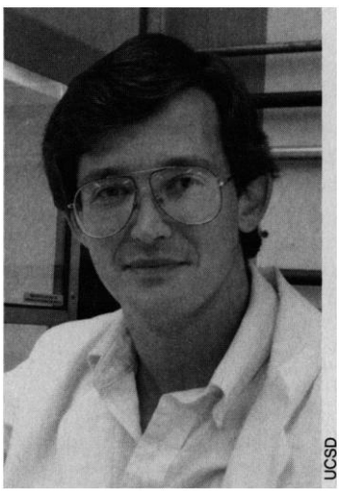
Manic Depression Gene Put in Limbo

A new study has failed to confirm that manic depression in the Amish is caused by a single gene on chromosome 11

When John Kelsoe began follow-up work on a landmark manic depression study, he didn't plan to author a paper that would turn that study on its ear. But that is what happened. The paper, published in the 16 November issue of *Nature*, reveals that the widely ballyhooed 1987 genetic study of inherited manic depression in the Amish of Pennsylvania was premature in its claim to have located the gene that causes the illness.



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Gene seeker. *John Kelsoe couldn't find the manic depression gene he was looking for.*

Kelsoe and his collaborators at the National Institute of Mental Health and the National Cancer Institute had originally set out to isolate the gene they presumed had been properly located by Janice Egeland of the University of Miami and her coauthors. But they found instead that they couldn't reproduce the published mapping study, dashing the hopes of many who felt Egeland's work would lead to better diagnosis of manic depression—even a cure someday. "I don't think any of us could have predicted what we're now publishing," says Edward Ginns, who was Kelsoe's postdoctoral adviser at NIMH when the current work began.

But there may be a silver lining to this story. Along the way to their surprising conclusion, all the researchers involved learned some important, if sobering, lessons about the pitfalls of doing genetic analyses of illnesses as complex as manic depression—lessons that may apply equally well to other illnesses, such as schizophrenia and Alzheimer's disease, that may have more than one cause. And they also made some encouraging discoveries about the value of scientific cooperation.

When Kelsoe, Ginns, and colleagues discovered that they couldn't reproduce the published mapping study, they made a fateful, and unconventional, decision. Rather than begin a long battle of contradictory claims, they called up principal author Egeland to see if the two research groups could put their heads together and work out the discrepancies. "We felt this finding was so important that we really wanted to present a clear message to the rest of the scientific community, and that's why we pursued it in this way," says Kelsoe, who is now an assistant professor at the University of California, San Diego.

In the original work, which was described in *Nature* on 26 February 1987, Egeland and her colleagues performed a genetic analysis of an inherited form of manic depression in an Amish family. The Amish are ideal subjects for such a study because of their large family size, detailed genealogical records, and genetic isolation. All 15,000 members of the religious sect descend from just 30 couples who migrated from Europe in the early 18th century.

And, while the overall incidence of manic depression in the Amish is the same as in the rest of the population, a genetic tendency toward the disease seems to run in some Amish families. Statistical analyses suggested a single, dominant gene was responsible, and the 1987 study used a genetic technique called linkage analysis to localize it to the tip of the short arm of chromosome 11.

Kelsoe stresses there was nothing inherently wrong with that study. "They did excellent work, and there were no errors in what they did," he says, "but a number of things developed after their study." It was those later developments that caused the conclusions to crumble.

Back in 1987, the Amish study provided a ray of hope in a murky field. About 1% of the population of North America and Europe suffers the violent mood swings of manic depression. The disease is not inherited in all cases, and even in those in which it is, different genes might be acting. But it seemed likely that in the case of the genetically isolated Amish a single gene was responsible and that Egeland's group had beaten a path to the gene's door. Its identification would likely lead to clues about the development of the disease in the Amish and other sufferers as well.

Kelsoe, a psychiatrist trained in molecular techniques, embarked on his search for the gene in the time-honored way, by attempting to repeat the work of Egeland and her co-workers. The Miami researcher had made cell lines derived from the Amish subjects available through the Coriell Institute for Medical Research, in Camden, New Jersey. But when the NIMH group used the cell lines to look for evidence that the depression gene was linked to DNA markers on chromosome 11, they couldn't find it.

"Our lod scores [a logarithmic measure of the likelihood of linkage] were not very robust," recalls Steven Paul, who worked with Ginns and Kelsoe. Indeed, their scores were a pitiful 1 or so, indicating a mere 10 to 1 odds that the association of the gene with DNA marker sites on chromosome 11 was due to genetic linkage rather than chance. That was 10,000-fold lower odds than the lod scores of nearly 5 reported in the original paper and 100-fold lower than the score of 3, which is generally accepted as the minimum required to show genetic linkage. That's when they called Egeland.

What followed was an intense examination of all aspects of both studies. Members of both groups rechecked their analysis of the DNA markers carried by each individual in the pedigree. They rechecked each clinical diagnosis, which had originally been established by a panel of psychiatrists. And they reran their computer analyses to determine the likelihood of linkage.

Once they had corrected several misunderstandings about which subjects had been included in the first study, the NIMH group had no trouble reproducing the original high lod scores. But since 1987, Egeland's group had added more than 40 new subjects to the 81 in their original study, and two of the original subjects had become newly ill. And therein came the rub: Those changes were what sent the lod scores plunging.

To begin with, one of the two newly diagnosed manic depressives did not carry the DNA markers thought to be linked to the depression. It was obvious that his illness would hurt the case for linkage. The other newly diagnosed subject did have the markers, yet his diagnosis had an equally devastating effect on the lod scores. Together the two new positive diagnoses lowered the lod scores by about 1.5.

That a positive diagnosis, coupled with the right DNA markers, would cause a drop in lod scores was a puzzle at first, says Yale geneticist David Pauls, who did the statisti-

cal analysis for the original study. The explanation, he says, lies in the fact that the person has several siblings who have the same DNA markers, but are healthy, making the appearance in the brother of both illness and the markers look like a chance event.

The addition of several other new subjects did little to affect the lod scores. But 31 of the 40 added make up what is called a lateral extention of the pedigree—that is, they are linked to the core family by a marriage. And they dealt the scores another devastating blow. This new branch clearly has an inherited form of manic depression, but when Egeland and Kelsoe's teams analyzed the group independently, the depression gene carried by its members showed no linkage whatsoever to the markers on chromosome 11. And when the group was analyzed together with the rest of the pedigree, it lowered the lod scores to 1.

These gloomy results raised two possibilities: either the gene for manic depression in the Amish is not on chromosome 11 or the Amish have two such genes, one of which may be on chromosome 11. But the evidence to date cannot distinguish these possibilities and so it is back to the drawing board for the researchers.

How did it feel to Egeland's team to have the rug pulled out from under their results? "I would much prefer to hear it over the phone than to see it in print," says Yale's Pauls. "They didn't have to [call us]," he says, "but by doing it they really strengthened the scientific finding."

The lessons the group learned are myriad. With the capriciousness of lod scores, and the drastic changes that can be introduced with just one new diagnosis, it is vitally important for the results in such a study to be checked and rechecked, and for the study group to be followed for a long time to pick up any new diagnoses. Egeland adds that it is crucial to carefully define the diagnosis criteria used, since even one false positive diagnosis of illness can be devastating to the results.

Egeland, whose commitment to understanding manic depression in the Amish goes back 30 years, to a time when she lived with one of the affected families, said that watching the data weaken was a little like the experience of manic depression itself. "There was the euphoria and the ecstasy [when we thought] we had made a significant step in trying to track a gene for this common and terrible illness, and then, wham . . . lod scores drop when you don't expect them to drop, and you're down into the depression." But she says she is proud of the collaborative effort of the two groups and grateful for the lessons learned.

■ MARCIA BARINAGA

Deep Water: "Phase B" Is Decoded

Not all of Earth's water lies in its oceans, rivers, and lakes. Much of it may be locked hundreds of miles underground. But even though this subterranean water could hold the key to several basic questions about the behavior of Earth's mantle and the evolution of its environment, no one knows exactly how much water is down there.

Now a team of seven geophysicists at the Carnegie Institution of Washington and the State University of New York at Stony Brook has solved one piece of the puzzle (*Nature*, 14 September, p. 140). The researchers have decoded the structure of "phase B," the only mineral known to retain water at the intense pressures of the earth's deep mantle. The work should give new insights into the complex chemistry that goes on in the mantle, and it has already led to the prediction of other water-bearing minerals that may exist in the mantle, perhaps even deeper than phase B.

One of the reasons that geophysicists want to know the structures of such minerals as phase B is that it may help them determine where Earth's water came from, says the Carnegie Institution's Robert Hazen. Although the planet as it was originally formed had plenty of water, some theories suggest that the early earth collided with a Mars-sized object to form the moon, a process that would have stripped away all the surface water. If so, then much of the water on Earth today may have been released by volcanic activity from water-bearing rocks deep in the mantle.

Because scientists can't obtain rock samples directly from the mantle, they have been forced to try to duplicate mantle conditions in the laboratory by building presses that subject mineral samples to high pressures and temperatures. That was how phase B was discovered in 1967 by researchers who mixed water with normal silicates, such as quartz or feldspar, at more than 100,000 atmospheres of pressure. However, because no one was able to grow single crystals of the material, scientists were unable to determine its complicated crystalline and chemical structures.

That changed this year when Jaidong Ko, Tibor Gasparik, and Donald Weidner at Stony Brook used a split-sphere, multi-anvil press to mimic conditions that exist at about 360 kilometers below Earth's surface. A mixture of water, magnesium, and silicon heated to 1200°C at 120,000 atmospheres yielded phase B crystals that were up to 0.1 millimeter across. Weidner attributed the success to a combination of high technology and human skill. The Japanese-made press is state of the art for large presses, he says, and "Jaidong has a green thumb for growing crystals."

But even with the crystals, the phase B structure is so complicated that the team needed a bit of luck to be able to decipher it. Claude Herzberg at Rutgers University provided that luck earlier this year when he discovered a mineral closely related to phase B, but without the water. The Stony Brook team also succeeded in making single crystals of this mineral, called AnhB, for anhydrous phase B.

Because AnhB has a much simpler makeup than phase B, Hazen, Larry Finger, Russell Hemley, and Charles Prewitt of the Carnegie Institution were able to determine its structure from x-ray diffraction experiments. This in turn gave them enough clues to infer phase B's structure from the diffraction data they had accumulated on that mineral. "Larry [Finger, who calculated the crystalline structures] says he couldn't have done it without anhydrous B, because the crystals of phase B weren't of high enough quality," Hemley says.

The chemical composition of phase B is $Mg_{12}Si_4O_{19}(OH)_2$, while that of AnhB is $Mg_{14}Si_5O_{24}$. Specifying phase B's crystalline structure involves 40 atom positions, Hazen says, which makes it the most complex silicate structure described.

Finger and Prewitt have now used what they learned about phase B to predict structures for other water-bearing magnesium silicates that would be stable in the deep mantle. They have received a partial confirmation from Gasparik, who had independently synthesized a new mineral, called superhydrous B, that seems to have one of the compositions they predicted. If this is confirmed, superhydrous B would contain more water than phase B and be formed at even hotter temperatures.

In addition to shedding light on the origin of Earth's water, the properties of phase B may help explain some of the intriguing seismic behavior that takes place in a region of the mantle called the transition zone, Hemley says. "The complex chemistry we're inferring from these experiments may or may not be consistent with [this behavior]," he says. The next step, he adds, will be to determine the mechanical properties of the mineral and see if they can be fitted to what is observed seismically. ■ ROBERT POOL