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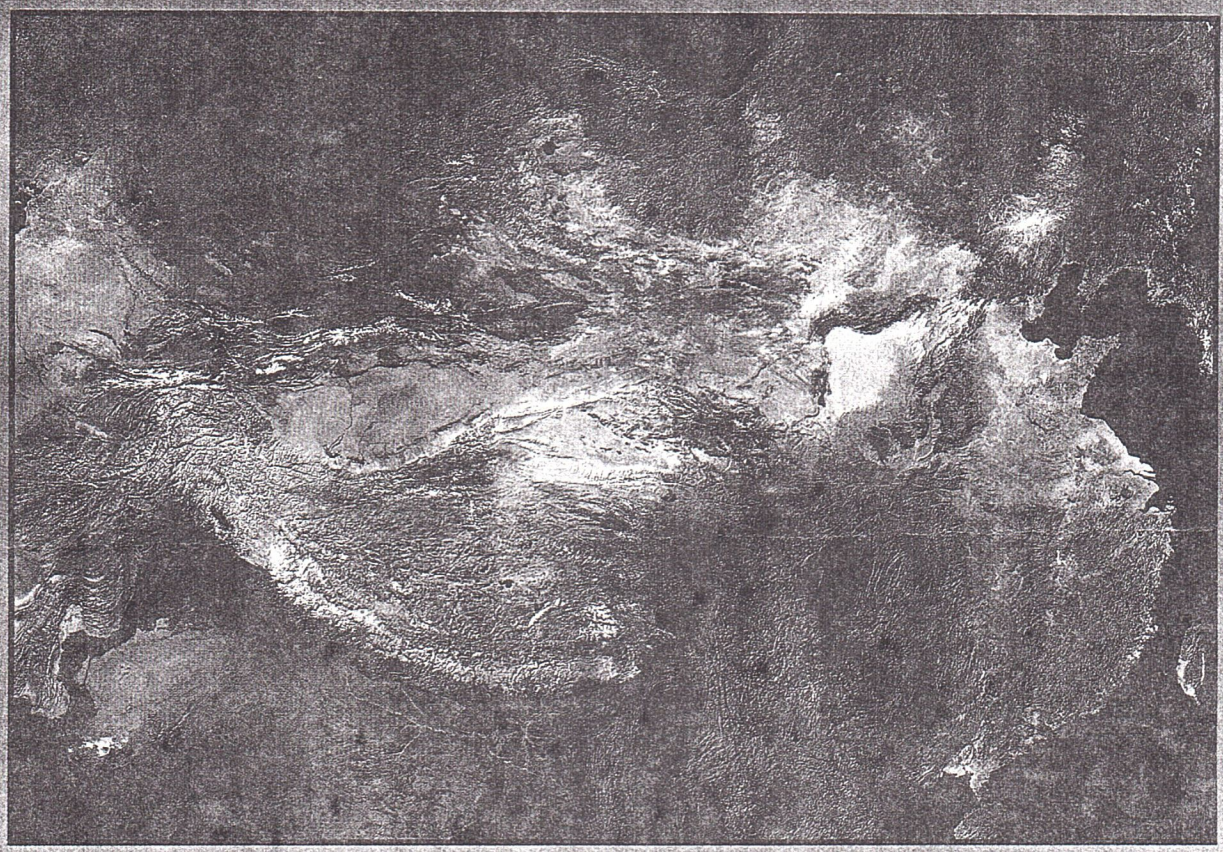

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## SCIENCE IN CHINA

## *A Great Leap Forward*

# Annual Genetics Meeting: Some Puzzles, Some Answers

**MINNEAPOLIS**—Over 3800 geneticists gathered for the 45th Annual Meeting of the American Society of Human Genetics (ASHG), held here from 24 to 28 October. Many were disappointed that a talk on the defective gene that causes Bloom syndrome was canceled at the eleventh hour—reportedly because of fears that advance publicity would jeopardize the publication of the new finding (*Science*, 10 November, p. 909). But most found plenty else to tickle a geneticist's fancy, as attested to by these two reports.

## Making Sense of Myotonic Dystrophy

With much aplomb, the gene sleuths announced in 1992 that they had identified the defect that causes the inherited disorder known as myotonic dystrophy (DM). It was, they said, an abnormal triplet repeat (a sequence of the same three nucleotides occurring again and again) in a gene encoding a protein kinase, a class of enzymes that controls the cell's metabolic machinery. It soon became clear, however, that a single defect even in a key enzyme like a protein kinase would be unlikely to account for everything about DM—a disease that afflicts one in 25,000 Europeans with a broad range of symptoms that may include respiratory distress in infants, early balding in men, diabetes, muscle wasting, and mental retardation. Indeed, says geneticist Eric Hoffman of the University of Pittsburgh School of Medicine (UPSM), "myotonic dystrophy just doesn't make sense. It sticks out like a sore thumb."

But a poster by Hoffman and his colleague Amelia Morrone, also of UPSM, may help solve at least some of the mysteries surrounding DM. Their findings suggest that the RNA produced by the mutant kinase gene somehow interferes with the cell's handling of RNAs produced by other genes, which may explain DM's dramatic range of symptoms.

"It's an absolutely novel idea," says molecular biologist Luba Timchenko of the Baylor College of Medicine in Houston. "So far everyone has tried to figure out the mechanisms that cause DM by looking at the DNA or the protein. If the [Hoffman] data are correct, it could explain many of the [disease's] contradictions."

Hoffman's group has not been alone in searching beyond the kinase gene to explain DM's range of symptoms. Earlier this year, a team led by Keith Johnson of the University of Glasgow in the United Kingdom reported that the DM triplet repeat may directly impinge on two genes, not just one (*Science*, 26 May, p. 1135). But even adding a second gene to the picture couldn't account for another anomalous observation. The DM defect is dominant—meaning that inheritance of a single defective copy of the gene can cause the disease. But while most dominant disease genes wreak havoc by creating defective proteins, the DM mutation lies in the 3′ end of the gene, which isn't translated into protein.

Clues to what else might be happening began accumulating over the past year. One came from cell biologist Robert Singer of the University of Massachusetts, Worcester, who found that the RNA made by the mutant protein kinase gene remains clumped in the nucleus of DM patients' cells instead of moving, as it should, into the cytoplasm to be made into protein. Then the Hoffman team, and one led by Michael Siciliano of the University of Texas M. D. Anderson Cancer Center in Houston, provided a possible reason why the RNAs get stuck in the nucleus. They found that fewer of the RNA copies of the mutant DM kinase gene acquire the poly-A tails (strings of adenine nucleotides) that are added to RNAs before they can move into the cytoplasm. Even more intriguingly, in DM patients, the same fate also befell the RNA made by the normal kinase gene.

Exactly why this happens is unclear, but the Hoffman team believes that they may be witnessing a previously undescribed method by which a dominant disease gene knocks out, or negates, the healthy gene copy. Whereas most "dominant-negative" disorders occur at the protein level, "the DM dominant-negative effect is unique; it wipes out the healthy gene at the RNA level," says Hoffman.

What's more, he says, the DM defect may have a widespread impact on RNA metabolism. In the work reported at the ASHG meeting, the Pittsburgh team found that muscle tissue from 11 patients with DM contains 40% less insulin-receptor RNA than muscle from four healthy people and four patients with other muscle disorders such as muscular dystrophy. A lack of insulin receptor RNA, and thus the protein, may explain why some DM patients have diabetes. Their cells may be unable to respond normally to insulin. And if RNAs from other genes are similarly affected, additional symptoms might be produced, Hoffman says: "We're suggesting that the dramatic variability in the clinical symptoms could be a consequence of different RNAs being altered at different times, in different cell types."

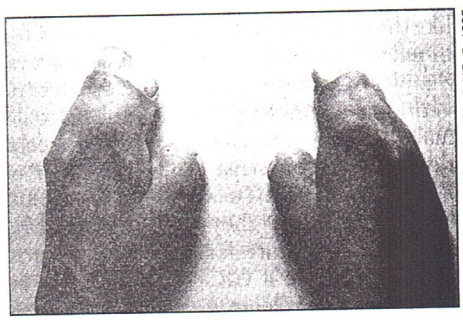
The next steps will be to work out exactly how the triplet repeat defect disrupts RNA metabolism, he says, and to try to establish whether RNAs produced by other genes are in fact affected. If they are, myotonic dystrophy may at last begin to make sense.

## Tracking the Origins of "Sporadic" Genetic Diseases

The best understood genetic diseases follow a clear pattern of family inheritance. But that's not always the case: Take the rare genetic disorder known as Apert's syndrome, whose features include misshapen heads and fused fingers and toes. Parents of Apert's syndrome children are healthy, and that's not because they carry a silent defective gene: Only one of the two copies of the gene needs to be defective to cause the disorder. Each case of Apert's syndrome is thus due to a fresh or "sporadic" mutation that arises during egg production in the mother, spermatogenesis in the father, or early embryo development.

Just which of those processes is the source of the mutations that cause Apert's syndrome and other sporadic genetic diseases has been difficult to discern. But at the ASHG meeting, geneticist Andrew Wilkie of the Institute of Molecular Medicine in Oxford, U.K., described how he and his colleague Dominique Moloney traced the origin of the Apert's syndrome mutation to confirm a long-held suspicion that it arises in the father. They think the same technique could give clues to the origin of other sporadic mutations, a prospect geneticist Max Muenke of the Children's Hospital of Philadelphia describes as "neat ... both for basic research and for clinical purposes, for counseling."

Until now, the only way to detect which



WILKIE *ET AL.*, *CURRENT BIOLOGY* VOL. 5, NO. 5, 1995

**Out of the blue.** Deformities like these can be caused by sporadic mutations.

parent harbored a sporadic mutation has been to isolate the chromosome carrying the defective gene in an animal cell, and then compare it to the corresponding parental chromosomes by screening for DNA "markers." But isolating an entire chromosome is cum-

bersome. The new method speeds the process by directly targeting a narrow region of the chromosome containing the defective gene.

Wilkie and Moloney already knew that Apert's syndrome is most commonly caused by one of two mutations in a copy of the gene encoding FGFR2, a membrane protein that picks up the chemical signals that prompt cell growth, division, or differentiation. To find out whether the gene came from the mother or father, Wilkie and Moloney used an established technique called the amplification refractory mutation system, or ARMS. ARMS, which is based on the polymerase chain reaction, is usually used to identify the strand of a gene containing a known mutation, but the two researchers used it instead to identify polymorphisms—harmless genetic variations—that had been inherited from each parent.

First, however, they needed to know what to look for. By screening a two-kilobase segment of the gene from each of 50 people, they found two suitable polymorphisms. Next, Wilkie and Moloney identified 30 Apert's syndrome families in which those polymorphisms would distinguish which of the child's two copies of the FGFR2 gene came from which parent. Finally, the researchers used ARMS to amplify the DNA from the children containing the two polymorphisms, and then restriction enzymes to pinpoint the strand that contained the mutated version of the *FGFR2* gene. In each of the 30 children, the mutated gene carried the father's polymorphism, showing that the defect must have been inherited from him.

That had been expected, Wilkie says, because earlier work had shown that men in their 50s have more than 20 times the risk of fathering Apert's syndrome children than men in their 20s: "The age effect had suggested that most of the mutations would be paternal in origin, but we didn't know whether to expect 80, 90, or 100%." The new results suggest, he says, that the vast majority of mutations come from the father, while the age effect suggests that the defect arises during sperm production rather than during formation of the sperm germ cells, as that happens before the father reaches puberty. So next, Wilkie says, "we are going to see whether we can find the same mutation in the sperm samples, and if so at what level it is present." If the mutation is detectable in sperm, then it might be possible to screen fathers of one Apert's syndrome child to see if they are at risk of having another.

Now that Wilkie and Moloney's technique has proven its mettle with Apert's syndrome, it may also help identify the origin of other sporadic genetic disorders—such as thanatophoric dysplasia, a lethal form of dwarfism. And just as in the case of Apert's syndrome, says Wilkie, that will allow geneticists to move on to two other questions: "The fundamental biological question of why these things arise? and, then, is there anything we can do to prevent them?"

–Rachel Nowak

AIDS RESEARCH

# New Drug Shows Promise in Monkeys

When Che-Chung Tsai and his colleagues first saw the results of their monkey experiments with a potential anti-HIV drug known by the acronym PMPA, they didn't believe them. They seemed just too good to be true. "We repeated and repeated the experiment," says Tsai, a veterinarian and pathologist at the University of Washington (UW) Regional Primate Research Center. Again and again, the data were positive. "Based on antiviral effects," concludes Tsai, "PMPA is the most effective drug we've seen."

Tsai and his colleagues at UW, the National Institutes of Health, and Gilead Sciences report the results of those tests on page 1197, and they are already attracting considerable interest. "There's a great deal of guarded optimism that this is very different from all the [anti-HIV] drugs we've tested," says Nava Sarver, a molecular biologist in the Division of AIDS at the National Institute of Allergy and Infectious Diseases (NIAID). Tsai and Sarver are quick to point out, however, that there is a big leap from monkeys to humans: For starters, HIV-1, the main AIDS virus that infects humans, differs significantly from SIV, the simian relative that was used in the tests. Still, PMPA's powerful effect in monkeys is raising hopes that it may one day help prevent and treat HIV infection in people.

Like the anti-HIV drug AZT, PMPA inhibits reverse transcriptase, a key enzyme that HIV and SIV use to copy themselves. In order to replicate, these viruses must first "reverse transcribe" their genetic material from RNA into DNA, using the RNA as a template to build the DNA one nucleotide at a time. These drugs block this process by acting as decoy nucleotides, which prematurely terminate the chain. PMPA acts faster and stays in cells longer than AZT does.

PMPA, and its older sister compound PMEA, was developed by investigators at Belgium's Rega Institute for Medical Research, and both are now licensed to Gilead, a biotechnology company in Foster City, California. Tsai came into the picture after he sent out a barrage of letters to companies developing AIDS drugs and asked whether he could test their compounds in monkeys. Gilead was one of the few companies that showed any interest, and Tsai first began testing PMEA, which is currently in human trials. He then became interested in PMPA, after test tube studies showed that it was 100 times less toxic than AZT and about 10 times less toxic than PMEA.

**Surprised by results.** Che-Chung Tsai.

Rather than evaluate whether PMPA could delay or prevent the onset of AIDS in SIV-infected monkeys, Tsai and his colleagues first tested it against what they believed was a lower hurdle: If given shortly before or after a monkey was exposed to SIV, could PMPA prevent the virus from establishing a permanent infection? To answer this question, the researchers injected PMPA into 15 long-tailed macaques 48 hours before they were inoculated with SIV. Five others started the drug 4 hours after inoculation, and another five began treatment after 24 hours. All the monkeys continued receiving PMPA for 4 weeks.

Eight months into the PMPA study, none of the animals has shown any sign of infection or drug toxicity. In contrast, 10 untreated animals given the same dose of SIV all became persistently infected.

These results provide no indication of PMPA's potential as a treatment for SIV infection once the virus has become established, but preliminary evidence from the California Regional Primate Research Center (CRPRC) in Davis shows promise in that regard as well. At a meeting in Monterey, California, last week, CRPRC's Koen Van Rompay, Marta Marthas, and colleagues reported that they infected eight rhesus macaques with SIV at birth and began treating four of the infants with PMPA 3 weeks later. After 6 months, these four animals remained healthy and had low levels of SIV in their blood. The four untreated newborns, by comparison, all had persistently high levels of SIV, and three died within 4 months. Veterinarian Van Rompay adds that they have yet to find any SIV in the treated animals that is resistant to the drug—a critical limiting feature of AZT and other anti-HIV drugs. Like Tsai, Van Rompay is stunned by his own results. "If I had not done the research myself, I would have doubted it," says Van Rompay.

In spite of the preliminary nature of the Tsai group's results, researchers are already speculating that PMPA might eventually find a role in preventing infection in people who have been accidentally exposed to HIV—a