Molecular genetics of age-related macular degeneration

Edwin M. Stone1,*, Val C. Sheffield2,3 and Gregory S. Hageman1

1Department of Ophthalmology and 2Department of Pediatrics, The University of Iowa College of Medicine, 200 Hawkins Drive, Iowa City, IA 52242, USA and 3The Howard Hughes Medical Institute, Iowa City, IA 52242, USA

Received July 17, 2001; Accepted July 24, 2001

The numerous conditions that clinicians group under the term ‘age-related macular degeneration’ (AMD) are collectively the most common cause of severe visual loss in the developed world. Moreover, the number of people affected by these diseases is expected to nearly double in the next 25 years. A growing body of data suggests that a large fraction of AMD is caused by genetic factors. As a result, numerous investigators have sought genes that contribute to this disorder. At least six genes have now been identified that cause heritable macular disease, but none of these seem to cause even a moderate fraction of AMD. Affected pedigree member studies suggest that some regions of the genome do harbor AMD predisposing genes, but none have yet been identified by this approach. Studies of human donor tissue have yielded important new insights into pathways associated with AMD. These studies, when combined with the power of genetic approaches, are likely to ultimately reveal a set of genes responsible for a sizeable fraction of AMD.

INTRODUCTION

The ability to distinguish fine details in an image, such as the differences among the various letters of the alphabet, is not distributed equally throughout the entire visual field. For example, if one looks at the edge of a printed page, the fact that words are printed there can be discerned but the words themselves cannot be read. This is because only a very small region of the retina, a circular area ~400 µm in diameter that is referred to as ‘the fovea’ by clinicians, has sufficient photoreceptor density, and sufficiently high bandwidth wiring to the brain, to subserve reading newsprint without optical magnification. Clinicians use the term ‘macula’ to refer to a circular zone of the retina 5–6 mm in diameter centered on the fovea. There are a number of significant anatomic differences between the macula and the remainder of the retina and some of these differences undoubtedly underlie the macula’s susceptibility to certain disease processes.

The term age-related macular degeneration (AMD) refers to a group of late onset conditions that affect the macula and that are collectively the most common cause of severe visual loss in the developed world (1–4). Although AMD has been a clinically recognized entity for >100 years, there is still no completely satisfactory definition for it. The main reasons for this are that (i) the clinical manifestations of the individual disorders of the AMD group only partially overlap (Fig. 1) and (ii) some non-AMD macular disease entities (e.g. central serous retinopathy, the presumed ocular histoplasmosis syndrome and a group of early onset Mendelian macular dystrophies; Fig. 2) also have clinical features that overlap AMD [so that a criterion of exclusion of these diseases has to be included in any AMD definition (5)].

With these limitations in mind, there is general consensus that the hallmark of AMD is the druse, an extracellular deposit of proteins, lipids and cellular debris that accumulates between the retinal pigment epithelium (RPE) and the pentalaminar structure known as Bruch’s membrane. Drusen can vary from single deposits that are smaller than a single RPE cell (and which are ophthalmoscopically invisible), to large collections that are several hundred microns in diameter. In addition to variations in size, drusen also vary in color, shape, margin sharpness, distribution in the fundus and presence or absence of calcium (Fig. 1). Many investigators suspect that different pathophysiologic processes are responsible for these different drusen phenotypes, but as will be discussed more fully below, careful biochemical, cell-biological and histopathological studies suggest that the overall composition of all drusen is similar (6–9). Another classic feature of AMD is the patchy loss of RPE cells, known as geographic atrophy. Geographic atrophy (Fig. 1F) can occur in regions that were previously extensively affected by drusen deposition, but it can also occur in eyes with very few, if any, clinically visible drusen. A third feature of AMD is a disruption of the normal distribution of pigment in the RPE (Fig. 1B). A severe complication that occurs in a subset of patients with AMD is the development of a network of new blood vessels that sprout from the underlying choriocapillaris and that grow through Bruch’s membrane into the subretinal or sub-RPE spaces (Fig. 1). Although this neovascular response occurs in only ~10% of all patients with macular degeneration, it is responsible for at least 90% of the severe visual loss associated with this disease (10).

Some classifications, including that of the International Working Group (5) make a distinction between ‘age-related maculopathy’ (the stages of disease that precede severe visual loss) and ‘AMD’ (the stages of disease characterized by geographic atrophy, disciform scarring and active choroidal neovascularization). For the purposes of this article we will use the term AMD to refer to all clinically detectable stages of all
of the disorders that cause drusen, geographic atrophy and choroidal neovascularization in individuals >55 years of age. With this type of broad application of the term, an extraordinary fraction of the population is at risk for the development of AMD. For example, the Beaver Dam Eye Study found that nearly 20% of the population between 65 and 75 years of age is affected with either early or late age-related maculopathy and also that >35% of the population >75 years of age is similarly affected (11). These numbers are especially alarming given that the US Census Bureau has predicted that the number of people in these two age groups will increase by 80% in the next 25 years (12).

GENETIC APPROACHES TO THE DISCOVERY OF AMD PATHWAYS

A substantial body of epidemiologic evidence has been assembled that suggests that AMD has a significant genetic component (13–20). These studies raise the possibility that molecular genetic methods can be used to identify AMD-causing genes and, in so doing, identify pathophysiological mechanisms that might be amenable to novel therapies. A variety of approaches have been used to try to identify genes involved in common complex human genetic disorders like AMD. These include candidate gene association studies, linkage disequilibrium studies, and the use of animal models. One strategy that has been utilized extensively is the use of genetic linkage analysis and positional cloning methods to identify genes that cause early-onset Mendelian macular diseases that share phenotypic features with AMD. A second approach has been to use affected sib pair methods to identify putative AMD loci in the hope that positional information will help to identify AMD candidate genes. The following sections summarize the main observations that have been made during the past decade using these two approaches.

RDS-associated pattern dystrophy

The RDS gene was first identified in the mouse as the gene involved in a photoreceptor degeneration known as ‘retinal degeneration slow’ (21). Shortly thereafter, the human RDS gene was shown to cause a similar photoreceptor degeneration phenotype known as retinitis pigmentosa (22,23). In 1993, three groups (24–26) made the observation that this gene could also cause a macular phenotype known as pattern dystrophy (Fig. 2A and B). Given that the RDS gene is expressed solely in photoreceptor cells, these observations were surprising for two reasons. First, the majority of the ophthalmoscopically visible disease in patients with pattern dystrophy occurs at the

Figure 1. Range of ophthalmoscopic phenotypes consistent with the diagnosis of AMD. (A) Normal fundus (for comparison); (B) RPE hyperpigmentation; (C) drusen intermixed with small pigment epithelial detachments; (D) large and small drusen confined to the macula; (E) flat, calcified drusen surrounding patches of geographic atrophy; (F) geographic atrophy; (G) large and small drusen throughout the posterior pole; (H) a wreath of large and small drusen surrounding a circular area of geographic atrophy; (I) subretinal hemorrhage secondary to a choroidal neovascular membrane; (J) numerous tiny drusen that spare the macula; (K) drusen limited to the temporal aspect of the macula; (L) RPE hyperpigmentation and subretinal fibrosis secondary to a choroidal neovascular membrane.
level of the RPE, not the neurosensory retina. Secondly, some patients with pattern dystrophy have normal retinal electrophysiology even in the seventh decade of life. This disease is an excellent example of the fact that the first steps in a pathophysiologic mechanism may be somewhat remote from the tissue whose dysfunction first causes a patient to be symptomatic. It is also a good example that multiple, quite different phenotypes can result from mutations in the same gene. For example, Weleber et al. (27) identified one family with a mutation in the RDS gene whose affected family members had been diagnosed previously with retinitis pigmentosa, pattern dystrophy and fundus flavimaculatus.

The RDS gene encodes a 346 amino acid protein that interacts with the product of a second gene (ROM1) to stabilize the flattened shape of the rhodopsin-bearing membranous discs in the outer segment of photoreceptor cells. In 1994, Kajiwara et al. (28) demonstrated that in rare cases, sequence variations in the RDS and ROM1 genes could be additive and result in retinitis pigmentosa through a true digenic mechanism. Despite the similarity of the ophthalmoscopic phenotypes of pattern dystrophy and AMD, when we screened 182 AMD patients for sequence variations in the RDS gene, none was found (unpublished data).

**Best disease**

Best disease (Fig. 2C and D) was mapped to chromosome 11 in 1992 (29,30) and the causative gene (VMD2) was identified by positional cloning in 1998 (31). Best disease is pathophysiologically interesting for at least three reasons. First, although large extracellular accumulations of “lipofuscin-like” material occur in the macula, these occur beneath the RPE and can leave a patient’s vision relatively unaffected for decades. Secondly, patients with Best disease exhibit a peculiar electrophysiologic abnormality in which the standing potential of the eye fails to respond to changes in light. It is amazing that this electrophysiologic response has been conserved from amphibians to man, but that its loss in Best patients results in no detectable loss of visual function. Finally, Best disease is incompletely penetrant. Some molecularly confirmed obligate gene carriers have a perfectly normal fundus appearance even in their sixth decade of life. This strongly suggests that some other permissive
factor, probably another gene, is required for the expression of the phenotype. Indeed, the identification of a factor that is capable of completely preventing the macular lesion has the potential of being of greater importance to the development of preventive therapies for lipofuscin-accumulating maculopathies than the discovery of the causative genes themselves. The function of bestrophin, the 585 amino acid product of the VMD2 gene, is at this point poorly understood. Marmorstein et al. (32) recently showed it to be localized to the basolateral plasma membrane of the RPE. It shares sequence homology to the so-called RFP family of proteins but this homology has not been sufficient to reveal the normal function of this protein. When we screened 321 AMD patients for sequence variations in the VMD2 gene, five were found (33). This was not statistically different from the frequency of such mutations found in controls (0/192).

**Stargardt disease**

Stargardt disease (Fig. 2E and F) is probably the most common of the Mendelian maculopathies, occurring in approximately 1 in 10,000 people in the population. It is the only autosomal recessive condition of the ones discussed in this article, the other five being autosomal dominant. The age of onset of Stargardt disease varies widely, with about one-third of affected individuals presenting in the first decade of life, whereas some patients first become symptomatic after 40 years of age. The disorder appears to be genetically homogeneous, and the causative gene was mapped to chromosome 1 in 1993 (34). This gene, now known as ABCA4, was identified by Allikmets et al. (35) and encodes a photoreceptor protein of 2273 amino acids. The function of this protein appears to be the translocation of N-retinylidene phosphatidylethanolamine, an intermediate of the visual cycle, from the intradiscal space to the cytoplasm of the photoreceptor cells (36,37). Failure of this translocation to occur results in the formation of A2E, a chemically stable amphipathic substance which, at low concentrations, can induce apoptosis (38–40) and, at high concentrations, can actually dissolve cell membranes (39,41,42), with subsequent loss of RPE cells and photoreceptors. Mata et al. (43) showed that light exposure was necessary for A2E accumulation in mice lacking the ABCA4 gene, suggesting that modification of light exposure might have some therapeutic benefit in humans with ABCA4-mediated retinal disease. Like RDS, mutations in the ABCA4 gene are capable of causing a wide range of phenotypes from typical Stargardt disease (Fig. 2E) to retinitis pigmentosa and cone–rod dystrophy. Some investigators believe that variations in the ABCA4 gene are responsible for a substantial fraction of AMD (44,45) although other studies have suggested that this is not the case (46,47). The main difficulty in assessing the role of this gene in any phenotype is the fact that it is highly polymorphic in the normal population. Webster et al. (48) found that 75% of the population harbors at least one departure from the ‘consensus’ ABCA4 sequence. Despite this extreme allelic diversity, a significant number of disease-causing mutations in the ABCA4 gene escape detection by most PCR-based assays in use today. This suggests that deletions and/or mutations in the non-coding regions of the gene may be quite common causes of Stargardt disease.

**Sorsby fundus dystrophy**

Sorsby fundus dystrophy (Fig. 2G and H) was mapped to chromosome 22 in 1994 (49). Affected patients develop night blindness in their twenties, but at 40 years of age most patients develop subfoveal choroidal neovascularization. Unlike
typical AMD, the visual loss continues to progress peripherally such that many affected patients progress to ‘hand motions’ vision, a distinctly unusual outcome for typical AMD. The gene associated with this disease encodes a tissue inhibitor of metalloproteinases (TIMP3) (49). Most of the mutations that cause SFD result in the creation of a new cysteine residue which presumably disrupts disulfide bond formation and hence the tertiary structure of the protein. The altered TIMP3 then causes an accumulation of collagenous material to develop beneath the RPE (50,51). Jacobson et al. (52) postulated that this layer of abnormal material caused the night blindness associated with SFD by blocking the passage of vitamin A from the choriocapillaris to the photoreceptors. When we screened 188 AMD patients for sequence variations in the TIMP3 gene, none was found (unpublished data).

**Malattia Leventinese**

Malattia Leventinese (ML) or Doyne’s honeycomb retinal dystrophy was the first of the Mendelian maculopathies to be clinically and histopathologically described (53,54). This condition was of significant interest to investigators who hoped that one or more of the heritable maculopathies would be allelic with typical late-onset AMD because the ophthalmoscopic features of ML (Fig. 2I and J) are more similar to typical AMD than any of the other Mendelian disorders discussed here. In addition, the time course of visual loss in patients with ML is similar to those with AMD. The median visual acuity of affected people remains 20/20 until 50 years of age, and then falls to 20/200 (legal blindness) by 70 years of age (55). The disease was mapped to chromosome 2 in 1996 (56) and the gene (EFEMP1) identified in 1999 (56). Surprisingly, a single mutation Arg345Trp is responsible for all cases of this phenotype in the world. Indeed, the lack of evidence of new mutations coupled with analysis of intragenic polymorphisms suggests that all affected individuals living today descended from a common ancestor. The gene product is a 493 amino acid extracellular matrix protein that is expressed most abundantly in the eye and lung, but also in the brain, heart, spleen and kidney. The single known mutation is positioned such that it is likely to disrupt one of the six calcium binding EGF-like domains of the molecule. When we screened 494 patients with AMD for sequence variations in the EFEMP1 gene, none was found (56).

**Stargardt-like dominant macular dystrophy**

Stargardt-like dominant macular dystrophy shares some clinical features with typical Stargardt disease (Fig. 2K and L) but is inherited in an autosomal-dominant fashion. The visual acuity of most patients affected with this disease falls to 20/200 or less by 20 years of age (57). The phenotype was originally mapped to both chromosome 6 (57) and chromosome 13 (58) but more recent studies have revealed the latter linkage to be an error, the ‘chromosome 13’ family is actually distantly related to most other families with this phenotype in North America (59,60). The disease gene (ELOVL4) was identified in 2001 (60). This gene encodes a protein of 314 amino acids that is expressed in photoreceptor cells. This gene has significant structural and topological similarity to members of the ELO gene family that are known to be involved in the elongation of fatty acids in yeast and mice. Thus, Zhang et al. (60) have suggested a similar function for ELOVL4 in the human retina.

**The affected pedigree member method**

The late onset of AMD makes it difficult to identify single families with enough individuals to permit classic linkage analysis. In the rare case in which this is possible (61), it is difficult to know whether the disease affecting the family is a frequent cause of AMD or another rare macular dystrophy such as the six described above. Non-parametric approaches such as the affected pedigree member method (62) have several advantages over classic linkage analysis including (i) the ability to utilize small kindreds; (ii) freedom from assumptions about the mode of inheritance; and (iii) the ability to estimate the fraction of a genetically heterogeneous phenotype that is determined by a given genetic locus. Weeks et al. (63) recently reported the results of a full genome (386 markers) scan of 364 families (2129 individuals) with age-related maculopathy. The region of the genome that this study most strongly suggested to harbor an AMD-causing gene was a portion of chromosome 10 near D10S1230. Of perhaps greater interest was the observation that none of 15 candidate loci (including the loci of ABCA4, EFEMP1, RDS, ELOVL4, VMD2 and TIMP3) exhibited any evidence of linkage. In fact, four of the latter loci (ABCA4, EFEMP1, ELOVL4 and TIMP3) were excluded from harboring an age-related maculopathy gene using multipoint analysis. These findings support the candidate gene screening results described above.

**FUNCTIONAL APPROACHES TO THE DISCOVERY OF AMD PATHWAYS**

One conclusion that can be drawn from the preceding section is that even when mutations in a specific macular disease-causing gene are not responsible for a significant fraction of AMD, they can nonetheless be important in revealing the roles of specific biological pathways in the disease process. Just as the phenotypic domain of macular degeneration (as illustrated in Fig. 1) is quite varied, it is likely that the domain of pathophysiological mechanism will also be quite diverse. Since drusen were first observed ophthalmoscopically, investigators have proposed a wide variety of mechanisms to explain their formation and many of the more prominent hypotheses are summarized in Table 1. The genetic progress of the past decade has shown that several of these mechanisms are involved in the pathogenesis of human macular disease. As successful as traditional molecular genetic approaches have been for identifying genes and pathways involved in early onset macular disease, the biochemical pathways involved in typical late onset macular degeneration remain completely unknown.

Just as the affected pedigree member method is potentially complementary to family-based genetic approaches, biochemical, molecular biological and cell biological approaches to the identification of disease pathways are likely to be synergistically complementary to the genetic approach. Access to human eye tissue within a few hours of a donor’s death (especially when good clinical information is available on the donor’s eye condition during life) makes it possible to ask specific structural, immunologic, cellular and biochemical questions using
CONCLUSIONS

In the past decade, several genes were identified that cause diseases with clinical features that overlap with AMD. Although none of these genes has been found to cause a significant fraction of AMD, they have at the very least provided clear evidence that a wide range of pathophysiological mechanisms are likely to be active in this group of diseases. This has turned attention away from a search for ‘the AMD gene’, and toward the identification of biological pathways that are likely to be important in both the causes and the cures of this group of diseases. Another important trend of the past decade has been the increased recognition of the importance of cell biology in the understanding of the true biological meaning of disease-causing genetic variation. With respect to AMD, such a multidisciplinary approach is yielding important, and somewhat surprising, new insight into the biogenesis of drusen, the extracellular deposits that are the hallmark of AMD. A thorough understanding of this process will be essential for developing preventive therapeutic strategies for this class of disorders and prevention in turn will be essential for stemming the tide of blindness that will otherwise envelope the population in the next 25 years.

ACKNOWLEDGEMENTS

This paper is dedicated to our colleague and friend, Dr Vera Soares. This work is supported in part by NIH grant EY10539, the Carver Endowment for Molecular Ophthalmology, The Grousbeck Family Foundation, The Foundation Fighting Blindness and an unrestricted grant from Research to Prevent Blindness.

REFERENCES


increasingly powerful molecular tools. As reviewed more extensively elsewhere, Hageman et al. (64) have used an integrated morphological, molecular biological and biochemical approach to look for specific pathways associated with the formation of drusen. A large number of new observations have resulted from these investigations, but two of them seem most relevant in the present context. First, analyses of drusen composition reveal that a specific array of proteins—including amyloid A, amyloid P component, vitronectin, C5 and C5b-9 terminal complexes, HLA-DR, fibrinogen, Factor X, prothrombin and, occasionally, immunoglobulin—are common to virtually all clinical phenotypes of drusen (6–9). Surprisingly, many of these components that have been thought to be synthesized primarily in liver are, in fact, also synthesized locally by RPE, retinal and/or choroidal cells. Such molecules include complement components 5 and 9, immunoglobulin λ and κ light chains, Factor X, HLA-DR, apolipoprotein E, amyloid A and vitronectin. This suggests that the various phenotypic differences illustrated in Figure 1 are not secondary to the composition of the drusen associated with them. It is also quite striking that a number of these drusen-associated molecules are important components in immune and/or inflammatory processes.

Another important recent observation is the discovery of ‘core’ domains of drusen which are made up largely of glycoproteins with O-glycosidically linked carbohydrate moieties. Careful evaluation of these core reveals that they occasionally include bulbous cell processes that breach Bruch’s membrane. These processes can be traced to cell bodies on the choroidal side of Bruch’s membrane that immunoreact with a subset of antibodies that suggest that these cells are of monocytic origin. Indeed, the presence of reactivity to specific CD antigens (CD1a, CD83 and CD86) strongly suggest that these cells are dendritic cells that belong to the DC1 lineage. DC1 cells are antigen-presenting cells that are thought to participate in the induction of immunity (65). Drusen cores of this type have been observed in all drusen phenotypes (6), which is another indication that the biogenesis of all drusen phenotypes may be similar. Collectively, these two observations seem to support an important role for immune-related processes in drusen development and the etiology of AMD.

Table 1. Proposed AMD mechanisms

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormality of the neurosensory retina</td>
<td>(66–68)</td>
</tr>
<tr>
<td>Abnormality of the RPE</td>
<td>(69,70)</td>
</tr>
<tr>
<td>Abnormality of Bruch’s membrane</td>
<td>(71,72)</td>
</tr>
<tr>
<td>Abnormality of the choriocapillaris</td>
<td>(73,74)</td>
</tr>
<tr>
<td>Immune system activation</td>
<td>(75,76)</td>
</tr>
<tr>
<td>Abnormal scleral rigidity</td>
<td>(77)</td>
</tr>
<tr>
<td>Mitochondrial dysfunction</td>
<td>(78,79)</td>
</tr>
<tr>
<td>Light toxicity</td>
<td>(80–82)</td>
</tr>
<tr>
<td>Nutritional deficiencies</td>
<td>(83)</td>
</tr>
</tbody>
</table>

Table 1. Proposed AMD mechanisms


(1899) Peculiar condition of choroiditis occurring in several members of the same family. Trans. Ophthalmol. Soc. UK, 19, 71.


