Health & SCIENCE

Guilt by association, nailing culprit genes

A relatively unused technique for finding common disease-predisposing genes should ultimately surpass the approaches currently in vogue, according to a statistical study by Neil Risch, professor of genetics at Stanford University School of Medicine.

This method — called association analysis — will be especially useful in understanding the genetic basis of complex disorders, if a particular gene increases disease risk only moderately or else mutiple genetic factors are involved.

"Using this technique, it should be feasible in the next decade to test each of the estimated 100,000 human genes for possible links with every disease," Risch said.

Risch will present his findings on Friday, Feb. 14, at the morning session (8:30 to 11:30 a.m.), "Complex Traits: Where Are the Genes?" at the annual meeting of the American Association for the Advancement of Science, held in Seattle.

In the study, he created mathematical models to compare association analysis with linkage analysis — the method used to map most genes until now. Risch compared the number of people who would have to be studied by each method in order to identify disease genes with varying properties. "The data showed that linkage analysis requires sampling far more people to generate the same amount of information," he said.Scientists have used linkage analysis to find genes for many disorders, including Huntington's disease and Alzheimer's disease.

This approach, which is part of a process called "positional cloning," identifies disease genes by their chromosomal location, gradually zeroing in on them among their neighbors in the genome.

Researchers compare the co-inheritance of known landmarks, or genetic markers, in the genome with the disease state of family members. They locate the disease gene by finding the markers closest to it — those most highly correlated with inheritance of the disease.

"The nice thing about linkage analysis is that you can find genes involved in disease by using only a limited number of genetic markers along the various chromosomes," said Risch. "Even if you're some distance away, you still detect a signal that's strong enough to see where the gene resides. With association studies, you could be at the gene next door and still have no signal."

But the problem with linkage analysis is that it depends on having that strong signal, which occurs only if the gene is the predominant cause of disease, Risch explained. "If the gene is one of many factors that lead to the disease, the signal won't be loud enough to detect, even if you're very close," he said.

Association tests are more powerful in such cases because they give clear-cut answers to specific questions, Risch explained. Scientists ask directly whether affected individuals have a certain characteristic, and get a yes or no answer. They find out whether there are relative differences in the frequency of a particular genetic variant, or allele, in people with and without disease.

"This approach gives a loud signal," said Risch. "It's much more powerful. You get more information than by trying to find some attribute that is shared by affected individuals. A limitation of association studies, however, is that you need to be on top of or very close to the implicated gene to have a signal."

Association studies have already been done when researchers have had an idea of what causes a particular condition. For instance, earlier this year scientists in New York used this approach to find a mutation that decreases a person's risk of HIV infection. They knew that a certain protein interacts with the cellular molecule that allows HIV to enter the T cells of the immune system. So they looked at the gene for this cofactor in different people and found something startling.

"What they saw was that a piece was missing from this particular gene in some people," Risch said. "Individuals who have two copies of this defective gene — and as a result lack the cellular protein it encodes — are resistant to HIV infection."

In this case, the mutation is beneficial. But the same approach can be used to detect genes that cause disease: Researchers can ask whether there is an association between a particular genetic variant and disease.

"The HIV story is a case in which one gene predisposes people to resistance," said Dr. David Cox, professor of genetics and co-director of the Stanford Human Genome Center. "But the same approach can be used for conditions with multifactorial causes. In these cases, certain combinations of genes predispose people to disease. The question is which genes differ between normal and diseased populations. That's how you figure out which alleles and patterns of alleles are associated with disease."

"The key to this method is that you need a candidate gene," Risch said. "Instead of gradually getting closer to the disease gene, you ask, 'Is this it?'"

Although linkage analysis has succeeded in identifying genes with a strong influence on disease, "there are a lot more genes with small effects on disease, and those are what we're turning to now," he explained.

The genetic variations that cause complex diseases are widespread. "These variations are very common, and they've been in the human population for a long time," said Richard Myers, professor of genetics and director of the Stanford Human Genome Center. "They probably contribute to common diseases like autoimmune disease, psychiatric disease and cancer, which often are not thought of as being genetic. Getting these diseases probably requires the inheritance of multiple genes and some interactions with the environment as well."

FDA calls "party drug" deadly

Americans should avoid a popular party drug called GHB because the concoction, often promoted to teen-agers over the Internet as an aphrodisiac or an easy high, can be deadly, the government warned Tuesday.

Criminal investigators from the Food and Drug Administration are tracking down laboratories that illegally produce the chemical, which was banned in 1991 but is experiencing a resurgence — particularly as a "date rape" drug that, when slipped into a woman's drink, can render her helpless.

GHB is blamed for dozens of hospitalizations and at least three deaths. A Texas high school student died in August after someone slipped the chemical into her soft drink at a dance club. A Winchester, Va.; woman in her 20s was thought to have been in a drunken-driving crash until an autopsy showed she had taken GHB instead of alcohol.

Just a week ago, three Massachusetts college students were hospitalized after trying GHB. Two fell into a coma, but all eventually recovered. The drug, known by the street names "cherry meth," "liquid X" and "liquid ecstasy," is believed one of several that sickened dozens of youths at a New Year's Eve concert in Los Angeles.

California doctors last fall reported having to resuscitate Hollywood nightclub patrons who stopped breathing after ingesting the drug.

"The individual consumer should not even be thinking about taking this drug," said Jim Dahl, assistant director of the FDA's criminal investigations office. "It is very bad for you, and it can certainly cause death."

GHB, or gamma hydroxybutyrate, is an odorless, nearly tasteless drug that produces a high. But it also can cause vomiting, tremors and seizures, side effects strong enough to send some people into comas.

The drug commonly is distributed as a white powder or clear liquid that can be mixed into a drink. It sells for about $10 a vial.

GHB was originally developed as a surgical anesthetic but had so many side effects that it was abandoned. In 1990, the FDA began investigating reports that body builders were abusing GHB as an alternative to steroids. The following year, the FDA declared it illegal to manufacture or sell GHB for any purpose.

But after an initial lull, the FDA says, GHB is on the rise again. Since 1995, FDA prosecutors have begun 45 investigations of underground GHB manufacturing or distribution, 12 of which have resulted in convictions so far.

Its inherent danger aside, doctors say GHB is made so haphazardly that people might think the dose that gave them a mild buzz once is safe to try again —only to have the same amount send them into a coma because the new batch is more potent.

"It's a very narrow margin of error," said Dr. Rossanne Philen of the Centers for Disease Control and Prevention, which is preparing to publish a study of GHB injuries in Texas and New York. "There's no easy way for an individual who is going to consume this substance to tell the purity of it."

GHB proponents actually declare it legal to possess because the Drug Enforcement Administration has not yet listed the chemical as a controlled substance like cocaine or marijuana. But two states, Georgia and Rhode Island, have separately declared GHB a controlled substance, and other states are considering the move.

Meanwhile, the FDA is urging police officers, emergency rooms and coroners to begin aggressively testing for GHB when young people wind up in emergency rooms with the chemical's symptoms, so the government can get a better sense of how widely it is abused.

Researchers who discover maleness gene win prize

The Amory Prize for Reproductive Biology was awarded today by the American Academy of Arts and Sciences to an American and two Britishers for discovering the single gene men carry that determines whether a woman's fertilized egg develops into a male.

According to their studies, instructions from the SRY gene cause a female's fertilized egg to develop testes and become a male. Without these genetic instructions, a woman's (and every mammal's) fertilized egg develops ovaries and blossoms into a female.

While it had been more or less conventional wisdom that sex determination occurs when chromosomes of men and women get together. the nature of the gene or genes involved and the mechanism by which this information is transferred was unknown.

For solving "one of the central problems in biology, namely how the two sexes form," the Academy bestowed its Amory Prize for the first time in five years. The award ceremony took place at the 1795th Stated Meeting of the Academy at Academy House here.

Presiding at the ceremony were Jaroslav Pelikan, President of the Academy and Sterling Professor of History at Yale University, and Paul R. Schimmel, Chairman of the Academy's Amory Prize Committee and Joan D. & Catherine T. Macarthur Professor of Biochemistry and Biophysics at Massachusetts Institute of Technology.

The three prize-winners are David C. Page of MIT, the Whitehead Institute and the Howard Hughes Medical Institute; Peter Goodfellow of SmithKline Beecham; and Robin H. Lovell-Badge of the MRC National Institute for Medical Research.

The scientists operated on the assumption that whatever gene causes maleness is missing in men with female (XX) chromosomes, and present in women with male (XY) chromosomes. They found that mutations of the SRY gene causes its instructions to the fertilized egg to go awry so that male development is prevented or incomplete. The incidence of human intersex is estimated at between 2 to 4 per 10,000 births.

Dr. Page was recognized for developing the first map of the Y chromosome that identified the approximate location of the SRY gene. This map was ultimately expanded into the first detailed map of a human chromosome. In subsequent work, Page's team has also characterized the region of the Y chromosome that exchanges with the X chromosome, and has identified regions of the Y chromosome that contain genes for stature and for development of testicular tumors and characterized a gene on the long arm of the Y chromosome responsible for one of the most common forms of male infertility. Page has also begun to characterize genes on the X chromosome essential for female development.

In 1990, using the gene map developed by Page, British teams led by Robin Lovell-Badge and Peter Goodfellow, then at the Imperial Cancer Research Fund (ICRF), used special cloning techniques to isolate what they suspected was the male-determining gene in both mice and men and then proved that it was responsible for testicular development. When they introduced the gene, which they named SRY, into newly fertilized mouse eggs, it caused the genetic female mice to develop into males.

How the SRY gene causes the sexually indifferent gonad of the embryo to develop into a testis is not entirely clear, but the subsequent work of Goodfellow and Lovell-Badge indicates that the product of SRY is a protein that binds to DNA and appears to bend DNA sharply.

Glaucoma diagnosis key found

UCSF eye researchers say a study published in the journal *Science* is "proof in principle" of their theory from several years ago that a particular gene — known as the TIGR gene — will be one of the keys to glaucoma diagnosis and that defects in the gene could account for more than 20 percent of adult glaucoma.

The Science article reports that mutations of the TIGR gene play a role in primary open angle glaucoma (POAG) — the most common form of glaucoma, affecting an estimated 3 million Americans, and the second leading cause of blindness in the United States. The study was conducted by University of Iowa researchers, who had been studying a rare form of glaucoma called juvenile glaucoma, and was co-authored by UCSF researchers.

The TIGR gene was originally cloned at the UCSF Department of Ophthalmology by molecular biologist Thai D. Nguyen, Ph.D., using a human cell culture system developed by Jon R. Polansky, M.D., UCSF associate professor of ophthalmology. UCSF has filed a series of patent applications beginning in 1994 covering the TIGR gene for diagnosis of glaucoma and other uses.

The University has exclusively licensed these patent rights for the development of diagnostic products to InSite Vision Inc. of Alameda, California.