Toward Gene Therapy: *Lesch-Nyhan Syndrome*

By JULIE ANN MILLER

As ethicists argue about the morality of engineering human heredity, they often distinguish between two potential opportunities. One is the tinkering with genes to make people smarter, stronger, taller or otherwise better matched to a general ideal. The other possibility, which many genetic engineering skeptics find presents a more difficult dilemma, is the possibility that genes may be changed to offer aid to victims of devastating diseases.

Lesch-Nyhan syndrome, a rare form of cerebral palsy, is such a ruinous diease. Victims of the most severe cases are mentally retarded, spastic and aggressive, and they compulsively mutilate themselves by biting their lips and fingers. Scientists now report success in experiments seen as preliminary steps toward correcting the genetic defect, or at least providing means of detecting it before birth.

Mutation of a single subunit nucleotide of one human gene is responsible, at least in some cases, for this complex of symptoms, scientists report. And in different families with Lesch-Nyhan members, different points within the gene are abnormal. The techniques of recombinant DNA research recently have allowed biologists to identify and examine this X-chromosome gene and move it into cells growing in laboratory culture.

Now the investigators report also that a normal gene inserted into cells taken from a Lesch-Nyhan patient can make a functional product and correct the cells' biochemical defect.

While scientists are not yet ready to begin gene transfers for Lesch-Nyhan patients, they consider the isolation of this gene to be an important advance. It may help researchers to learn how the genetic defect leads to severe abnormalities in the nervous system. And it may open the way for investigation of other genes on the X chromosome responsible for human disease.

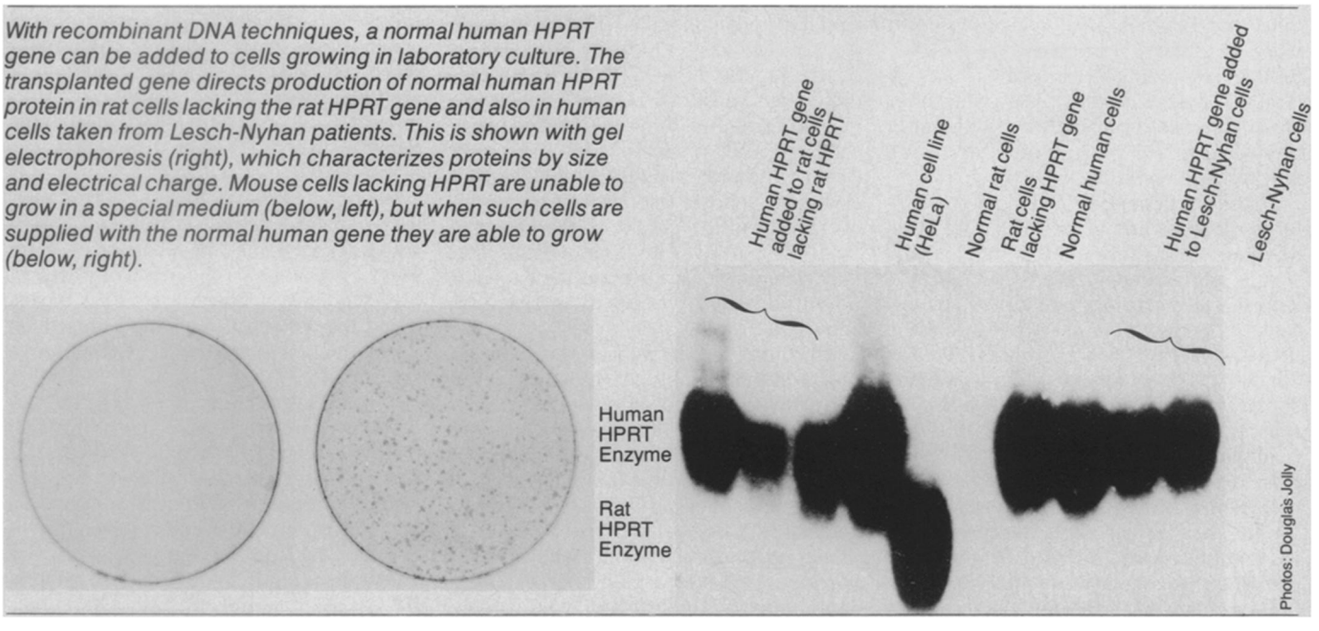
Lesch-Nyhan disease is due to an absence of, or serious defect in, a single enzyme called hypoxanthine-guanine phosphoribosyltransferase (HPRT), which is present at its highest level in the brain. This enzyme adds a sugar group to ring-shaped compounds, purines, as a step in their metabolism. Accumulation of unmetabolized purines appears to be the primary problem in Lesch-Nyhan syndrome. But it is still a mystery how this biochemical abnormality leads to the bizarre neurologic signs.

Lesch-Nyhan syndrome is not the only genetic disorder linked to the HPRT gene. A quite different set of symptoms, constituting a severe but treatable form of gout, is the result of less serious deficiencies of the enzyme HPRT. In these cases, overproduction of purines leads to an excess of their degradation product, uric acid. William N. Kelley of the University of Michigan in Ann Arbor estimates that about 0.15 percent of the U.S. population (or 0.5 percent of gout patients) has this type of gout.

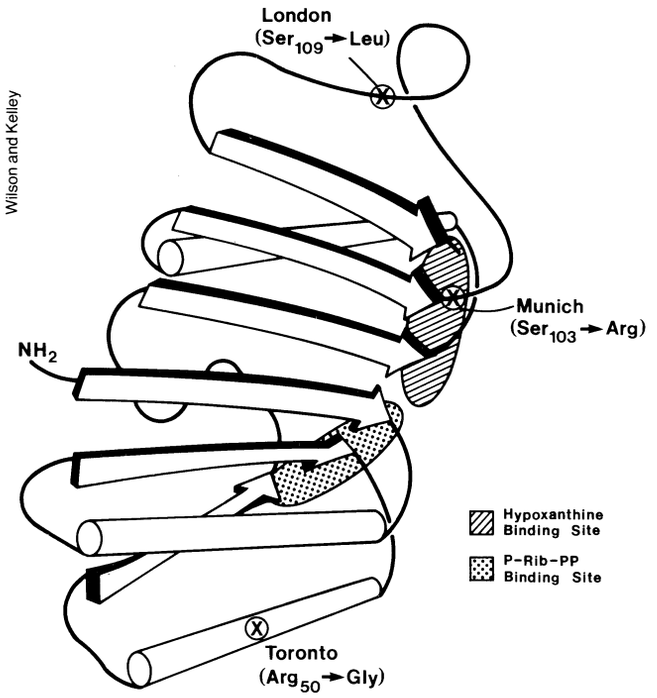
The first specific mutations to be determined in patients with Lesch-Nyhan syndrome, or with the related gout, were reported at the recent meeting in San Francisco of the American Society of Biological Chemists. Kelley and colleague James M. Wilson characterized the abnormal HPRT of four patients. In each case only a single amino acid was changed and that difference appears to be due to a change of a single nucleotide in the gene. In three of these cases the location of the mutation is in the part of the enzyme thought to bind the substrates upon which it acts.

A different type of genetic change has been implicated in experiments at Baylor College of Medicine in Houston. C. Thomas Caskey reports finding an altered pattern of DNA pieces, cut by a specific enzyme, from the DNA of one Lesch-Nyhan syndrome patient. The unusual pattern appears to be due to a deletion of a stretch of DNA from the HPRT gene.

At first, each unrelated patient studied showed a different specific defect in the HPRT gene. Now the most recent work by Wilson and Kelley suggests a second instance of a mutation observed earlier. Kelley says that the occurrence of a variety of mutations for a single disease contrasts with some other genetic disorders, such as sickle cell anemia, in which a single mutation is widespread. In these cases a person having one copy of the specific mutant gene is better able to survive and reproduce under some conditions than if he or she had two copies of the normal gene. (In the case of sickle cell, carriers of the gene in single copy have increased resistance to malaria.) But if no specific mutation confers such an advantage, an assortment of rare mutations will be present in the population.

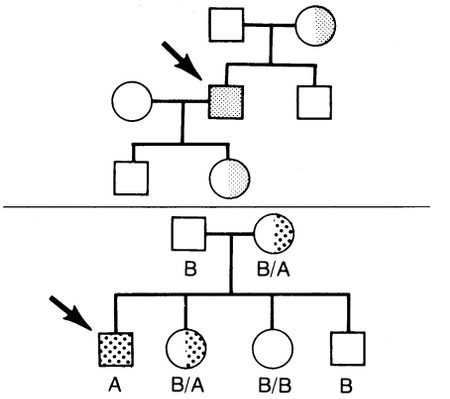


With recombinant DNA techniques, a normal human HPRT gene can be added to cells growing in laboratory culture. The transplanted gene directs production of normal human HPRT protein in rat cells lacking the rat HPRT gene and also in human cells taken from Lesch-Nyhan patients. This is shown with gel electrophoresis (right), which characterizes proteins by size and electrical charge. Mouse cells lacking HPRT are unable to grow in a special medium (below, left), but when such cells are supplied with the normal human gene they are able to grow (below, right).



Wilson and Kelley

The mutation responsible for gout in a Munich patient falls in the region of the HPRT enzyme where one of the substrates binds, according to this model of the protein's structure. The enzyme from this patient shows profound abnormalities in its binding of substrate and reaction rate. The enzyme from two gout patients from London and Toronto has more normal characteristics, and the mutations fall outside the binding sites.



New genetic methods enable determination of which normal members of a patient's family carry the defective HPRT gene on an X chromosome. Top: DNA analysis of the family of a gout patient (arrow) reveals the defective gene in his mother and daughter (half-shaded circles). Bottom: DNA patterns (A and B) in regions near the HPRT gene confirm that the mother and one sister (half-shaded circles) of a Lesch-Nyhan patient (arrow) each carry the defective gene.

The identification of specific mutations has led to a method for detecting defective genes in relatives of a patient. An example is the case of a Toronto gout patient whose HPRT has the amino acid glycine in place of an arginine. The DNA code for the arginine (CGA), but not for the glycine (GGA), forms part of the site cut by a specific DNA-snipping enzyme (the Taq I restriction enzyme). Therefore when DNA of people with this mutation is exposed to the restriction enzyme, a different pattern of DNA pieces results.

Using this procedure scientists were able for the first time to tell which members of a gout patient's family had inherited one copy of the defective gene. From the cut DNA pattern Wilson and Kelley made a family pedigree showing that the mother and daughter of the patient each have one normal and one abnormal copy of the gene. Thus there is a 50 percent chance that any sons of the daughter will have the disease. The scientists are now working to develop similar mutation-detecting techniques for two of the other mutations.

Another technique for making pedigrees of families of Lesch-Nyhan patients has just been reported by Robert L. Nussbaum, William E. Crowder and C. Thomas Caskey of Baylor College of Medicine in Houston and William L. Nyhan of the University of California at San Diego, the scientist whose research in 1963 led to identification of the genetic disorder. These investigators used a different DNA-cutting enzyme, called BamH1, to snip the chromosomes taken from normal subjects and Lesch-Nyhan patients.

Three different patterns of cut DNA were observed, but both normal subjects and patients had each type. Therefore, these variations are not dependent on the mutations responsible for Lesch-Nyhan syndrome, but are so closely associated with the HPRT gene on the chromosome that they can be used to make pedigrees.

Suppose a patient (who is male and thus has only one X chromosome) displays a cut-DNA pattern, call it A, and his mother shows two different patterns (one for each X chromosome), call them A and B. Then all sisters with at least one copy of the chromosome giving pattern A are carriers of the syndrome and have a 50 percent chance of passing it on to each of their sons.

In the July PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, (Vol. 80, No. 13) the researchers demonstrate the success of their technique with pedigrees of three families in which all females proven to be carriers have the same cut-DNA pattern displayed by the Lesch-Nyhan patient, but other females and unaffected males do not.

Two approaches have been used to study and isolate the gene underlying Lesch-Nyhan syndrome. Theodore Friedmann and colleagues at the University of California at San Diego put pieces of human DNA into mouse cells lacking HPRT. They then used a solution called HAT medium, which allows only cells with functional HPRT to grow, to select mouse cells containing the relevant human gene. Then the human genetic material was identified in the mouse cells by detecting neighboring stretches (called Alu sequences) characteristic of human DNA. The fragment of human DNA retrieved from the mouse cells was used to isolate the entire gene from a set of unidentified pieces of human DNA.

The gene for HPRT was also obtained by Caskey and colleagues. They started with mouse tumor cells containing repeated copies of the HPRT gene. The messenger RNA from such cells was used as a template to make complementary DNA. These laboratory-made DNA molecules were then employed to pick out the natural genes from sets of normal pieces of human DNA.

Both teams of scientists have been able to insert the normal genes into defective cells and to observe HPRT function. Each group put the normal genes in plasmids (rings of DNA) with control elements of RNA viruses. These plasmids can restore HPRT activity to HPRT-deficient rodent cells or to cells taken from Lesch-Nyhan patients and maintained in laboratory culture. In addition, by introducing both the HPRT-containing plasmids and RNA viruses into rodent cells, Friedmann and colleagues have obtained infectious agents capable of correcting HPRT-deficient rodent cells, making them able to grow in HAT medium.

"These developments promise rapid advancement in our knowledge of the structure and expression of the HPRT gene," Caskey and co-workers say. But the scientists are not ready to begin considering gene transfers for Lesch-Nyhan patients. Friedmann says there is a problem with the viruses used to carry the genes, or donate control regions, in the laboratory experiments. These viruses are associated with animal cancers, so should not be used in human transfers. Friedmann also is reluctant because so little is known about how the lack of HPRT leads to the nervous system abnormalities observed. "I'm not convinced that replacement of this enzyme willy-nilly will affect central nervous system function," Friedmann says.

Although he believes there is still a long way to go before researchers find a way to correct the deficiency in cells in Lesch-Nyhan patients, Friedmann says the isolation of the human HPRT gene opens the way for many important studies. It may help scientists learn how genes affect neurological function and behavior, and, because it is one of the few genes isolated from the X chromosome, it may aid in study of other X-linked genes responsible for color blindness, hemophilia and some forms of muscular dystrophy and mental retardation. □