GENETICS

Manic-Depression Findings Spark Polarized Debate

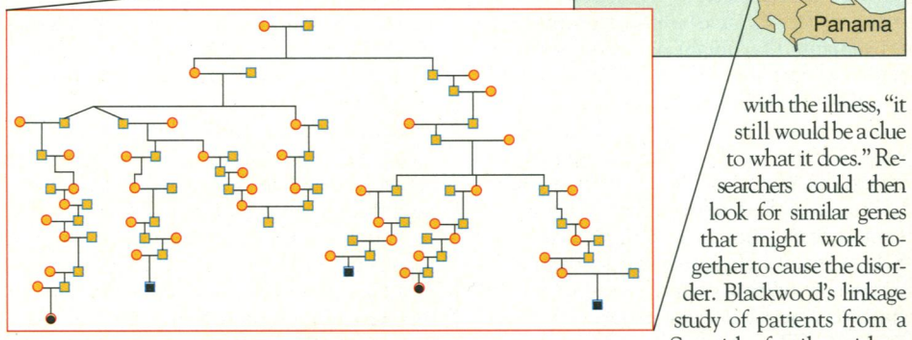
For researchers trying to decipher the genetics of manic depression, the past decade has been a long "down" period with few "up" interludes. The search for a genetic basis for the disease—a serious, often fatal psychiatric condition characterized by dramatic mood swings—has turned up a variety of candidate chromosomes, which have been announced, disproved, and cast aside with dispiriting frequency (*Science*, 17 June 1994, p. 1693). The reason, critics say, was that disease traits were often ill-defined, and researchers were looking for a single responsible gene when the illness likely involves interactions among many of them.

Now two research groups have published some of the first studies designed to search for multiple genes. But—in a debate mirroring the polarized nature of the disease—some researchers see their results as signs of an upswing, while others take a darker view. These investigations, which appear in the April issue of *Nature Genetics*, involve painstaking scans of the whole genome, searching for multiple genetic markers associated with the disease. One study identified several possible loci, the most promising being a mutation on the long arm of chromosome 18. A second report points to candidate loci on chromosomes 6, 13, and 15. "This is a paradigm for the field which people are beginning to take more seriously," says Ed Ginns, a neurologist at the National Institute of Mental Health and an author of this second paper. A third paper in the same issue used a variation on the older "single-gene" methodology and found evidence for a marker on chromosome 4.

Among those who see real progress, particularly in the chromosome 18 work, is Nicholas J. Schork, a statistical geneticist at Case Western Reserve University in Cleveland. "It's a clever study," he says. Both of the multiple genes studies, other researchers say, used a strict definition of the disease likely to have the strongest genetic basis. But others look at the divergent results—each study points to different regions on different chromosomes—and are dismayed. In a harshly worded commentary accompanying the papers, David Botstein and Neil Risch, geneticists at Stanford University, write that none of the reports differs much from the earlier studies that could not be replicated, and none show much statistical significance. "To tell you the unvarnished truth," Botstein told *Science*, "not one is convincing. They read as if they have strong results, when at best they are weak."

Those are the kinds of criticisms that felled earlier manic-depression studies and, ironically, led to the multigenic hypothesis behind two of the current reports: When the early work failed to pan out, researchers began to suspect that more than one gene was involved. Nelson B. Freimer, a human geneticist at the University of California, San Francisco, and his colleagues saw a chance to find those genes in two extended, genetically isolated families in Costa Rica's Central Valley.

Both families have a high incidence of extreme manic depression—also called bipolar disorder 1 (BP-1)—and both were established by a small number of founders in the 17th century, which increases the probability that current patients inherited their disease genes from a common ancestor. In such groups, researchers have shown that extended chromosomal regions ("marker haplotypes") are inherited with disease genes more often than in a wider population—a phenomenon called "linkage disequilibrium." To find these haplotypes, Freimer's team screened the patients' entire genomes via traditional genetic linkage analysis, which pointed them to promising regions. The most promising was on chromosome 18, where they noted marker haplotypes that were identical among almost all the BP-1 patients, but which were rare in the general Costa Rican population. "This suggests a relatively narrow region where one of the genes might be," says Freimer, who hopes to confirm these findings by identifying the same linkage disequilibrium in randomly selected BP-1 patients from the Central Valley.



Family ties. In Costa Rica's Central Valley, individuals with extreme manic depression (dark circles and squares) can be traced back to a common ancestor, yielding genetic clues to the disease.

The second multigenic study, by Ginns and several colleagues, screened 551 markers in 207 individuals in 17 Old Order Amish families that have a high incidence of BP-1. The researchers looked for alleles (gene variants) that were inherited more often than is expected by chance in the affected individuals. They used the results to generate "lod" scores (a measure of an event occurring merely by chance). For the loci on chromosomes 6, 13, and 15, Ginns's produced scores that ranged from 1.5 to 2. In a study looking for single genes, contributions are generally considered meaningful only if they are greater than 4. But Ginns regards his lower scores as positive, for if genes on several chromosomes are involved in the disorder, "the individual contributions need not be so large." He notes that another study relied on similar scores to locate several genes that may be involved in insulin-dependent diabetes.

The third study, by Douglas H. R. Blackwood, a psychiatrist at Edinburgh University, used traditional linkage analysis, a method that allows scientists to home in on rare dominant genes that could cause disease. Although Blackwood agrees with the other researchers that BP-1 is probably not passed along this way, he notes that although the technique is designed to pinpoint just one gene associated with the illness, "it still would be a clue to what it does." Researchers could then look for similar genes that might work together to cause the disorder. Blackwood's linkage study of patients from a Scottish family with a high incidence of the disease produced a high lod score of 4.8, pointing to a region on chromosome 4. Their finding was strengthened when they discovered the same association in 11 other Scottish families vulnerable to the disorder.

Robert Elston, a genetic statistician also at Case Western, says publication of these three papers will help the field along. "Any time you put a testable hypothesis and the relevant data forward, it helps," he says. But Botstein and Risch believe the results might simply prove to be yet another diversion. Despite Ginns's explanation, they are particularly troubled by the low lod scores in his work. "Lod scores in that range," says Risch, "are what you'd expect from background noise. And without more data, it's difficult to

tell the difference." Even Blackwood's high lod score may be misleading, he continues. "Results like these have been presented before using the same approach" and did not hold up. "So until the research is replicated, I can't jump up and down about these scores." Moreover, the sample sizes are small enough to warrant caution. Finally, Risch and Botstein note that some of Freimer's markers don't seem strongly associated with the illness, and they fault him for not performing a formal statistical analysis that might indicate whether the haplotype frequency in patients with the disease was merely the result of chance.

"I just wonder how many times you can cry wolf without damaging the public's perception of this research," says Risch. At least 14 different chromosomal regions (including the five new ones, as well as one that Botstein's own group recently announced) have now been reported to contain manic-depression genes, he says. "We have four genes for Alzheimer's, two for breast cancer, yet not one for manic depression despite all of these intensive searches. Why is that? Now we have five more loci being claimed to harbor genes. Given the past history of the field, it's not clear to me which if any of these is real."

The three researchers counter that Risch and Botstein are far too pessimistic. Their emphasis on lod scores is misleading, Blackwood says. "It's only part of the picture. We use it to pinpoint interesting regions, then go to these sites to see if there's something going on, such as chromosomal rearrangement" that might account for the genetic malfunction. "What we've done is put forward a testable hypothesis," says Freimer, "which we are now testing." That test, via the independent Costa Rican sample, should reveal the statistical significance of the inheritance of the haplotypes his team discovered. Ginns, too, is pushing ahead. And Schork thinks these papers do the field a service by "pointing out the need to develop statistical methods for assessing complex disease traits. They shouldn't be chided because these tools don't yet exist."

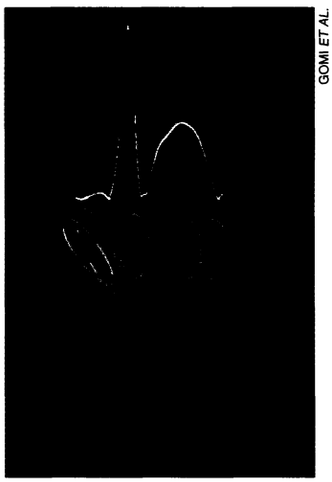
One area that all the researchers do agree on is that pooling data—both positive and negative—would speed the process along. "One of the biggest problems is that these studies and others only publish their positive findings," says Risch. "Maybe these studies have turned up some genes that seem to be only of modest effect, but if we merged our data, we'd see they are significant." Blackwood says he would be happy to provide data which did not appear in *Nature Genetics* because of space limitations to anyone who asks, while Freimer says his are under review at another journal. The Amish cell lines are available at a National Institute of General Medical Sciences repository in New Jersey. Such sharing might smooth out these research ups and downs.

–Virginia Morell

NEUROSCIENCE

Tilting Against a Major Theory Of Movement Control

At first glance, Hiroaki Gomi and Mitsuo Kawato's invention at the ATR Human Information Processing Research Labs in Kyoto, Japan, looks like a computer setup for arm-wrestling. But it's not. They use their apparatus—a semicircular steel table with a complex system of levers and shafts supporting an armrest above the mirror-smooth surface—to test the strength of the reigning theory about how the brain controls voluntary movements of the arm and other limbs. And as Gomi and Kawato report on page 117, the theory, known as the equilibrium point hypothesis, seems to have lost a round.



GOMI ET AL.

Out of sync. The ellipses show the subject's arm movements, with the predicted velocity profile in yellow and the actual profile in red.

The hypothesis implies that the brain does not compute all the forces required to move a limb from one place to another, but simply launches the limb, depending on reflexes and the intrinsic elasticity of the muscles to get it to its destination. That's possible, the theory holds, because that intrinsic springiness makes opposing muscles seek an equilibrium, or balance, whenever the brain perturbs the system by setting the limb in motion—hence the name equilibrium point hypothesis. But by tracking a moving arm and measuring its stiffness along the way with their invention, Gomi and Kawato have undermined the hypothesis, providing evidence that the brain is in fact in control throughout a movement, doing all the calculations necessary to figure out what muscles to move and when.

The Japanese findings join an earlier challenge to the equilibrium point hypothesis, reported in the October *Journal of Neurophysiology* by James Lackner's team at Brandeis University in Waltham, Massachusetts. If the U.S. and Japanese work is right—and not everyone agrees that it is—the researchers will have overcome an idea so entrenched that "it's becoming the folklore of motor-control science," says neuroscientist Gerald Gottlieb of Boston University.

Gomi and Kawato's experiment focused on a variant of the equilibrium point hypothesis that implies that the brain picks the endpoint for a movement and then specifies the trajectory the arm will follow by triggering a sequence of muscle contractions that ensure it reaches that final position. Based on their calculations, Gomi and Kawato predicted that an arm following an equilibrium point trajectory should accelerate rapidly, slow down, and then speed up again before coming to its final destination. Previous work by other scientists had also indicated that the arm should remain stiff throughout the movement.

Designing an apparatus to test those predictions was not easy. "It's a major technical problem to measure stiffness during movement without disrupting the movement," says neurophysiologist Allan Smith of the University of Montreal. It requires a lightweight apparatus that can move as fast as the human arm while being strong and rigid enough to measure the arm's stiffness and movement. To achieve this, Gomi and Kawato designed a setup consisting of an arm brace linked to a motor-driven spindle controlled by a computer, all suspended above the table by flowing air to minimize the effects of extraneous forces on the arm. Even Emilio Bizzi of the Massachusetts Institute of Technology (MIT), who helped develop the version of the equilibrium point hypothesis Gomi and Kawato tested, describes their apparatus as "ingenious."

For the experiments now being reported, three people performed a series of arm movements that each repeated eight times. The subject sat in the chair and extended his or her right arm over the chest-high table, setting it into the arm brace and grasping a handle. The subject then moved the arm to a target displayed on a computer screen at the end of the table as sensors in the brace and handle relayed position and force data back to the computer, which plots the actual trajectory of the arm.

Sometimes, the computer allowed the limb to proceed unimpeded. But at other times, it caused the apparatus to push on the arm a little, too quickly for the brain to adjust for the perturbation. Such perturbations check whether the arm is indeed following the trajectory calculated by Gomi and Kawato.

As the researchers soon learned, the actual trajectories differed from what they pre-