# Molecular Genetics and Heterogeneity in Manic Depression

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## Abstract

Recent research has shown that there are X-linked and possibly chromosome 11-linked forms of manic depression as well as at least one other autosomal form. Segregation analyses of large affected families and the finding of genetic linkage between chromosome specific markers and manic depression mutations provide strong evidence that bipolar as well as unipolar forms of manic depression (MD) within the same family are inherited as a dominant gene disorder. This clarification of the etiology of certain types of depression should bring changed attitudes within psychiatry and may serve to stimulate discusssion of the role of evolutionary

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mechanisms. From a clinical point of view, it has now become possible to determine whether clinical (phenotypic) variation reflects the underlying genotypic heterogeneity of linkage. A preliminary analysis of data from four recent studies shows that there is no clear correlation between such clinical features as the ratio of unipolar to bipolar cases and the genotypic form of manic depression. Further recombinant DNA research, proven to be successful in other genetic diseases, can soon be applied to manic depression. The specific problems posed by manic depression for these techniques are discussed.

**Index Entries:** Manic depression; heterogeneity, genotypic; X-linkage; chromosome 11-linkage; variation, phenotypic.

## Manic Depression and Genetic Analysis

Manic depression is a severe mental illness that has a lifetime prevalence of about one percent. It can be expressed as either a bipolar disorder with elated phases and grandiose delusions, followed by depression, or simply as a recurrent unipolar depression. The depression that occcurs as part of the bipolar illness, as well as the recurrent unipolar depression, may be severe with suicidal ideas and delusions of self blame and sometimes with auditory hallucinations of voices denigrating the patient. Many other types of depression exist, and it is sometimes not possible to differentiate a mild unipolar "manic depressive" depression when there is no evidence of mania or hypomania from an environmentally-caused, unipolar depression. It has been well known for a long time that the recurrence risk of unipolar and bipolar disorders is high in the first- and second-degree relatives of manic depressives. However, modern methods of segregation analysis have only recently shown that the pattern of recurrence is that of dominant Medelian inheritance with age of onset variation (Pauls, 1985). The most convincing proof of dominant gene transmission has come from the recent genetic linkage research (Egeland et al., 1987; Baron et al., 1987) in which a standardized diagnostic system was employed (Spitzer and Endicott, 1978).

The first positive statistical investigation of X-linkage in manic depression was carried out nearly 20 years ago (Reich et al., 1969), and it has taken many years for this preliminary result to be confirmed by the statistical criterion of a log score ($\log_{10 \text{ and } 3}$ of the odds for linkage between marker and disease loci against nonlinkage) above 3 in two separate studies. This has been achieved by Baron and coworkers in Israel (Baron et al., 1987) using the color blindness and gluscose-6-phosphate-dehydrogenase loci as linkage markers and also by Mendelwicz et al. (1987) in Belgium who used the taq I DNA polymorphism at the factor IX locus. The chromosome-11 linkage for manic depression has been reported by Egeland et al. (1987). In contrast, work in our own laboratory (Hodgkinson et al., 1987) and in the USA (Detera Wadleigh et al., 1987) also using the same diagnostic instruments has failed to confirm chromosome 11-linkage and has rejected this locus as being responsible in six extended kindreds. Unpublished work on an Irish family in Dublin (Gill, 1988) has produced another negative chromosome-11 result. We have also shown that the X-chromosome mutation near the factor IX gene is not responsible for causing manic depression in any of the five large families we have studied so far (Sherrington et al., unpublished data).

It is possible that modern cultural and social factors may have an influence on the expression of the MD genotype that has made it more or less penetrant than before. Some evidence that penetrance for manic depression has increased since 1940 has emerged recently (Gershon et al., 1987). A simple evolutionary explanation for the survival of the MD phenotype is that it arises as a number of random mutational events at various loci with relatively late expression in the

life cycle that have not affected fertility and fitness very much. The phenotype as a whole could be the result of such a mutation interfering with the brain systems controling mood and activity, but in itself the mutation has not played a role in the evolutionary development of these behavioral systems as a whole. A proportion of cases may of course be new mutations and chromosomal "hot spots" for such mutations may exist at specific loci.

The effect of a specific mutation will be modified by other genes, an effect known as epistasis. Such epistasis may involve many genes or a single gene. Recent work in *Drosophila* genetics has found the unexpected effect that the variance of a trait may increase rather than decrease after there has been an experimentally induced restriction of the number of alleles (genetic bottleneck) in a breeding population (Bryant, 1986). Using the definition of epistasis current in population and quantitative genetics when it refers to all non-allellic gene interactions. It seems possible, therefore, that relatively isolated populations, such as the Amish, may demonstrate epistatic effects not seen in more heterogeneous populations.

## Clinical Heterogeneity and Genotypic Forms of Manic Depression

The finding of linkage heterogeneity for manic depression has complicated the outlook for future linkage studies. It will now be necessary to apply statistical tests for heterogeneity when conducting studies on relatively small family units when no single family provides enough statistical power. Diagnosis in the recent genetic linkage studies was uniformly made using the Research Diagnostic Criteria (RDC) of Spitzer and Endicott (1978). The system includes the categories of Bipolar I (mania) and Bipolar II disorder (hypomania) as well as major depressive disorder (unipolar depression). Unipolar depressions may be psychotic or non-psychotic. In order to determine whether certain clinical features of manic depression are specific to the recently determined genetic subtypes, we carried out a comparison across the published linkage studies. For the purposes of comparison, we counted schizo-affective cases together with bipolar I and bipolar II cases. This comparison is shown in Table 1. Only those individuals over the age of 18 have been counted as being at risk.

The largest sample is that of the Israeli study. The proportion of those at risk who received affective disorder diagnoses is highest in Iceland and lowest in the Amish ($chi2 = 6.71$, d.f. = 1, $p < 0.01$). This could be a result of differences in age distribution within the families or because of environmental and polygenic background effects. The proportion of cases of depression who are schizo-affective or bipolar as opposed to unipolar is highest in the Amish (74%) and lowest amongst the Icelandic (46%), but the differences do not reach statistical significance ($chi2 = 3.24$, d.f. = 1, $p < 0.1$) even when cases of unipolar depression complicated by alcoholism are excluded. It is noteworthy that alcoholism, combined with unipolar depression, is confined to the Icelandic populations. When the rates of unipolar depression, uncomplicated by alcoholism, are compared between the Icelandic populations and the nonalcohol drinking, Sephardic Jewish families, similar incidences are found. Schizo-affective mania is found rarely in both the X-linked as well as the non-11/non-X forms. It is interesting to note that the pedigrees published by Hodgkinson et al. (1987) and by Baron et al. (1987) show specific families both in Iceland and Israel in which the bipolar rather than the unipolar type of expression is predominant. It is possible, therefore, that there may be genetic or environmental familial factors influencing penetrance that are more important than the role of a specific mutation. The analysis is preliminary and we are currently carrying out statistical work to examine differences in age related penetrance for the genotypically defined

Table 1
Comparison of Clinical Diagnoses
in Genetic Subtypes of Manic Depression

| | Chromosome 11 Linked, Amish | Non 11 and Non X, Iceland | Non 11 and Non X, USA[a] | X linked, Israel |
|---|---|---|---|---|
| Number at risk | 66[b] | 73 | 26 | 122 |
| Number with no diagnosis of depression | 47 (71%) | 29 (40%) | 11 (42%) | 75 (62%) |
| Number of all affective disorder cases | 19 (29%) | 44 (60%) | 15 (58%) | 47 (38%) |
| Number of schizo-affective and bipolar cases | 14 (78%)[d] | 20 (46%)[d] | 9 (60%)[d] | 24 (52%)[d] |
| Number of unipolar cases | 5 (26%)[e] | 18 (41%)[c,e] | 4 (27%)[e] | 20 (43%)[e] |
| Number both unipolar and alcoholic | 0 | 6 | 0 | 0 |
| Number alcoholic | 0 | 0 | 1 | 0 |
| Number cyclothymic | 0 | 0 | 1 | 3 |
| Number schizo-affective | 1 | 0 | 1 | 4 |

[a]Larger number of individuals studied, but details not published (Detera Wadleigh et al., 1987).
[b]Number over the age of 18 (K. Kidd, personal communication).
[c]Excluding alcoholic unipolar affective disorders.
[d]Percentage of all affective disorder cases.
[e]Percentage of all affective disorder cases.

forms of manic depression. Even though genetic linkage has not yet brought about a subclassification of manic depression on clinical grounds, considerable benefit could be obtained from precisely defining the mutations causing the illness. Once this has been achieved the abnormal protein(s) could be identified, and as a result more new effective treatments could be designed. The problems in reaching this goal are discussed below.

## From Linkage to Locus: Recombinant DNA Approaches to Define the Mutations Causing Manic Depressive Psychosis

The mutations responsible for manic depression may be deletions, base pair mutations, or some type of genetic rearrangement such as gene conversion. Techniques to define deletions have successfully been used in the study of Duchenne Muscular Dystrophy (DMD) and Chronic Granulotomatous Disease (CGD). Kunkel et al. (1985) made use of an unusual DMD patient who had an interstitial deletion on the short arm of the X chromosome at Xp 21.1. As a result of using the Phenol-Enhanced Reassociation Technique (PERT), one clone detected genomic DNA deletions in some DMD patients and was closely linked to the disorder. Chromosome "walking" identified further DNA in this region. Nonrepetitive DNA segments, which were highly conserved in many species, were identified using "zoo" blots by Monaco et al. (1985) and these were used to find candidate mRNA transcripts for the DMD gene. Such an approach could work for manic depression if such a deletion was identified. Our own survey of the cytogenetic studies of manic depression has not revealed a single reported instance of a case associated with a chromosomal abnormality. Chronic granulotomatous disease is another disorder with a chromosomal deletion mapping to this area of the X chromosome. The deletion was identified cytogenetically in a combined CGD/DMD patient by Francke et al. (1985). Genomic clones generated by Monaco et al. (1985) that mapped to the Duchenne Muscular Dystrophy deletions also mapped to the deletion found in CGD. B-Cell lymphoblastoid lines created from a patient with CGD and a cytogenetic deletion were used to prepare mRNA. After subtractive screening with cDNA prepared from mRNA from normal phagocytic cells, the resultant cDNA clones were hybridized with the deletion clones. One clone identified a phagocyte-specific mRNA that was absent from some patients and which exhibited a deletion in others (RoyerPokora et al., 1986). Such an approach could not easily be accomplished in manic depression because of the complexity of brain mRNA and also because mRNA is not readily available except at postmortem where it may have degraded.

An example of a molecular approach when there was no apparent cytogenetic deletion, but where the disease locus was shown to be on chromosome 7 by the DNA linkage markers MET and J3.11 is that which has been applied to Cystic Fibrosis (Escivill, 1987). In this instance, the critical finding that enabled successful fine mapping was the observed linkage disequilibrium between one of the linkage markers and the disease mutation. Normally, linkage marker polymorphisms are randomly associated with mutations even when they are close enough to show linkage in genetic studies. However, if they are sufficiently close to each other so that very little recombination between the two has taken place during evolution, then both mutation and polymorphism will remain linked together and show up as a population association beween marker and disease among unrelated affected individuals. This is known as linkage disequilibrium or allelic association. Estivill et al. (1987) used physical mapping procedures to show that MET and J3.11 were very close to the disease locus and created cell lines that contained transgenomes including them. From these lines, a nonmethylated CpG

rare cutter cosmid library was created. This enabled the isolation of Hpa II tiny fragments (HTF) islands that identified coding sequences. Clones were then selected on the basis of showing strong linkage disequilibrium with CF. "Zoo" blots were used to select clones that were highly conserved across species and these, in turn, were used to screen lung cDNA libraries for candidate genes.

Such an approach is, of course, only feasible in MD when the localization of the disease locus is known and where markers sufficiently close to be in linkage disequilibrium with MD are identified. The problems of gentic heterogeneity must be considered carefully when attempting to establish such disequilibrium. One approach is the prior establishment of a manic depressive individual's genetic subtype by linkage analysis of the patient's family, followed by association analysis across unrelated cases who share a common subtype. Another approach is to obtain a sufficiently large sample of affected cases from many different families and then to hope that linkage disequilibrium for a particular subtype will show through the noise. The success of the latter approach depends on just how heterogeneous manic depression turns out to be. At the present time, probably only one in three or less of all families are X-linked (Baron et al., 1987), and only one in seven families so far are of the chromosome-11 subtype. Confirmation of the chromosome 11-linkage has not yet been achieved either by extending the analysis to further affected cases in the Amish, or by finding chromosome 11-linkage in other families. Therefore it is still possible that the Amish result is spurious and that there are simply two forms of manic depression. This latter eventuality will make linkage disequilibrium more easily established.

Work in our laboratory is underway using highly polymorphic markers (Jeffreys et al., 1985; Nakamura et al., 1987) that provide linkage information from every family in order to localize the non-11/non-X type of manic depression (Hodgkinson et al., 1987). We have previously described some of the technical and methodological problems associated with this approach (Hodgkinson et al., 1987). The hypervariable markers may be localized or unlocalized to specific chromosomes. In the latter case, it is necessary to obtain high resolution autoradiographs of restriction fragments and to study families with at least 10 or so affected cases.

This is necessitated by the fact that the unlocalized markers are very numerous and because each family will have fragments of different sizes potentially linked to a disease locus. Hypervariable clones that have been localized to specific chromosomes are less problematic but only provide information about single loci rather than the "shotgun" linkage information obtained from the unlocalized clones.

Other workers are investigating the role of candidate genes such as that encoding a subunit of the beta adrenergic receptor clone in manic depressive families (Wright and Reich, personal communication). Pharmacogenetic approaches such as the one employed by Dewar and Reading (1971) before the days of gene cloning may also be rewarding if modern methods are employed. These researchers found changes in brain messenger RNA concentrations in response to lithium carbonate treatment (a treatment for manic depression) in rat brain. Now that cDNA clones can be constructed and identified qualitatively rather than by the quantitative analysis of total mRNA, then this approach can be repeated with a greater chance of success in identifying genes involved in either the treatment or the causation of manic depression. The problem with this approach is that it depends on using an animal model that may not be applicable to humans. Secondly, drugs that are used to treat manic depression may not in fact alter brain mRNA levels and have other sites of action.

Despite the problems of heterogeneity, molecular biology seems poised to increase our understanding of manic depression much more both at the level of the genome and also in terms of gene expression.

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