Closing In on the Muscular Dystrophy Gene

Two groups of researchers are very close to the gene for Duchenne muscular dystrophy and expect to have it isolated within a year

Two groups of investigators, using different strategies, are closing in on the gene for Duchenne muscular dystrophy—the most common X-linked genetic disease. They are so close that they are likely to isolate the gene within 6 months to a year. Already they have developed tightly linked genetic markers for the gene that can detect carriers with 98 percent accuracy. Moreover, the methods they used are expected to be applicable to the search for genes associated with other inherited disorders.

Although molecular geneticists are becoming more and more adept at finding genes, the search for the muscular dystrophy gene breaks new ground. In the past, genes were found because investigators knew in advance what their protein products were. Only recently have they begun looking for genes without knowing what the genes do. About 2 years ago, a group of researchers announced that they were close to finding the gene for Huntington's disease, a dominant inherited disease, but they used different methods than the investigators looking for the muscular dystrophy gene and their methods are not so generally applicable to rare and recessive genetic disorders (*Science*, 25 Nov. 1983, p. 913).

The attempts to locate the Duchenne muscular dystrophy gene began in earnest several years ago when researchers learned from two separate lines of evidence approximately where it is located on the X chromosome. Then Louis Kunkel and his colleagues at Children's Hospital in Boston proposed looking for the exact location of the gene by analyzing in detail that region of the X chromosomes in boys who have deletions of the muscular dystrophy gene. At the same time, Ronald Worton and his colleagues at the Hospital for Sick Children in Toronto proposed looking for the gene in children with translocations that interrupt the muscular dystrophy gene (*1*). Now it is clear, says Donald Wood, associate director of research at the Muscular Dystrophy Foundation in New York, that "both approaches work."

Although there are ten forms of muscular dystrophy, the Duchenne form is by far the most common childhood type. It occurs about once in every 3000 to 4000 male births, and one-third of these cases arise from new mutations. Hemophilia, another well-known X-linked disease, occurs only once in every 10,000 births. Other X-linked disorders are even less frequent.

Duchenne muscular dystrophy is a muscle-wasting disease. It begins slowly but, by age 5 or earlier, afflicted boys have difficulty standing and will get up from the ground by walking their hands up their legs. They will find it hard to climb stairs and may not be able to jump. Invariably, by the time they are 12 years old, they will be in wheelchairs.

The muscle wasting progresses upward from the boys' legs to the rest of their bodies. The muscle cells actually die, although no one has any idea why or how this occurs. Eventually, the diaphragm muscles are affected and the boys die of respiratory failure. Nearly all Duchenne muscular dystrophy patients die by their mid-20's.

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Muscular dystrophy has long been thought to affect only males but, geneticists now realize, sometimes, very very rarely, females do get the disease. It is this realization that helped initiate the search for the muscular dystrophy gene. In 1977, R. H. Lindenbaum of Oxford University diagnosed the disease in a young girl and determined that the child's X chromosome was broken at position Xp21, a dark band near the middle of the short arm. Usually in human females one or the other X chromosome is active in each cell, and which X that is seems to be determined at random. But this girl with muscular dystrophy had the broken X active in every one of her cells.

Once this girl was diagnosed, several others with the disease were found and now, says Worton, he knows of 12 girls with Duchenne muscular dystrophy. In every case, the girls have a break in their X chromosome and, in every case, the break is in approximately the same area. Because the break probably causes muscular dystrophy by inactivating a normal X chromosomal gene, the "muscular dystrophy gene" is almost certainly a normal gene that no longer functions properly.

At about the same time as the physical evidence indicating the general location of the muscular dystrophy gene was reported, it was confirmed by evidence of another sort. Kay Davies of Oxford University, Robert Williamson of St. Mary's Hospital in London, Peter Pearson of the University of Leiden, and others completed traditional linkage analysis studies, and their results also pointed to Xp21 as the site of the gene. So, as of 3 years ago, geneticists had narrowed down the position of the gene to this general area, which constitutes about 10 million base pairs and represents about 20 percent of the short arm of the X chromosome.

Early this year, Kunkel's group got the lead it needed to start searching for the gene itself. Kunkel wanted to find a boy who had Duchenne muscular dystrophy and who had a deletion of the muscular dystrophy gene. He planned to pinpoint the deleted area, which should put him very close to, if not within, the actual gene. The problem was to find a patient who clearly had a deletion.

Kunkel and his associates found this patient last year when Uta Francke of Yale University and Hans Ochs of the University of Washington in Seattle described a boy who had three X-linked diseases: Duchenne muscular dystrophy, retinitis pigmentosa, which causes blindness, and chronic granulomatous disease, which affects the immune system. Francke and Ochs saw that an area of the boy's X chromosome was deleted and proposed that the deletion caused his diseases. They confirmed their suspicions by testing more than 30 fragments of the Xp area of the X chromosome and eventually finding that a fragment sent by Pearson was absent from the boy's chromosome and, therefore, was deleted.

Francke and Ochs supplied samples of the boy's cells to Kunkel and his colleagues Anthony Monaco and William Middlesworth. They added fragments of the boy's DNA with its deletion to fragments of X chromosomal DNA from an individual with no deletion to find pieces of DNA in the normal X chromosomes that are missing from the X chromo-

somes of the boy's cells. They found seven small regions of normal X chromosomal DNA that are absent from the boy's X chromosome and, by continuing to analyze the DNA in this area, they have found a 38,000–base pair segment that is missing from patients with muscular dystrophy who are known to have deletions in the area of the muscular dystrophy gene (*2*). This area, they propose, may contain all or part of the muscular dystrophy gene.

While Kunkel's group was doing its analysis of the DNA from the deletions, Worton and his colleagues Peter Ray and Margaret Thompson were pursuing a slightly different strategy. Their idea was to study a female patient whose muscular dystrophy was caused by a translocation within the muscular dystrophy gene itself. The junction of the translocated DNA in the X chromosome must be within or very close to the muscular dystrophy gene, Worton proposed.

One of the female patients had been studied in Worton's laboratory 3 years earlier by Christine Berellen, a research fellow from Belgium. The patient, who also is from Belgium, had a translocation involving a block of ribosomal RNA genes on chromosome 21. This made her an ideal candidate for the kind of studies that Worton wanted to do. Roy Schmickel of the University of Pennsylvania had cloned the ribosomal RNA genes, so Worton reasoned that he could use fragments of the cloned genes to pull out the region on the X chromosome that was at the junction of the translocation. He did this and, like Kunkel, has cloned DNA fragments from the region he isolated. "We're probably about as close to having the muscular dystrophy gene as Kunkel is," says Worton, "although we may be on the other side of the gene."

Both Kunkel's group and Worton's group are now trying to isolate the gene itself and to find out what it does, which may have profound clinical implications. It is not a straightforward task, however, to go from the isolated DNA segments to the gene. "It's a backwards approach to genetic diseases," Worton remarks. Yet there are several obvious strategies, and both groups are trying all the strategies at once.

One approach is to look for gene products. If there were a piece of messenger RNA that is normally transcribed from the regions isolated by Kunkel and Worton and if that mRNA were missing from cells from muscular dystrophy patients or present only in an altered, possibly shortened form, then the gene coding for that mRNA would be a prime candidate for the muscular dystrophy gene. But it is not clear where to look for such an mRNA. An obvious place is muscle tissue, yet it is possible that the muscular dystrophy gene product is produced elsewhere. Both groups are hedging their bets by looking in a variety of tissues. Kunkel, for example, is looking in brain cells, fibroblasts, lymphocytes, and liver cells as well as in muscle. "We are looking at as many kinds of cells as we can," he says, but speculates that he still might not find the mRNA he is looking for if the muscular dystrophy gene turns out to be one that is turned on only briefly during development.

Another strategy is to identify a set of deletions that span the muscular dystrophy gene. By looking at the DNA from a large number of patients, Kunkel's group and now, more recently, Worton and his colleagues expect to find a number of patients with different deletions that, taken together, define a common region deleted in patients. This might be a likely candidate for the gene itself.

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Even though they do not yet have the exact location of the gene, Kunkel and Worton are close enough for their work to have real clinical significance. The areas of DNA that they have pinpointed can be used to detect genetic markers that can identify carriers of the gene and also can be used for prenatal diagnosis. Recognizing the importance of a really accurate set of markers, Kunkel has offered his probes for the muscular dystrophy gene to clinical investigators who wish to use them to detect carriers. He began sending them out several months ago, and so far he has sent them out to about 40 researchers, all of whom agreed not to use them to look for the gene in the same way that Kunkle is looking and also to send him samples of any patients who turn out to have deletions in the region where the gene is.

But those who are using Kunkel's probes are delighted with them. "It has really improved diagnostic accuracy. It's a *remarkable* improvement," says C. Thomas Caskey of Baylor University. Previous probes had a 10 to 15 percent error rate, whereas the new probes are 2 percent or less inaccurate. Caskey, whose laboratory is a national referral center, says his group has 15 families under study and has completed its analysis for nine of them. The researchers have made prenatal diagnoses for three pregnancies and, in each case, predicted that the children did not inherit the muscular dystrophy gene. They are currently conducting prenatal diagnoses of four other pregnancies. Others, including Worton's group, Kunkel's group, and the group headed by Davies, are also doing prenatal diagnoses.

Two of the three couples who went to Caskey and were told that their male fetuses are normal have elected to continue their pregnancies. The third couple decided to terminate theirs. These parents, says Caskey, were unwilling to trust so totally in his analysis and chose to abort their male fetus rather than risk having a child with muscular dystrophy. Although Caskey is quite confident that his predictions are accurate, the proof of his diagnoses will not be in until the boys are between 2 and 5 years of age and are still symptom free.

In addition to doing prenatal diagnoses, Caskey is able to tell women who have already had a child with muscular dystrophy whether the child inherited the muscular dystrophy gene from the mother—in which case any other boys she had would have a 50 percent chance of getting the gene—or whether the muscular dystrophy arose as a new mutation—in which case any other boys she had would not be at risk. So far, says Caskey, his group has found both situations.

Caskey is one of the handful of researchers in this country who hope soon to try gene therapy. Obviously, the possibility of treating muscular dystrophy patients with gene therapy is very much on his mind. He predicts that within the next 2 years, gene therapy will be attempted, but muscular dystrophy patients will probably not be among the first to have this new treatment. Still, he says, if gene therapy works and if—as everyone expects—the muscular dystrophy gene is isolated soon—there is, Caskey remarks, "every reason to think of gene therapy for Duchenne muscular dystrophy."

But for now, Caskey notes, his lab has all it can do to just keep up with the increasing demands from families with at least one child with the disease who want to know who carries the gene and who does not.—**GINA KOLATA**

References

1. C. H. Verellen *et al.*, *Human Genetics* **67**, 115 (1984).
2. A. P. Monaco *et al.*, *Nature (London)* **316**, 842 (1985).