service SK, et al. A complete genome screen for genes predisposing to severe bipolar disorder in two Costa Rican pedigrees. *Proc Natl Acad Sci USA* 1996; 93:13060–13065.
- McMahon FJ, Hopkins PJ, Xu J, et al. Linkage of bipolar affective disorder to chromosome 18 markers in a new pedigree series. Am J Hum Genet 1997;61:1397–1404.
- De Bruyn A, Souery D, Mendelbaum K, et al. Linkage analysis of families with bipolar illness and chromosome 18 markers. *Biol Psychiatry* 1996;39:679–688.
- 55. Freimer NB, Reus VI, Escamilla MA, et al. Genetic mapping using haplotype, association and linkage methods suggests a locus for severe bipolar disorder (BPI) at 18q22-q23. *Nature Genet* 1996;12:436–441.
- Ewald H, Degn B, Mors B, et al. Significant linkage between bipolar affective disorder and chromosome 12q24. *Psychiatr Genet* 1998;8:131–140.
- Blackwood DHR, He L, Morris SW, et al. A locus for bipolar affective disorder on chromosome 4p. *Nature Genet* 1996;12: 427–430.
- Ewald H, Degn B, Mors B, et al. Support for the possible locus on chromosome 4p15 for bipolar affective disorder. *Mol Psychiatry* 1998;3:442–448.
- Nothen MM, Cichon S, Franzek E, et al. Systematic search for susceptibility genes in bipolar affective disorder—evidence for disease loci at 18p and 4 p. Am J Hum Genet 1997;61(S):A288.

- 60. Ginns EI, St Jean P, Philibert RA, et al. A genome search for chromosomal loci linked to mental health wellness in relatives at high risk for bipolar affective disorder among the Old Order Amish. *Proc Natl Acad Sci USA* 1998;95:15531.
- 61. Gershon ES, Badner JA, Ferraro TN, et al. Maternal inheritance and chromosome 18 allele sharing in unilineal bipolar illness pedigrees. *Neuropsychiatr Genet* 1996;67:1–8.
- 62. Goldin LR, Gershon ES, Berrettini WH, et al. Description of the Genetic Analysis Workshop 10 bipolar disorder linkage data sets. *Genet Epidemiol* 1997;14:563–568.
- Lin JP, Bale ŚJ. Parental transmission and D18S37 allele sharing in bipolar affective disorder. *Genet Epidemiol* 1997;14: 665–668.
- 64. Schwab SG, Hallmayer J, Albus M, et al. Further evidence for a susceptibility locus on chromosome 10p14-p11 in 72 families with schizophrenia by non-parametric linkage analysis. Am J Med Genet 1999;81:302–307.
- Schwab SG, Albus M, Hallmayer J, et al. Evaluation of a susceptibility gene for schizophrenia on chromosome 6p by multipoint affected sib-pair linkage analysis. *Nature Genet* 1995;11: 325–327.
- Straub RE, MacLean CJ, O'Neill FA, et al. A potential vulnerability locus for schizophrenia on chromosome 6p22-24: evidence for genetic heterogeneity. *Nature Genet* 1995;11: 287–293.
- Moises HW, Yang L, Kristbjarnarson H, et al. An international two-stage genome-wide search for schizophrenia susceptibility genes. *Nature Genet* 1995;11:321–324.
- Faraone SV, Matise T, Svrakic D, et al. Genome scan of European-American schizophrenia kindreds: results of the NIMH Genetics Initiative and Millennium consortium. Am J Med Genet 1998;81:290–295.
- Straub RE, MacLean CJ, Martin RB, et al. A schizophrenia locus may be located in region 10p15-p11. Am J Med Genet 1998;81:296–301.
- 70. Aita VM, Liu J, Knowles JA, et al. A comprehensive linkage analysis of chromosome 21q22 supports prior evidence for a putative bipolar affective disorder locus. *Am J Hum Genet* 1999; 64:210–217.
- 71. Lachman HM, Kelsoe JR, Remick RA, et al. Linkage studies support a possible locus for bipolar disorder in the velocardiofacial syndrome region on chromosome 22. *Am J Med Genet* 1996;74:121–128.
- 72. Edenberg H, Foroud T, Conneally M, et al. Initial genomic scan of the NIMH genetics initiative bipolar pedigrees: chromosomes 3,5,15,16,17, and 22. *Neuropsychiatr Genet* 1997;74:238–246.
- 73. Pulver AE, Nestadt G, Goldberg R, et al. Psychotic illness in patients diagnosed with velo-cardio-facial syndrome and their relatives. *J Nerv Ment Dis* 1994;182(8):476–478.
- 74. Papolos DF, Faedda GL, Veit S, et al. Bipolar spectrum disorders in patients diagnoses with VCFS: does a hemizygous deletion of chromosome 22q11 result in bipolar affective disorder? *Am J Psychiatry* 1996;153:1541–1547.
- Carlson C, Papolos D, Pandita RK, et al. Molecular analysis of VCFS patients with psychiatric disorders. Am J Med Genet 1997; 60:851–859.
- Harada S, Agarwal DP, Goedde HW. Aldehyde dehydrogenase deficiency as cause of the flushing reaction to alcohol in Japanese. *Lancet* 1982;2(8253):982.
- 77. Thomasson HR, Edenberg HJ, Crabb DW, et al. Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *Am J Hum Genet* 1991;48:677.
- Mayeux R, Stern Y, Ottman R, et al. The apolipoprotein e4 allele in patients with Alzheimer's disease. *Ann Neurol* 1993; 34:752–754.
- 79. Saunders AM, Schmader KE, Breitner JC, et al. Apolipoprotein

- E4 allele distributions in late-onset Alzheimer's disease and in other amyloid-forming diseases. *Lancet* 1993;342:710–711.
- 80. Martin ER, Lai EH, Gilbert JR, et al. SNPing away at complex diseases: analysis of SNPs around APOE in Alzheimer disease. *Am J Hum Genet* 2000;67:383–394.

1038

- 81. Berrettini WH. On the interpretation of association studies in behavioral disorders. *Mol Psychiatry* 1997;2:274–275.
- 82. Falk CT, Rubenstein P. Hablotype relative risks: an eary reliable way to construct proper control samples for risk calculations. *Ann Hum Genet* 1987;51:227–233.
- 83. Ott J. Statistical properties of the haplotype relative risk. *Genet Epidemiol* 1989;6:127–130.
- Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 1993;52: 506–516.
- 85. Spielman RS, Ewens WJ. A sibship test for linkage in the presence of association: the sib transmission/disequilibrium test. *Am J Hum Genet* 1998;62:450–458.
- Lazzeroni LC, Lange K. A conditional inference framework for extending the transmission/disequilibrium test. *Hum Hered* 1998:48:67–81.
- 87. Monks SA, Kaplan NL, Weir BS. A comparative study of sibship tests of linkage and/or association. *Am J Hum Genet* 1998;63: 1507–1516.
- 88. Craddock N, Daniels J, Roberts E, et al. No evidence for allelic association between bipolar disorder and MAOA gene polymorphisms. *Am J Med Genet* 1995;60:322–324.
- Lim LC, Powell J, Sham P, et al. Evidence for a genetic association between alleles of MAOA gene and bipolar affective disorder. Am J Med Genet 1995;60:325–331.
- 90. Nothen MM, Eggermann K, Albus M, et al. Association analyses of the MAOA gene in bipolar affective disorder by using family based controls. *Am J Hum Genet* 1995;57:975–977.
- 91. Parsian A, Todd RD. Genetic association between monoamine oxidase and manic-depressive illness: comparison of relative risk and haplotype relative risk data. *Am J Med Genet* 1997;74(5): 475–479.
- 92. Furlong RA, Ho L, Rubinszstein JS, et al. Analysis of the MAOA gene in bipolar affective disorder by association studies, meta-analyses and sequencing of the promoter. *Am J Med Genet* 1999; 88:398–406.
- 93. Turecki G, Grof P, Cavazzoni P, et al. MAOA: association and linkage studies with lithium responsive bipolar disorder. *Psychiatr Genet* 1999;9:13–16.
- 94. Preisig M, Bellivier F, Fenton BT, et al. Association between bipolar disorder and MAOA gene polymorphisms: results of a multicenter study. *Am J Psychiatry* 2000;157:948–959.
- 95. Kawada Y, Hattori M, Dai XY. Possible association between MAOA gene and bipolar affective disorder. *Am J Hum Genet* 1995;56:335–336.
- Muramatsu T, Matsushita S, Kanba S, et al. MAO genes polymorphisms and mood disorder. Am J Med Genet 1997;19: 494–496.
- 97. Kunugi H, Ishida S, Kato T, et al. A functional polymorphism in the promoter region of MAOA gene and mood disorders. *Mol Psychiatry* 1999;4:393–395.

- 98. McInnis MG, McMahon FJ, Chase GA, et al. Anticipation in bipolar affective disorder. *Am J Hum Genet* 1993;53:385–390.
- Grigoroiu-Serbanescu M, Martinez M, Nothen MM, et al. Patterns of parental transmission and familial aggregation models in bipolar affective disorder. *Am J Med Genet* 1998;81:397–404.
- Kadowaki T, Kadowaki H, Mori Y, et al. A subtype of diabetes mellitus associated with a mutation of mitochrondrial DNA. N Engl J Med 1994;330:962–967.
- Prezant TR, Agapian JV, Bohlman MC, et al. Mitochondrial ribosomal RNA mutation associated with both antibioticinduced and non-syndromic deafness. *Nature Genet* 1994;4: 289–294.
- 102. Tilghman SM. The sins of the fathers and mothers: Genomic imprinting in mammalian development. *Cell* 1999;96: 185–193.
- 103. Bunzel R, Blumke I, Cichon S, et al. Polymorphic imprinting of the serotonin-2A (5-HT2A) receptor gene in human adult brain. *Brain Res Mol Brain Res* 1998;59(1):90–92.
- Margolis RL, McInnis MG, Rosenblatt A, et al. Trinucleotide repeat expansion and neuropsychiatric disease. Arch Gen Psychiatry 1999;56:1019–1031.
- 105. Lipp O, Souery D, Mahieu B, et al. Anticipation in affective disorders. *Psychiatr Genet* 1995;5:S8.
- Nylander PO, Engstrom C, Shotai J, et al. Anticipation in Swedish families with bipolar affective disorder. *J Med Genet* 1994; 31:686–689.
- Gershon ES, Hamovit JH, Guroff JJ, et al. Birth-cohort changes in manic and depressive disorders in relatives of bipolar and schizoaffective patients. *Arch Gen Psychiatry* 1987;440: 314–319.
- 108. Penrose LS. The problem of anticipation in pedigrees of dystrophia myotonica. *Ann Eugenics* 1948;14:125.
- Hodge SE, Wickramaratne P. Statistical pitfalls in detecting age-at-onset anticipation: the role of correlation in studying anticipation and detecting ascertainment bias. *Psychiatr Genet* 1995;5:43–47.
- O'Donovan MC, Guy C, Craddock N, et al. Expanded CAG repeats in schizophrenia and bipolar disorder. *Nature Genet* 1995;10:380.
- O'Donovan MC, Guy C, Craddock N, et al. Confirmation of association between expanded CAG/CTG repeats and both schizophrenia and bipolar disorder. *Psychol Med* 1996;26: 1145–53.
- 112. Lindblad K, Nylander P-O, De Bruyn A, et al. Detection of expanded CAG repeats in bipolar affective disorder using the repeat expansion detection (RED) method. *Neurobiol Dis* 1995; 2:55–62.
- 113. Oruc L, Lindblad K, Verheyen GR, et al. CAG repeat expansions in bipolar and unipolar disorders. *Am J Hum Genet* 1997;60: 730–732.
- 114. Schalling M, Hudson TJ, Buetow KH, et al. Direct detection of novel expanded trinucleotide repeat in the human genome. *Nature Genet* 1993;4:135–139.
- 115. Vincent JB, Klempan T, Parikh SS, et al.Frequency analysis of large CAG/CTG trinucleotide repeats in schizophrenia and bipolar affective disorder. *Mol Psychiatry* 1996;1:141–148.
- 116. Sidransky E, Burgess C, Ikeuchi T, et al. A triplet repeat on 17q accounts for most expansions detected by the repeat-expansion detection technique. Am J Hum Genet 1998;62:1548.