

AUGUST 30, 2001

## Epilepsy Gene Identified in Mice

Researchers have identified a novel gene that is mutated in mice that develop spontaneous epileptic seizures in response to loud noises. The brain protein affected by the mutation is unlike any other known to cause epilepsy in mice or humans.

In an article published in the August 30, 2001, issue of the journal *Neuron*, a research team led by Howard Hughes Medical Institute investigator Louis J. Ptacek at the University of Utah reported cloning and sequencing the gene that is responsible for an audiogenic form of reflex epilepsy in the *Frings* mouse strain. Although this mouse strain has been used in research for half a century, the genetic defect underlying the disorder had been unknown.

---

"The *Frings* mouse shows a form of audiogenic reflex epilepsy precipitated by sound. This is a common form of epilepsy in mice and is similar to the human reflex epilepsies triggered by stimuli such as strobe lights."

— Louis J. Ptáček

---

"The *Frings* mouse shows a form of audiogenic reflex epilepsy precipitated by sound. This is a common form of epilepsy in mice and is similar to the human reflex epilepsies triggered by stimuli such as strobe lights," said Ptacek. "Since the same anti-epileptic drugs that work in other forms of epilepsy also work in this form, we are hoping that this will be a generalizable and useful model of epilepsy."

In earlier work, Ptacek and his colleagues narrowed the search for the *Frings* defect to a region of DNA, or locus, on mouse chromosome 13. Compared to other mouse models of audiogenic epilepsy, where mutations were thought to reside at multiple loci, the *Frings* mice appeared to be unusual because their mutation was in a single locus.

After narrowing their search, Ptacek and his colleagues then set out to map this locus finely enough to identify the specific causative gene. "It was just a matter of brute force, in which we generated a colony of twelve hundred mice and performed genotyping on them to home in on the gene," said Ptacek.

"Eventually, we localized the gene to thirty six thousand base pairs and just sequenced that whole region." DNA sequencing showed that only one gene lay within the locus, said Ptacek. And within that gene, Ptacek and his colleagues found a single deletion of a DNA base pair that created a stop signal that truncated the normal protein, and rendered it nonfunctional. The scientist named the gene *mass1*, for "monogenic audiogenic seizure-susceptible."

"We did encounter one fascinating twist that complicated things," said Ptacek. "In comparing other mouse strains closely related to *Frings*, we found one that had many of the same genetic polymorphisms, but not the deletion. However another strain, called BUB/BnJ, did have the same deletion but was not known to suffer audiogenic seizures. So, we thought we might have a serious problem in demonstrating that this deletion caused epilepsy."

The scientists found that due to hearing loss, these mice lose their susceptibility to seizure during maturation and, thus, would not respond to seizure-inducing sound. When the researchers tested young BUB/BnJ mice, all of them showed such seizures. This finding, said Ptacek, demonstrated clearly that the *mass1* gene mutation caused audiogenic epilepsy.

Analysis of the MASS1 protein structure revealed that it did not resemble any known proteins, particularly ion channels that are known to malfunction in other forms of epilepsy. However, said Ptacek, the protein has multiple regions that resemble those of calcium-binding proteins, suggesting a possible role in regulating calcium flow in neurons.

"We're at the early stages of studying the protein and where it is found, and it has been a very difficult problem," said Ptacek. "An interesting complication is that the messenger RNA that is encoded by the *mass1* gene is so low in quantity in the mouse that it is undetectable."

Ptacek and his colleagues were unable to detect the gene initially by the usual technique of creating a library of complementary DNA to the cell's messenger RNA and searching that library. The paucity of messenger RNA (mRNA) could be due to a number of factors, said Ptacek. "It may be that the cell needs only small amounts of MASS1 protein or that it is highly stable and doesn't need to be produced in great amounts," he said. Another theory is that MASS1 mRNA might rapidly degrade or might be present only during embryonic brain development to wire brain circuitry properly.

The scientists are now developing antibodies to the MASS1 protein to use in tracing its location and interactions with other proteins. The scientists have also identified the human gene homologous to *mass1*, said Ptacek. In addition, they are collaborating with other scientists who have identified a family whose epilepsy appears to be caused by a mutation at the same locus as the human *mass1* gene. The researchers hope to be able to determine whether human MASS1 is the cause of this epilepsy.

"We believe that this protein represents an exciting opportunity to understand a completely different mechanism of epilepsy," he said. "The protein could

potentially be a novel therapeutic target, since all of the current anti-convulsant medications act against ion channels. And although these medications work very well on many kinds epilepsy there are still many patients whose epilepsy does not respond to them."