NEUROBIOLOGY

Role of Gene Defect in Hereditary ALS Clarified

Earlier this year research on the devastating neurodegenerative disease known as amyotrophic lateral sclerosis (ALS) got a big boost when a multi-lab team identified the defective gene that causes one hereditary form of the condition. The discovery was particularly promising because the gene turned out to encode an enzyme called superoxide dismutase (SOD) that had already been widely studied. This stimulated hopes that the knowledge already accumulated on SOD would soon lead to a better understanding of what brings about the neuronal degeneration in patients who have the mutant gene —and perhaps also in the much larger number who have the nonhereditary form of the disease (*Science*, 5 March, p. 1393). When the initial report appeared (in the 4 March *Nature*), however, the researchers who had identified the gene were still in the dark about a key question: how the mutations they detected might affect the function of the SOD enzyme and cause nerve cell damage.

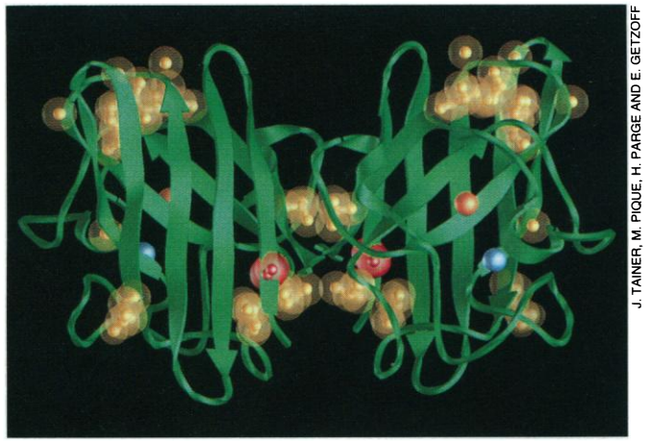
Now, another large team, including many of the same researchers who found the gene in the first place, has taken a big step toward answering that question. On page 1047, neurologist Teepu Siddique of Northwestern University Medical School in Chicago and crystallographers John Tainer, Elizabeth Getzoff and Hans Parge of the Scripps Research Institute in La Jolla and their colleagues report new work suggesting that the SOD mutations disrupt the three-dimensional structure of the enzyme, reducing both its stability and activity.

Norine Stirpe, director of research development for the Muscular Dystrophy Association which provided some funding for the study, describes the work as "very encouraging" because it provides an immediate guide for pursuing therapeutic strategies for ALS. If reduced SOD activity causes ALS, it might be possible to treat patients by giving them a good SOD gene or drugs that mimic SOD's action.

Beyond that, the finding provides direct evidence for a hypothesis long favored by neurobiologists—that ALS and other neurodegenerative disorders may be caused by damage done to nerve cells by free radicals. The job of SOD in the body is to help cells get rid of superoxide free radicals. These highly reactive molecules are given off by a variety of normal cellular activities and are very toxic. A decrease in SOD activity could therefore lead to the buildup of nerve-damaging superoxide radicals.

Paradoxically, however, an increase in SOD activity could also increase free radical levels. The reason: while SOD gets rid of superoxide free radicals, it also generates certain other types. At the time the *Nature* paper came out, says Siddique, who was one of its co-authors, "we speculated that maybe the activity of the enzyme is decreased—or maybe it's increased."

But the current work suggests that SOD overactivity is not the problem. In the new studies, Siddique and his colleagues have further analyzed the mutant gene in ALS families and found that any of 12 different amino acids in the SOD enzyme can be altered, although one change—the replacement of the alanine normally located at position four with a valine—was especially common, occurring in eight of the 23 families with SOD mutations. Then, the researchers mapped the locations of the mutated amino acids on the three-dimensional SOD structure determined by the Scripps group. The results, Tainer says, are "really striking."



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Getting bent out of shape. SOD mutations cluster in regions critical for maintaining the enzyme's three-dimensional structure.

None of the mutations is anywhere near SOD's active site, which is the part of an enzyme that performs its biochemical work. Instead, all are located in regions critical for maintaining the enzyme's three-dimensional structure. (Joseph Beckman of the University of Alabama, Birmingham, and his colleagues make a similar point for the original mutations in the 12 August *Nature*.) Take, for example, the predominant mutation at alanine four. To get an active SOD enzyme, two copies of the SOD protein chain must join together in a dimer; alanine four is located right where the two chains make contact. As a result of this and the other amino acid changes, the enzyme protein either can't fold up properly or can't form normal dimers, causing it to be destabilized, Tainer says.

The consequences of that destabilization can be seen in measurements made of the SOD activity in red blood cells taken from ALS patients. "We looked at six different mutants," Siddique says, "and they all have decreased activity," averaging about 40% of normal. To Stanley Appel of Baylor College of Medicine in Houston, who is also studying ALS, that finding is the most significant advance. "This really provides the data as to what the difference might be in the [mutated] enzyme," he says.

Still, this result may not be the whole story, cautions neurologist Robert Brown Jr. of Harvard's Massachusetts General Hospital, a collaborator in the original study but not in this one. Brown says his group has confirmed Siddique's observation that SOD mutants have decreased activity but notes that other alterations in the enzyme's activity haven't been ruled out.

Indeed, a great many questions remain about SOD's role in ALS. Among them: why the results of the mutations don't become apparent until mid-life and why they affect predominantly motor neurons. Especially important, notes Appel, is the question of whether the SOD work will also clarify the cause of the 90% of the ALS cases thought not to be hereditary. He's hopeful that it might.

Because the hereditary and nonhereditary forms of the disease are clinically indistinguishable, many ALS experts think that the final pathway of nerve cell destruction is the same in both. And Appel sees a possible connection between the SOD work and his own group's recent results indicating that sporadic ALS may be caused by antibodies that react with calcium channels, abnormally raising the internal calcium ion concentrations in nerve cells and bringing about their death. He points out that damage done to cells by free radicals might also increase intracellular calcium ion levels.

Much more work will clearly be required to clarify the origins of ALS. But the linkage of the SOD gene to the disease has had another encouraging effect, says Robert Abendroth, who chairs the research committee of the ALS Society. It's sparked a great deal of interest in researchers who want to follow up on the observation. The new work, he predicts, will provide a further stimulus. With enough researchers entering the field, some answers to longstanding questions might be forthcoming.

–Jean Marx