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? Alternatively, 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. 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.
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 bezugziffer, 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 ~1%, then the risk to first-degree relatives of BPD probands is ~10%. Similarly, the first-degree relatives of BPD probands are at increased risk of RUP disorders: if the general population risk if ~8% (1,2), the RUP risk to first-degree relatives of BPD probands is ~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 first-degree 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 monozygotic (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 = \[
\frac{[\text{MZ Concordance} \% - \text{DZ Concordance} \%]}{[100 - \text{DZ Concordance} \%]}
\]

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 ~18%. When one parent is UP (and the other parent is unaffected), there is ~16% risk of RUP illness and a ~3% risk of BPD. These risks are elevated compared to the general population risk of ~1% for BP and ~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 ~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 BP 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 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:

<table>
<thead>
<tr>
<th>No. of alleles shared</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of all sibling pairs</td>
<td>25%</td>
<td>50%</td>
<td>25%</td>
</tr>
</tbody>
</table>

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). 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 summed to provide evidence that a region contains a susceptibility gene.
with each other and the environment to produce these well-known 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 greater than 2 in a large fraction of ill people have been detected: one is human leukocyte antigen (HLA) for insulin-dependent diabetes mellitus [increased risk = \( \sim 3 \) (32)], and the other is apolipoprotein E in late-onset Alzheimer’s disease (33–35, 79, 80). Substantial sample sizes are required to detect initially (LOD \( \geq 3.6 \) or \( p \leq .00002 \)) loci that increase risk by a factor of 2. Nearly 400 affected sibling pairs are needed to have >95% power to detect initially (LOD \( \geq 3.6 \) or \( p \leq .00002 \)) 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 (\( 2 \times 2 \times 2 = 8 \)). 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 \( 10^{-5} \)). Independent evidence of confirmation of this finding was reported.

### TABLE 71.1. CONFIRMED LINKAGES IN BIPOLAR DISORDER

<table>
<thead>
<tr>
<th>Genomic Location</th>
<th>Principle Report</th>
<th>Independent Confirmations</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>18p11.2</td>
<td>Berrettini et al., 1994 (38) and 1997 (39)</td>
<td>Stine et al., 1995 (40); Nothen et al., 1999 (41); Turecki et al., 1999 (42)</td>
<td>Paternal parent-of-origin effect; see Schwab et al., 1998 (43)</td>
</tr>
<tr>
<td>21q22</td>
<td>Straub et al., 1994 (44)</td>
<td>Detera-Wadleigh et al., 1996 (45); Smyth et al., 1996 (46); Kwok et al., 1999 (47); Morissette et al., 1999 (48)</td>
<td>Velocardiopial syndrome region; possible overlap with a schizophrenia locus</td>
</tr>
<tr>
<td>22q11–13</td>
<td>Kelsoe et al., 2001 (49)</td>
<td>Detera-Wadleigh et al., 1997 (50) and 1999; (51)</td>
<td>See Freimer et al., 1996 (55)</td>
</tr>
<tr>
<td>18q22</td>
<td>Stine et al., 1995 (40)</td>
<td>McInnes et al., 1996 (52); McMahon et al., 1997 (53); De Bruyn et al., 1996 (54)</td>
<td>Principal report in a Canadian isolate</td>
</tr>
<tr>
<td>12q24</td>
<td>Morissette et al., 1999 (48)</td>
<td>Ewald et al., 1998 (56); Detera-Wadleigh et al., 1999 (51)</td>
<td>See Ginns et al., 1998 (60)</td>
</tr>
<tr>
<td>4p15</td>
<td>Blackwood et al., 1996 (57)</td>
<td>Ewald et al., 1998 (58); Nothen et al., 1997 (59); Detera-Wadleigh et al., 1999 (51)</td>
<td>See Ginns et al., 1998 (60)</td>
</tr>
</tbody>
</table>
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 ~1,200 samples, were assembled for metaanalyses (62). An affected sibling pair (N = 382 sibling pairs) metaanalysis yielded $p = 2.8 \times 10^{-8}$ 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 ~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. For example, J. Detera-Wadleigh et al. (45), who employed multipoint nonparametric analyses. 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 ~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 18p22. 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 is 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 4p4.1 region has been reported in a paper by Noten 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 ~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):

<table>
<thead>
<tr>
<th>Model/genotype</th>
<th>DD</th>
<th>Dd</th>
<th>dd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant</td>
<td>3R</td>
<td>3R</td>
<td>R</td>
</tr>
<tr>
<td>Recessive</td>
<td>3R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Additive</td>
<td>6R</td>
<td>3R</td>
<td>R</td>
</tr>
<tr>
<td>Multiplicative</td>
<td>9R</td>
<td>3R</td>
<td>R</td>
</tr>
</tbody>
</table>

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 ~4 (at age 75), whereas homozygosity for E4 increases risk by a factor of ~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.
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, 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, case-control 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 as 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-HT2A) gene (103), which is imprinted in specific brain regions. Imprinting has been described for

![FIGURE 71.3. An example of paternal imprinting.](image-url)
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 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 brain-expressed 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 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...
inheritance, and trinucleotide repeat expansion. These non-mendelian 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

Chapter 71: Bipolar Disorders: Review of Molecular Genetic Linkage Studies

of bipolar affective disorder to chromosome 18 in a sample of 57 German families. Mol Psychiatry 1999;4:76.


