

A Clearer View of Macular Degeneration

Genes tied to age-related macular degeneration confirm the notion that inflammation helps destroy the central area of the retina in this vision disorder

More than 10 million people in the United States and an estimated 50 million worldwide suffer from age-related macular degeneration (AMD). This deterioration of the retina wipes out their central vision, robbing them of the ability to perform essential activities such as reading or driving a car. Until recently, researchers had few clues to what causes AMD. But a series of recent gene discoveries has gone a long way toward solving the mystery.

The work has shown that variations in two genes encoding proteins in the so-called complement cascade account for most of the risk of developing AMD. This complex molecular pathway is the body's first line of defense against invading bacteria, but if overactive, the pathway can produce tissue-damaging inflammation.

A tough disease to crack

The causes of AMD have been hard to pin down partly because the disease develops late in life, usually after age 60. In addition, AMD is a complex disease, caused by an interaction between multiple genes and environmental factors such as diet and smoking. That's made it hard to do studies aimed at tying particular gene variants to the disease. "Until last year, we just didn't have a clue about the etiology [of AMD]. It's been very frustrating," says Gregory Hageman of the University of Iowa in Iowa City, one of the field's pioneers.

In their search for clues, researchers have looked at hereditary eye diseases that develop early in life and mimic some features of AMD pathology, such as the development of drusen: abnormal deposits of proteins and other materials in the retina. They identified the

or viruses by poking holes in their membranes, but it can also damage normal cells if not controlled.

Based on these and other results, Hageman and his colleagues proposed about 5 years ago that drusen growth begins when some as-yet-unknown insult damages cells in the retina. The leftover cell debris provides the seed for drusen formation and triggers complement activation and local inflammation. Over time, the drusen grow as they accumulate inflammatory proteins and other materials, and the inflammation persists, causing additional damage to the retina and, in the worst cases, blindness.

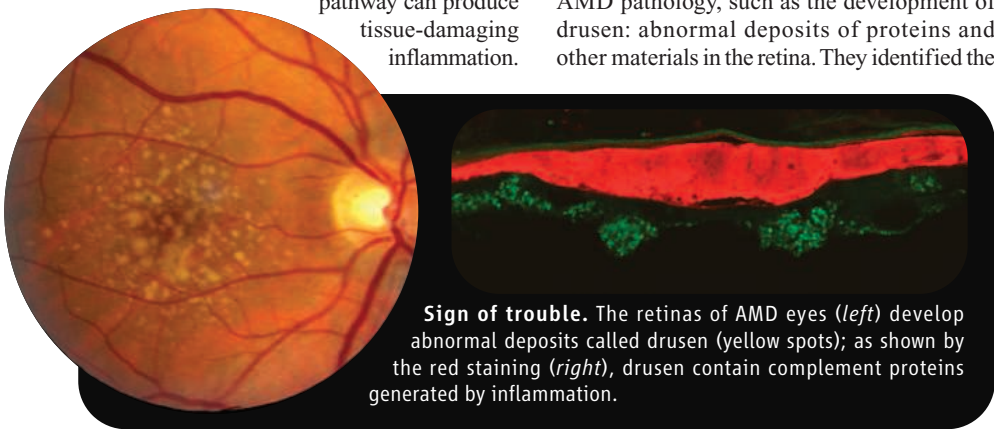
At the time, this idea "was not met with a lot of positivity," Hageman wryly recalls. "He pushed the hypothesis for many years, and nobody believed him," says Rando Allikmets of Columbia University, a recent collaborator. Allikmets notes that he, like many other AMD researchers, acknowledged that there could be an inflammatory component in AMD but "thought this was secondary or tertiary" to whatever was actually causing the retinal damage.

A genetic link to inflammation

The turning point in the inflammation story came just over a year ago, thanks mainly to new gene-hunting tools, including the human genome sequence as well as the growing library of human single-nucleotide polymorphisms (SNPs), subtle DNA sequence changes that can be used to pin down the gene variants at fault in a disease. Over the past few years, researchers have used these SNPs to identify several chromosomal regions likely to contain genes that influence the risk of getting AMD and then zeroed in on the genes themselves.

Last March, three independent groups—led by Josephine Hoh of Yale University School of Medicine; Margaret Pericak-Vance of Duke University Medical Center in Durham, North Carolina; and Albert Edwards of the University of Texas Southwestern Medical Center in Dallas and Lindsay Farrer of Boston University School of Medicine—reported online in *Science* that they had uncovered a gene on chromosome 1 that greatly increases the risk of getting AMD. The gene encodes a protein called complement factor H that helps keep the complement system under tight control so that it doesn't attack the body's normal cells. The researchers found that people bearing a particular variant of the factor H gene were much more likely to get AMD than were people with other variants (*Science*, 15 April 2005, pp. 362, 385, 419, and 421). Their calculations showed that the high-risk variant could explain up to 50% of the cases, presumably because the protein product of that gene is less effective in inhibiting the complement pathway.

Hageman describes this confirmation of complement involvement in AMD as "pretty



Sign of trouble. The retinas of AMD eyes (left) develop abnormal deposits called drusen (yellow spots); as shown by the red staining (right), drusen contain complement proteins generated by inflammation.

The new links between AMD and the complement genes suggest that excessive inflammation resulting from uncontrolled complement activity underlies the vision-destroying changes that particularly strike the macula, the central region of the retina. Indeed, such inflammation-promoting gene variants contribute to the development of perhaps as many as 75% of AMD cases. Several other genes have also been implicated recently, including one of as-yet-unknown function that may also make a substantial contribution to AMD risk.

At long last, say researchers, they have the kind of information needed to find ways to prevent or treat AMD. "I think we're headed for a period of time when we're going to come up with possible therapies," says AMD researcher Michael Gorin of the University of Pittsburgh School of Medicine in Pennsylvania. This might be accomplished, for example, by finding ways to inhibit complement activity in the eye.

genes at fault in some of these early developing diseases and for a time hoped that the same genes might also be major contributors to AMD. "It turns out that for the most part that idea was wrong," says Gorin.

About 15 years ago, Hageman began pursuing a different tack, collecting donated eyes from both people afflicted with AMD and those who were not. His analyses of those eyes suggested that inflammation is a key player in the etiology of AMD.

For example, working with Iowa colleague Robert Mullins and Don Anderson and Lincoln Johnson of the University of California, Santa Barbara, Hageman found that proteins associated with immune system activity are located in or near the drusen in eyes with AMD. These proteins included various activated components of the complement system, such as the membrane attack complex, which is the business end of the complement cascade. It destroys cells infected with bacteria

wonderful”—even though the other teams got their work into print before his own. In the 17 May 2005 *Proceedings of the National Academy of Sciences*, Allikmets and Hageman, working with Bert Gold and Michael Dean of the National Cancer Institute in Bethesda, Maryland, and colleagues reported that they, too, had linked the same high-risk variant of the factor H gene to AMD and also identified other variants that appeared protective. In addition, they showed that factor H is made in the macula and is present in drusen. In other words, it's right where it should be to influence AMD development.

The case for a link between complement activity and AMD risk got another major boost earlier this month. In the March issue of *Nature Genetics*, Allikmets, Hageman, Gold, Dean, and their colleagues report linking the gene for a second component of the complement cascade to the eye disease. This gene, located on chromosome 6, produces a protein called factor B involved in complement activation; the researchers again found both high-risk and protective variants.

The earlier factor H studies showed that some people with healthy eyes also carry the AMD-predisposing variant, although it is present in more AMD patients. Allikmets says that the existence of protective variants of the factor H and B genes can help explain why some people with the “bad” variants don't get AMD. By looking at the variants of both genes that people have, he says, “you get a much clearer picture. Seventy-four percent of the patients had no protective [gene variants], while 56% of the controls had at least one.”

Still unclear, however, is the nature of the initial trigger that touches off complement activation. An infection is one possibility. About a year ago, Joan Miller of Harvard Medical School in Boston and her colleagues reported evidence indicating that the eyes of patients with the so-called wet form of AMD, characterized by blood vessel growth in the macula, had been infected by the bacterium *Chlamydia pneumoniae*. A causative link between *Chlamydia* infection and AMD needs to be confirmed, however.

More AMD genes

A third gene recently tied to AMD doesn't fall neatly into the complement story. In work published last summer in the *American Journal of Human Genetics*, Gorin and his colleagues followed up on previous studies placing an AMD gene on chromosome 10. Gorin says that his group's analysis homed in on two tightly linked genes (designated *PLEKHA1* and



Missing faces. As illustrated by the simulated picture at right, AMD robs its victims of their central vision and can leave them legally blind even though a rim of peripheral vision may remain.



LOC387715) but couldn't discern which is the culprit. However, in a paper that appeared a few months later in *Human Molecular Genetics*, a team led by Bernhard Weber of the University of Regensburg, Germany, reported that the strongest AMD risk seemed to be associated with a single amino acid change in the protein predicted to be encoded by *LOC387715*. In work published online early this month in the *American Journal of Human Genetics (AJHG)*, Pericak-Vance and her colleagues confirmed that conclusion.

The genetic analyses indicate that *LOC387715*'s effect on AMD is independent of that of the factor H gene but is almost as strong, contributing to perhaps 40% of AMD cases. Still, the function of the gene's protein is a mystery. “Until one knows what it does, you can't really say it's the gene,” Gorin cautions.

In their *AJHG* paper, Pericak-Vance and her colleagues also point out an intriguing

connection between the high-risk variant of *LOC387715* and cigarette smoking, one of the strongest environmental risk factors for AMD. The researchers found that the combined risk of smoking and carrying the AMD-promoting gene variant was more than the sum of the risk of the two individually. This indicates, Pericak-Vance says, that the two factors interact to foster AMD development.

The factor H and B genes and *LOC387715* are likely not the only ones that affect AMD risk. For example, in a study published in January in *Investigative Ophthalmology and Visual Science*, Pericak-Vance's team looked at eight candidate genes that were suspected of involvement in AMD. Their analysis implicated three of them, including the *VEGF* gene and two involved in lipid metabolism. The product of the *VEGF* gene stimulates blood vessel growth, suggesting it might be involved in wet AMD, which is the most severe form.

But the complement genes and *LOC387715* are certainly the major contributors to AMD risk, and establishing that, Hageman says, is good news for people who might develop AMD. Having to look at just a few genes could make it easier to identify high-risk individuals, who could then take preventive steps such as avoiding smoking, decreasing their fat intake, and increasing their intake of antioxidants and carotenoids. Many of these steps, suggested by epidemiology studies, are the same ones prescribed to reduce a person's risk of heart attack and stroke. “There is a very similar risk profile for cardiovascular disease and AMD,” says epidemiologist Johanna Seddon of Harvard Medical School.

If just a few genes account for almost all of the risk of getting AMD, that should also help in devising therapies that can slow or prevent vision loss in people with the disease. “If each gene contributed just 4% or 5%, developing a therapy [based on those genes] would be pretty much impossible,” Hageman says. However, the new studies suggest a much brighter outlook for efforts to beat this devastating disease.

—JEAN MARX



Pioneer. Despite early skepticism from his colleagues, Gregory Hageman's view that inflammation plays a causal role in AMD is proving correct.