ability, a second for PKC levels. "When we broke the code, we found a very significant correlation between activity of PKC in the hippocampus...and performance," Wehner says. And that finding suggests the PKC gene is one of the many genes that influence spatial learning.

RI mice are convenient to use because they can be ordered from Jackson Lab, sparing the researcher the laborious job of making and analyzing genetic crosses. And since all the mice in any RI strain are genetically identical, researchers from different teams can easily compare their work to see if they are on the trail of the same genes. The drawback of using RI mice, however, is that there are only a few dozen RI strains derived from any two parent strains such as C57 and DBA. And that severely limits the statistical power of any analysis that uses the mice to link genes or regions of DNA to behavioral traits.

So before drawing any firm conclusions, Wehner plans to take her analysis further using another new technique, quantitative trait locus (QTL) analysis, proposed 5 years ago by Eric Lander and David Botstein, then both of the Massachusetts Institute of Technology. QTL makes it possible for researchers to identify multiple regions of DNA that contain genes that contribute to a single trait in rats, mice, or any other species for which inbred laboratory strains are available (see box on page 1691).

Wehner's QTL analysis will involve crossing DBA and C57 mice and genetically characterizing hundreds of second-generation mice with random assortments of DBA and C57 genes, then testing them for learning ability and enzyme levels. That's a daunting amount of work, says Wehner, but she thinks that in this field it's better to be safe than sorry. "Learning is very difficult to study because of both genetic and environmental influences, and I don't want to have to say, 'Oh, those were putative loci, [but] none of them panned out'"—as has happened repeatedly with candidate behavioral genes both in animals and in humans.

The caution Wehner is showing seems to come with the territory in studying genes and behavior. Though many behavioral genes have been identified in animals, the indirect relationship between genes and behavior, along with the influence of the environment, makes hunting for behavioral genes a risky business. And once researchers manage to find a gene or group of genes that shape behavior, they may still be a long way from knowing precisely how those genes exert their influence. Given the difficulties, it is safe to say that persistence is a quality (perhaps even a genetically determined one) that will be required of behavioral geneticists for many years to come.

–Marcia Barinaga

MANIC DEPRESSION

Highs and Lows on the Research Roller Coaster

A decade ago, many researchers were confident they would soon have proof that a mutation in a single gene can have a dramatic effect on complex human behaviors. They had found many mutations that can influence at least some forms of behavior—those that cause profound retardation, for example—but they wanted to find a gene more directly involved in causing subtler effects. Specifically, they thought they would be able to find a gene for manic depression. Encouraged by the triumphs of internal medicine, several groups set out in the 1980s in high hopes of tracking down the putative manic-depression gene, using techniques that had proven spectacularly successful in locating marker DNA for Huntington's disease and Duchenne muscular dystrophy in 1983 and markers for retinoblastoma and cystic fibrosis 2 years later.

Among the behavioral disorders, manic depression was at the top of the list of new targets because its symptoms are clear-cut. The roller-coaster ups and downs of manic depression, researchers believed, would make it relatively easy for clinicians to identify patients with an inherited illness. Through "linkage analysis"—looking for common genetic markers among patients from families with a high incidence of the disorder—they expected to find a gene. Not everyone in the field agreed; some thought a disorder as complex as manic depression must be produced by multiple genetic traits interacting with one another and the environment. But the medical model had been so successful that by the mid-1980s the hunt was on in earnest.

Since then, researchers looking for a manic depression gene have been riding an emotional roller coaster of their own. The high came early: Between 1987 and 1988, psychiatric geneticists published several reports linking manic depression to specific regions of the human genome. By 1989, however, that exhilarating "up" gave way to a vertiginous decline. Authors began revising their work and challenging others' findings. One by one, the statistical results linking manic depression with a particular region of the human genome began melting away, leaving a residue of failure.

Today, there are signs that optimism is building again, as gene hunters acquire better maps of the chromosomes and faster methods of testing DNA. The field also has received a vote of confidence from the Charles A. Dana Foundation, which last year awarded $2.5 million to a new consortium searching for a manic-depression gene. This infusion of cash and enthusiasm hasn't yet overcome all the disappointments of the past 10 years, however. Psychiatric researchers still hope to find a gene for manic depression, but they're less confident than they were in the 1980s. Indeed, like their colleagues looking for genes for alcoholism (see story on p. 1696), they've become somewhat gun-shy, although there's little doubt that the disease has a genetic component.

Elliot Gershon of the National Institute of Mental Health (NIMH) acknowledges that he and his peers have become hesitant to publish. When Gershon spoke with *Science*, he was awaiting publication of his new finding in the *Proceedings of the National Academy of Sciences*—a report with Wade Berrettini of Thomas Jefferson University in Philadelphia linking manic depression to a region of human chromosome 18. Gershon says they "had a lot of fear and trepidation" about sending the paper out. Caution and

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Demons of depression. A 19th-century engraving shows depression (or melancholia, as it was then known) in the form of blue devils.

self-doubt have become a way of life, Gershon said, largely because "this field could not survive" another round of unconfirmed findings like those of the 1980s.

Caution was not the mood on 26 February 1987, the day *Nature* published an article by psychiatrist Janice Egeland of Miami University Medical School in Florida. Co-authored and vetted by many geneticists and statisticians, Egeland's report made a groundbreaking claim: It linked a stretch of DNA on chromosome 11 with manic depression in members of a large Old Order Amish family in Pennsylvania. Susceptibility appeared to be passed by a single, dominant gene.

If correct, this would have been the first evidence that a complex behavioral disorder could be inherited in a simple, Mendelian pattern. In the words of Egeland and her colleagues, the discovery had "broad implications for research in human genetics and psychiatry." It promised "significant clinical benefits," perhaps enabling doctors to pinpoint those at risk in families afflicted with a high incidence of manic depression and provide them with preventive care.

The research looked solid. Egeland, who lived among the Amish for years, had identified patterns of inherited disease in 32 families. She and her colleagues followed up with independent genetic and psychiatric analyses. A panel of psychiatrists—ignorant of personal identities or family ties—reviewed the diagnoses, labeling individuals as affected or unaffected. A separate team drew blood samples and extracted DNA. Egeland's collaborator Daniela Gerhard of Washington University in St. Louis studied several markers that occur at known locations on chromosome 11. The team found that in one family, many individuals affected by the illness carried a particular variant of a DNA marker on the short arm of chromosome 11. This suggested that a gene for manic depression might be located close by.

In the last phase of analysis, the researchers tested the significance of the apparent linkage between this DNA marker and the disease. The results, calculated by computer programs known as LINK-MAP and LIPED, showed that the likelihood of a linkage being due to chance was extremely small. Expressed as a "lod" score (a logarithm of the odds of an event occurring by chance), the statistical significance came out at between 4 and 5. This meant the odds of the association being accidental were minute: between 1 in 10,000 and 1 in 100,000.

It took only a few months, however, for the euphoria over the Amish study to turn to disappointment and depression. Other scientists who hoped to clone the gene began churning through the data, and what they found was discouraging. Led by John Kelsoe, then a postdoc at NIMH and now a research psychiatrist at the University of California, San Diego, and Edward Ginns, Kelsoe's supervisor at NIMH, this team failed to find evidence of any linkage to chromosome 11. They joined up with Egeland and her colleagues to dissect the study, and in the process uncovered several problems.

The biggest was the familiar challenge of getting a correct diagnosis of patients—always difficult in behavioral studies. But in this case, the error didn't occur because researchers made a wrong diagnosis; rather, two key subjects changed category after they had been classified. One young man who had shown no signs of illness and had therefore been classified as nonaffected began to show those signs in his late 20s, soon becoming a full-fledged manic-depressive. A similar change occurred in another subject. Both had originally been classified as healthy, and neither carried the DNA marker that had been linked with the disease. When their status "flipped," as Kelsoe says, the impact on the study was devastating. It ripped through the odds calculations, dropping the lod score to near 2. Later analysis lowered the score further. The linkage between the short arm of chromosome 11 and manic depression began to look like genetic "noise."

JERRY IRWIN/UNIPHOTO

Raised high. A barn raising among the Amish, the group that provided the setting for a 1987 study linking manic depression to a region on chromosome 18; the finding failed to hold up.

The reversal of the Amish study, says Berrettini, "spawned a whole subfield" of testing for statistical vulnerabilities in genetic linkage studies, led by Susan Hodge and David Greenberg at Columbia University in New York. Hodge reanalyzed the Amish pedigree, person by person, to see how changing the diagnosis for each subject would affect the final lod score. The statistics, she found, are often highly dependent on the health or illness of just a few key individuals—or even a single one. The important lesson, says geneticist Kenneth Kidd of Yale University, is that researchers learned that their statistical methods were not foolproof.

Other problems had come out in the reanalysis of similar studies of manic depression and schizophrenia in the 1980s. A group led by Hugh Gurling of University College, London, had reported a high lod score linking schizophrenia among Icelandic and English families to a region of chromosome 5. This finding was set aside when others failed to replicate it, and Gurling's group itself announced lower lod scores on reanalysis. Likewise, Miron Baron of Columbia University, who with colleagues in Israel published a report linking manic depression and a region of the X-chromosome, eventually withdrew the finding as well.

Some of the confusion in the late 1980s arose not from errors in classifying individuals, but from misinterpreting the significance of DNA variations. In their eagerness, researchers may have read too much into interesting-looking regions of the genome. For instance, Ginns of NIMH concedes that it was premature to zero in on the short arm of chromosome 11. The experience "told us that it's dangerous to focus on a particular region" unless you've already identified it as significant with a "robust" lod score. By that, he means higher than 2 or 3. Gershon simply says that a few research groups jumped the gun, rushing to publish before they had enough genetic information to establish a positive finding.

All of these methodological problems are soluble, and researchers are now putting into place solutions to most of them. But there is another problem that is not so easy to resolve, and it continues to haunt the community of manic-depression researchers: the possibility that no single gene plays a major role in the illness. That possibility was largely discounted in the earlier work. For example, the LINK-MAP and LIPED programs used by Egeland assume that the trait under investigation is passed from one generation to the next as a single dominant gene on a non-sex chromosome. Yet if the disease is the result of the interaction of multiple genes, the standard analytical programs may not produce meaningful results.

If disorders such as manic depression are the result of many genes acting in concert, the pattern of inheritance would be extremely difficult to decipher using today's analytical tools, most of which rest on single-gene strategies that were so successful in rooting out the cause of cystic fibrosis. Ginns now argues—and many of his peers agree—that the best way to proceed is to scan as much of the genome as possible, looking for linkage with markers scattered throughout all the chromosomes before zeroing in on any

one locus. At least that should ensure that the gene with the highest lod score is the best candidate for further investigation. But what if several genes are at work?

There are no efficient methods at present for pinpointing the sources of multi-gene disorders, although some geneticists like Berrettini hope that new techniques that have worked with lab animals (see box on page 1691) will pan out in human studies. For now, the more ambitious gene hunters have little choice but to combine familiar methods of analysis with a brute-force search strategy called the whole-genome scan. Armed with detailed maps of the genome and versatile "PCR-able" marker systems, they are combing through each subject's complement of 23 chromosomes, looking for markers that stand out from the background noise. Once the genome has been surveyed, these genetic surveyors hope to zero in with detailed studies of multiple hotspots.

This change in approach poses some difficult questions for researchers: Is it reasonable to publish claims of linkage based simply on the results of a high-scoring linkage analysis of one gene? Or would it be more responsible to complete a full genome scan first? Finally, considering that genetic marker maps are growing more complex and sophisticated every week, how thorough must a scan be before it is considered adequate? At the moment, the research community is spread out along a spectrum. At one extreme are those who plug along as in the past, targeting single genes for analysis, testing one promising candidate after another. At the other are those who insist that whole-genome scans are the only credible approach. In the middle are those who use a bit of both techniques.

One strong advocate of the whole-genome sweep is David Cox, a geneticist who collaborates with David Botstein at Stanford University. Botstein belongs to the Dana Foundation consortium, along with James Watson, director of the Cold Spring Harbor Laboratory in New York, and clinical director Kay Jamison and psychiatrist J. Raymond DePaulo of Johns Hopkins University in Baltimore. During a meeting of experts sponsored by the Foundation at Cold Spring Harbor last December, Cox urged researchers to speed up the pace of whole-genome scanning. He thinks too many are still focusing on individual genetic loci that give some appearance of linkage to manic depression. This strategy, he says, is like using a small net to snag a fish out of a pool, when it would be better to drain the pool and collect all the fish from the bottom. Doing whole-genome scans—though more time consuming—is like draining the pool, Cox says. But he feels that some investigators would still rather take their chances at "winning the lotto"—hitting one gene that is responsible for manic depression.

While whole-genome scans are more labor intensive than the "lotto strategy," many research projects have begun to take the more difficult path, from necessity. One group taking the laborious route includes Ginns, the NIMH geneticist who tests markers in the Egeland collaboration on the Old Order Amish. Ginns says, "We have now looked at 40% to 50% of the genome" in 200 Amish subjects, about 30 of whom are affected by the disease. Ginns has been conducting his scan with 300 markers. That means he must do 200 people times 300 markers, or 60,000 individual genotypings. Ginns thinks it could take another 300 or more markers to finish the scan—assuming nothing turns up along the way—requiring another year or two of tedious lab work. Ginns says, "I don't know of any group that has finished a whole-genome scan yet." Until that's been done, he argues, it's premature to conclude that there is no single major gene for manic depression.

While several groups are moving forward on whole-genome scans in limited populations, none has reported a strong finding (see table). Some have signaled that they are close, however. For example, Berrettini says he has received support for his chromosome 18 work from peers at Indiana and Johns Hopkins Universities. And Ginns says he thinks that another project led by Nicholas Barden of Lavalle University in Montreal may soon have something to report. Barden is working with two large families in an isolated French-speaking community in Quebec. Aided by a national health-care system which maintains computerized medical files, Barden says, "for the last couple of years, we have just been doing genotyping" of 130 individuals. He began with a candidate-gene approach, because "it seemed such a vast undertaking to go through the entire genome." But now he recognizes that the broader approach is "much more logical." In fact, the scan has already located a "particular area" of the genome which Barden is excited about. But he declines to say more.

Gershon also hopes to do whole-genome scans on his collection of manic-depressive families. He belongs to a collaboration that includes John Nurnberger at Indiana University, Theodore Reich at Washington University, and DePaulo. In Europe, another team under the aegis of the European Science Foundation includes Peter McGuffin at the University of Wales, Jacques Mallet of the French Centre Nationale de la Recherche Scientifique, Jean Weissenbach of Généthon, and others. Gershon concedes that the European group has a strong lead in the race because it has devoted less time to standardizing diagnostic regimes and to organizational issues.

Whether the new investments in technology and whole-genome scanning will pay off with a breakthrough is impossible to say. "I don't actually know how optimistic we should be," says Botstein. He warns that "all the false starts of the 1980s" suggest that the genetic etiology of manic depression is unlikely to be as simple as that of cystic fibrosis. But, like many other researchers, he is encouraged by the fact that "we now have a rough-and-ready map of the human genome" with markers at regular intervals that gives more "resolving power" than old maps based on fragmentary data, available 2 years ago.

It is "definitely worthwhile," Botstein says, to scan the genome in the hope of hitting pay dirt. The dimensions of the task are unknown, however. It all depends on whether the puzzle consists of 17 pieces or 17,000 pieces, says Botstein. "If it's 17,000 pieces, I'm not going to be able to put it together." But if there is a single important gene, he is confident the new methods will find it.

–Eliot Marshall

SCANNING THE WHOLE GENOME

Researcher*	Affiliation	# of Subjects	# of Markers	% Completed
Nicholas Barden	Lavalle U.	130	600–800	30%
Miron Baron & T. Conrad Gilliam	Columbia U.	1500	300–400	45%
David Botstein & J. Raymond DePaulo	Stanford U. Johns Hopkins U.	400	200–400	30%
William Byerley	Univ. of Utah	96	200–500	90%
Wade Berrettini & Elliot Gershon	Thomas Jefferson U. NIMH	400	500	50%
Janice Egeland & Edward Ginns	Univ. of Miami NIMH	200	300–600	45%
Nelson Freimer	UC San Francisco	125	500–600	80%
Daniela Gerhard	Washington U.	300	400	60%
John Kelsoe	UC San Diego	660	400	15%
Jacques Mallet	European Sci. Fdn.	1000+	400	30%
John Nurnberger	Indiana U.	660	300–400	33%
Theodore Reich	Washington U.	225	400	40%

*Names represent groups; numbers represent goals. Groups and databases overlap.