Factor H complements AMD

By Kai-Jye Lou, Staff Writer

Researchers in the EU and the U.S. have found what could be the mechanistic underpinnings of the increased risk for age-related macular degeneration in individuals carrying a common polymorphism in the gene for complement factor H.¹ The mutant form of the protein, unlike the wild-type version, has an impaired capacity to bind and neutralize proinflammatory malondialdehyde structures.

Malondialdehyde (MDA) is a toxic byproduct of lipid peroxidation and can modify DNA and proteins. MDA has been associated with multiple chronic inflammatory diseases including atherosclerosis² and AMD.³

"We've previously found that a large number of naturally occurring antibodies, which are proteins of the innate immune system, bind to MDA," said Christoph Binder, a professor of atherosclerosis research at the **Medical University of Vienna** and a principal investigator at the

Research Center for Molecular Medicine of the Austrian Academy of Sciences. "That result suggests that our bodies need to defend against the accumulation of structures that contain MDA."

Now, Binder's group has identified nonantibody proteins in plasma that bind MDA. In both mouse and human plasma, the researchers detected complement factor H (CFH) on beads linked to MDA but not on control beads coupled to polylysine. A series of competition assays confirmed that CFH specifically binds to MDA on proteins.

The team uncovered the mechanistic link between a CFH polymorphism and AMD when it was mapping the sites on CFH that bind to MDA. "One of the most well-studied SNPs associated with age-related macular degeneration is known to result in a single amino acid substitution, and we found that this substitution occurs in one of the two MDA-binding domains we identified on factor H," noted Binder. "Past studies have shown that those individuals who are homozygous for this SNP have about a sevenfold increased risk for developing the disease."

The researchers assayed plasma from subjects heterozygous and homozygous for the AMD risk allele and found 23% and 52% decreased CFH binding to MDA, respectively, compared with binding in plasma from those without the risk allele ($p < 0.001$ for both).

In human cell lines, the team found that CFH could inhibit the binding of MDA to cells

and its ability to stimulate the release of proinflammatory cytokines from those cells (see Figure 1, "Model for complement factor H-mediated protection in AMD"). In mice, an intravitreal injection of CFH lowered the ocular inflammatory response to MDA compared with

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Vanderbilt University Medical Center**

Figure 1. Model for complement factor H-mediated protection in AMD. Malondialdehyde (MDA) is a marker for oxidative stress and is associated with multiple diseases including age-related macular degeneration (AMD). As reported by Weismann *et al.*, the binding of complement factor H (CFH) to MDA epitopes on proteins could help protect against oxidative stress in AMD.

In the study, researchers found that wild-type CFH (crescents) could bind to MDA epitopes (circles) on proteins (I[a]) and neutralize their ability to stimulate proinflammatory cytokine production in cell populations involved in AMD pathology (II[b]).

The researchers found that a polymorphism in the CFH gene strongly linked to increased AMD risk results in a mutant CFH protein with decreased binding to MDA epitopes (II[a]). This reduced binding could impair the mutant CFH's ability to neutralize the production of proinflammatory cytokines triggered by MDA (II[b]).

injections lacking CFH.

Binder was corresponding author on the paper, which was published in *Nature*. The team included researchers from the **University of California, San Diego**, the **University of Jena** and **The Johns Hopkins University School of Medicine**.

"The researchers have clearly identified a link between CFH and the highly reactive endogenous aldehyde MDA," said Sobha Sivaprasad, a consultant ophthalmologist at **King's College Hospital**. "They show clearly that CFH could block the proinflammatory effect of MDA, which is an important finding and explains a novel function of CFH."

"Since the original paper from 2005 linking the factor H polymorphism to AMD risk, there has been an active effort in the field to explain the how and why behind this genetic link," said Milam Brantley Jr., an assistant professor of ophthalmology and visual sciences at the **Vanderbilt University Medical Center**. "The findings reported in the current study lay out a very reasonable and plausible mechanism to explain this key genetic risk factor in AMD. These findings also bring oxidative stress into the forefront of AMD research."

In 2005, an international team of researchers led by Rando Allikmets and Gregory Hageman found that the rs1061170 SNP is associated with increased AMD risk and manifests as a tyrosine to histidine substitution at amino acid 402 in CFH.⁴

"The big step now is to figure out how the observed differences in CFH binding to MDA are related to disease etiology," Brantley told *SciBX*.

Allikmets is research director of the Edward S. Harkness Eye Institute at the **Columbia University College of Physicians and Surgeons**. Hageman is director of the Moran Center for Translational Medicine and a professor in the Department of Ophthalmology and Visual Sciences at **The University of Utah School of Medicine**.

The H factor

Binder said the reported findings provide mechanistic support for evaluating CFH-based molecules in AMD and other chronic inflammatory diseases like atherosclerosis.

"For example, we could consider the use of a neutralizing peptide based on CFH or a recombinant CFH protein to inhibit the proinflammatory response mediated by MDA," Binder told *SciBX*.

Sivaprasad thinks a more effective strategy would be to go upstream and develop a therapy that lowers the generation of reactive oxygen species (ROS) such as MDA rather than dealing with the sequelae of inflammation and angiogenesis induced by just MDA.

"A comparative study of CFH and other antioxidants on the MDA concentration and the inflammatory profile is also worthwhile," she told *SciBX*.

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Research Center for Molecular
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of Sciences**

At least two companies have explored the use of CFH-based molecules in chronic inflammatory diseases, including AMD. **Optherion Inc.** had a recombinant form of a protective CFH variant in preclinical development for dry AMD and orphan renal diseases. Optherion out-licensed its AMD therapeutics business to an undisclosed company in 2010.

Alexion Pharmaceuticals Inc. is developing TT30, a fusion protein that consists of a complement receptor 2 (CR2) fragment linked to a CFH fragment. The compound, which Alexion gained from its January acquisition of Taligen Therapeutics Inc., is in preclinical testing for AMD.

In addition to AMD, Taligen previously reported preclinical data for TT30 in models of autoimmune disease, inflammation and graft rejection showing that the fusion protein reduced markers of disease pathology.

Binder said his group is trying to understand how the inflammatory responses to MDA are mediated. "So far, we have shown that MDA can initiate inflammatory responses, but we haven't yet identified the requirements for such responses. We are also investigating whether this pathway would be important in

atherosclerosis and other cardiovascular diseases that have an oxidative stress component."

The Research Center for Molecular Medicine has a pending patent covering the use of CFH for oxidative stress disease conditions. The work is available for licensing.

Lou, K.-J. *SciBX* 4(41); doi:10.1038/scibx.2011.1136
Published online Oct. 20, 2011

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