Age-Related Macular Degeneration: A Complementopathy?

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Key Words

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Abstract

Age-related macular degeneration (AMD) is a progressive eye disease affecting many elderly individuals. It has a multifactorial pathogenesis and is associated with numerous environmental (e.g. smoking, light and nutrition) and genetic risk factors. A breakthrough in the mechanisms causing AMD is emerging; the involvement of the alternative pathway of the complement system appears to play a pivotal role. This has led to the statement that AMD is a disease caused by a hyperactive complement system, allowing the term 'complementopathy' to define it more precisely. Abundant evidence includes: the identification of drusen components as activators of complement, immunohistochemical data showing the presence of many species of the complement system in the retinal pigment epithelium-Bruch's membrane-choroidocapillary region of AMD eyes, a strong association of AMD with certain genetic complement protein variants, raised complement levels in blood from AMD patients and the preliminary successful treatments of geographic atrophy with complement factor D (FD) inhibitors.

FD is the rate-limiting enzyme of the alternative complement pathway, and is produced by adipose tissue. Recent findings suggest that nutrition may play a role in controlling the level of FD in the circulation. Addressing modifiable risk factors such as smoking and nutrition may thus offer opportunities for the prevention of AMD.

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Introduction

Age-related macular degeneration (AMD) is the third leading cause of global blindness after cataract and glaucoma [1]. Aging is the major risk factor, but environmental (e.g. smoking, light and nutrition) and genetic factors also play an important role [2]. Aging was long seen as the most critical factor but did not explain the exact pathology of the disease. Although immunological mechanisms were for some time implicated in the pathogenesis of AMD, definitive proof of this came only in the last decade [3–5]. In this review, we will show that AMD is not a disease mediated by classic immune mechanisms, but that a peculiar dysregulation of one of the powerful effector mechanisms of the immune system plays a pivotal pathological role.

Age-Related Macular Degeneration

Patients with AMD irreversibly lose their central vision, which markedly affects their quality of life [6–9]. The perceived impact on a patient's life is comparable to other major illnesses like stroke, cancer or HIV infection [10, 11]. In view of the growth of the aging population and the development occurring in third-world countries, it is expected that AMD prevalence will further rise and become a major global public health issue in the coming decades [12].

The buildup of debris within retinal pigment epithelial (RPE) cells and under the RPE layer is considered the initial cause of AMD [13, 14]. RPE cells phagocytize worn-out photoreceptor outer disks and, in view of the large amounts of material processed, these cells are considered the most active phagocytizing cells of the body [14]. It is therefore not surprising that the machinery of these cells and their capacity to completely degrade all material becomes faulty with age. Since most of the light entering the eye focuses on the fovea [15], it is the photoreceptors at this location that show the highest degree of wear and tear including the generation of photooxidation products. The human fovea almost exclusively contains cones, and the lipoprotein content of these photoreceptors is higher than that of the rods, which may also add to the higher lipoprotein deposition under the RPE layer of the fovea. On the other hand, the cone outer segments in the fovea are longer than at the periphery, and the amount of visual pigment in the fovea is higher, offering protection against light [16]. The cellular debris under the RPE cells is thought to activate the complement system, leading to the damaging of the RPE cells and further malfunction of this cell layer, propagating a vicious cycle of local deterioration [17]. Details about the complement system will be described in one of the following paragraphs.

During a person's lifetime, the choroidal capillary area becomes thinner and Bruch's membrane may also accumulate lipoprotein material from the choroidal capillaries, which adds to the undergraded material delivered by the RPE cells and the deficiency of nutrient transfer between RPE cells and photoreceptors. The accumulated material under the RPE cells is termed 'drusen'; drusen size can predict the development of AMD [18]. A 5-stage classification scheme for AMD using drusen size and the presence of pigmentary abnormalities has recently been introduced by the Beckman Initiative for Macular Research Classification Committee [18]. In this scheme, the early stage of AMD is characterized by the presence of intermediate-sized drusen (≥ 63 and < 125 μm) in the ab-

sence of pigmentary abnormalities, and is accompanied by a mild loss of vision. Intermediate AMD refers to when the drusen are larger (≥ 125 μm) and/or the patient has any pigmentary abnormalities; it is associated with a moderate loss of vision. Late AMD is represented in patients with new vessel formation in the macular area (wet AMD) or with gross pigment abnormalities (dry AMD or geographic atrophy) which cause a severe loss of vision. Having intermediate-sized drusen in one eye predicts a 5% rate of developing late AMD after 10 years. Having intermediate drusen in both eyes predicts a 15% risk of developing late AMD. Individuals with no/only small drusen (< 63 μm) have a risk of 1% of developing late AMD 10 years later.

The final pathway leading to blindness consists of 2 major routes. In approximately 20% of the patients with drusen, the buildup of debris is followed by a neovascular response and is classified as wet AMD. The formed vessels are leaky and lead to a rapid loss of visual function of the macular area [19, 20]. In the early phases of the neovascular response, there is not yet a large degree of RPE or photoreceptor cell loss, and controlling the neovascular response by interfering with the growth factors governing this response, e.g. VEGF, has revolutionized the treatment of patients with the wet form of AMD [1, 21, 22]. Patients without the neovascular response tend to slowly develop RPE cell loss and photoreceptor death, which leads to irreversible blindness, classified as geographic atrophy. Why some individuals develop wet AMD and others develop geographic atrophy remains unknown, but, as mentioned above, the presence of drusen is considered to be the trigger leading to this condition. Elegant studies assessing the composition of drusen have been the start of elucidating the exact role of these deposits in the pathogenesis of macular disease [23].

The Complement System

The complement system has emerged as an effector mechanism of the immune system that is considered the pivotal player in the development of AMD [17]. The system was originally considered to be a first line of defense against microbes entering the body, but has also emerged as an important player in the immune response, thereby bridging innate and adaptive immunity [24]. It consists of over 40 proteins and regulators circulating in the blood and other body fluids. It represents an important amplifier of the immune system, and its activation products are among the most important mediators of inflammation

[25]. It plays an important role in clearing cellular debris and contributes to various steps of inflammation, such as the increase in vascular permeability, leukocyte chemoattraction and extravasation.

The complement system has 3 routes of activation: the classic, lectin and alternative pathways. It has been established that the alternative pathway shows the strongest association with AMD development. A crucial step in the alternative pathway is when the activated protein C3 interacts with certain permissive surfaces. An example of a permissive surface is, for instance, Bruch's membrane or the endothelial lining of the choroidal capillaries. Activation of C3 starts with a process called autoactivation, whereby spontaneous hydrolysis of an ester bond in the C3 molecule results in an active form of C3 ('tick over' of C3). Autoactivation of C3 leads to the formation of C3b that can covalently bind to a suitable surface. The activation of C3 is the central event where the 3 complement pathways described above join together, and it runs on 2 cycles, named the feedback and breakdown cycles [26]. In the so-called breakdown cycle, surface-bound C3b can be inactivated by the action of complement factor H (CFH) and factor I (FI), whereby the degree of inactivation depends on the ability of CFH to bind to this surface [27]. The inability of CFH to bind to this surface allows a further binding with the complement protein, factor B (FB). This C3bB complex can then be cleaved by factor D (FD), forming the alternative pathway, C3 convertase (C3bBb). C3bBb is quite unstable and is stabilized by the binding of properdin. Stabilized C3bBb can cleave more C3, thus generating a feedback loop. The additional binding of a second C3b molecule leads to the formation of the C5 convertase (C3bBbC3b) and the subsequent activation of the terminal complement pathway, along with generation of the effector molecules C5a and the membrane attack complex (MAC), C5b-9. The MAC results in cell lysis.

The degree of terminal complement activation thus depends on the final C3 feedback and breakdown pathways, and involves a balance between the levels of the various complement proteins, the functional genotype activity of each player and the local tissue environment (activator surface composition).

Parainflammation

The immune system was generally considered to be a biological system that developed during evolution to combat microbial intruders. Strict control of 'self' versus 'foreign' had to eliminate a so-called detrimental autoim-

mune response. This idea has now been amended, and a low-grade autoimmune response is considered beneficial in the removal of worn-out cells and proteins [28]. The spectrum of immune reactivity was scaled by Medzhitov [29, 30]. On the left side of the scale, one can observe a situation whereby an exaggerated self-response leads to autoimmunity; on the right side, a low-grade removal of cellular debris may lead to what has been termed 'parainflammation'. As mentioned earlier, the complement system plays an important role in clearing cellular debris, thereby creating a low-grade inflammatory reaction that may not always be detrimental for the tissue in which it is occurring. If, however, this takes place for ≥ 5 decades in the retina, it may lead to AMD. A homeostatic control of tissue function may, in the long run, thus lead to local pathology in the RPE-Bruch's membrane-choroidocapillary region [31].

Drusen as the Culprits

As mentioned above, drusen are the first signs of early AMD [18, 31]. The observation that drusen contain proteins of the complement system was first made by 2 independent European groups from France and the Netherlands, and was later confirmed in extensive immunohistochemical studies by the Hageman group in the USA [5, 32, 33]. The significance of these findings was unknown at first, but it soon emerged that the photooxidation of products generated during the visual cycle were able to activate the complement system; this can lead to a deposition in the drusen [34]. Lipofuscin normally accumulates inside RPE cells and this site is not readily accessible to complement. Oxidized lipofuscin is, however, considered to be excreted by RPE cells into the subretinal space, where it can interact with complement proteins [34]. Activation of complement by oxidized lipofuscin in the subretinal space can lead to local binding of several activation products such as C3b/iC3b/C3dg/C3d. Locally deposited C3b/iC3b/C3dg/C3d can act as a ligand for various leukocyte receptors [26].

Complement activation is under the strict control of a variety of complement regulators, and, depending on the balance between activation and regulation, this might eventually lead to the generation of the MAC. This may, in turn, cause the death of cells onto which it is attached [35]. It has been suggested that RPE cells might be prone to complement-mediated lysis [36].

Generation of the fluid-phase anaphylatoxins C3a and C5a, which show powerful chemoattractant activity, will

lead to an influx of inflammatory cells [26]. Activated macrophages may release VEGF, causing neovascularization. C5a has also been shown to stimulate RPE cells to produce VEGF, resulting in neovascularization, even in the absence of an overt macrophage infiltration response [37, 38]. Macrophage influx from the choroidal side is most likely, but evidence is emerging that microglial cells from the inner retina may start migrating towards the outer retina [39].

Complement Activation by Drusen Constituents

It is difficult to isolate drusen; to show complement activation, another approach has been taken to show that drusen contain complement activators. The individual components of drusen have been shown to activate complement. In vitro experiments have shown that oxidized lipofuscin, oxidized ApoE or β -amyloid protein can activate complement [40]. Further evidence that drusen can activate complement comes from immunohistochemical evidence showing the accumulation of complement components with drusen [5]. Although various techniques could be envisioned to detect complement in drusen (e.g. immunohistochemistry; electron microscopy, SDS-PAGE and immunoblotting), the difficulty of isolating drusen has prompted research groups to use only immunohistochemistry. With this technique, it has been shown that complement proteins such as C5, C6, C8 and C9 are associated with drusen [5]. Activation products of C3: (C3a, C3b, C3dg) and C5 (C5a and sC5b-9) in drusen have also been identified on immunohistochemistry [5]. It has been found that drusen also contain the regulatory protein, CFH [5].

Experiments on drusen being incubated with plasma to allow complement activation have not yet been performed, and there are alternative explanations for the presence of complement proteins in drusen. One explanation is that, with the exocytosis of debris by RPE cells, these cells might also release complement proteins [41, 42].

Activators of the complement system other than drusen may also be present in the posterior segment of the eye. Analysis of human donor eyes has shown that complement activation in the choroidocapillary region already starts early in life and is associated with choroidal thinning and the development of AMD [43]. In this study, it was shown that choroidal deposition of the MAC sC5b-9 in humans is already evident at the age of 5 years and that it increases during a lifetime. Quantitative analysis of

sC5b-9 in retinal samples on ELISA has shown young donors to have relatively low levels of sC5b-9. Retinal samples from AMD patients have displayed variable but significantly higher sC5b-9 levels when compared to age-matched control eyes. These findings suggest that the choroidal capillary endothelial cells are under chronic attack from the complement system and that this may explain choroidal thinning. The activator of the complement system in the choroidocapillary region has not been identified. It is possible that, like the glomerular capillaries, the choroidocapillary bed is a site prone to immune complex deposition [44], and that this leads to activation of the classic pathway of the complement system.

Association of AMD with Common or Rare Genetic Variants of the Complement System

Although initial studies focused on the deposition of complement proteins in or around drusen, a major breakthrough came in 2005 when different groups independently showed a significant association with a common variant in the gene coding for the important complement regulatory gene CFH [45]. A single-nucleotide polymorphism, rs1061170, resulting in a histidine at position 402 instead of a tyrosine in the CFH protein was associated with an odds ratio (OR) of 2.3 and 5.2 in heterozygotes and homozygotes, respectively [46]. Smoking has a large influence on the risk of AMD related to CFH. Current smokers homozygous for the CFH Y402H variant were found to have an OR of 34 for late AMD when compared to nonsmokers not carrying the risk allele [47].

The histidine variant of CFH is quite common in Europeans, with approximately 30% being heterozygotes for this risk allele. Further studies have shown that the CFH risk allele has functional consequences, in that it affects the binding of CFH to various substances such as C-reactive protein, bacterial proteins, glycosaminoglycans, sulfated polyanions and malondialdehydes [45]. CFH regulates the alternative complement pathway by binding to complement-activating surfaces and enhancing the breakdown of the C3 convertase, thereby terminating this pathway before inflammatory byproducts such as C3b, C3a, C5a and C5b-9 can be formed [26]. Genetic studies and further research concerning the biological consequences of the risk variants provide firm clues as to the statement that AMD is a disease caused by an inadequate control of complement activation. Further evidence for this statement will be provided below.

Rare variants of CFH (allelic frequency <0.1% in healthy controls) such as R1210C showed a strong association with AMD and onset of the disease 6 years earlier (a median of 65 vs. 71 years) [48]. This rare variant shows a lack of binding to complement activation sites and is thus considered a 'loss-of-function allele'. Of interest is the fact that the R1210C variant is also associated with atypical hemolytic uremic syndrome, a kidney disease caused by hyperactive complement activation [49]. Recently, other rare variants of CFH, such as R53C and D90G, were shown to be associated with AMD and to have a decreased ability to control complement activation [50].

The CFH gene is located on chromosome 1, and other downstream genes encoding the so-called FH-related proteins also show an association with AMD. Preliminary evidence suggests that variants in this region may compete with FH to complement activation sites, although further confirmation is needed [51, 52].

Both common and rare genetic variants of C3 show a significant risk for AMD development and these variants were shown to affect FH binding, thereby leading to a decreased control of complement activation [53, 54].

Various polymorphisms in a gene region encoding FB and C2 have been shown to be associated with AMD [55]. It is probable that several of the single-nucleotide polymorphisms tested are in strong linkage disequilibrium with a variant of FB (R32Q). This variant is a so-called type I mutation that affects the production and release of FB. Lower levels of FB result in dampened feedback activation of the alternative pathway [56].

A common single-nucleotide polymorphism (rs10033900) in the CFI gene region has been reported to be associated with AMD, although a biological function of this noncoding variant is not yet known [57–59]. An analysis of rare variants of FI showed that their frequency was much higher in AMD cases than in controls (7.8 vs. 2.3%) [45, 60]. Some of these variants affect the concentration of FI in the blood (type 1 mutation) whereas others influence the ability of FI to inactivate C3b (type 2 mutation). A missense mutation in CFI encoding a Gly119Arg substitution was shown to be strongly associated with AMD (OR 22.2) and had a lower ability to degrade C3b, but was also expressed and secreted at a lower level than the wild-type protein (combined type 1 and type 2 mutation) [61].

As discussed above, various genetic variants of complement system proteins affecting either their function or circulating levels may predispose to AMD. An individual with a certain complotype (combination of polymor-

phisms) is thus expected to have a hyperactive complement activation profile, which, in combination with a certain permissive target residing in the region of the RPE-Bruch's membrane-choroidocapillary region, will lead to excessive complement activation [56, 62].

Smoking

Apart from aging, smoking is one of the most important modifiable risk factors for AMD [63]. Chronic exposure of experimental animals to smoke leads to RPE damage and abnormalities resembling early AMD [64]. Experimental animal models have also shown that nicotine, the major component of cigarette smoke, leads to a lower expression of one of the important complement regulators, CD59, and the laser-induced choroidal neovascularization (CNV) model showed an increased MAC deposition [65]. Exposure of cultured human RPE cells to cigarette smoke resulted in an increased expression of complement activation products (including C3a and lytic C5b-9) and a reduced expression of complement regulators [42]. Enhanced C3a release resulted in the generation of IL-1 [42], which is a powerful cytokine that can generate further production of inflammatory cytokines by RPE cells [66]. Smoking leads to a decrease in choroidal thickness, but whether this is due to increased local complement activation has still to be investigated [67].

Raised Complement in the Blood of AMD Patients

Various independent studies have shown that the concentration of several complement proteins such as C3, C4, FB and FD as well as the activation products Ba, C3a, C3d, C5a and sC5b-9 are greater in the blood of AMD patients compared to in age-matched controls [62, 68–72]. A recent study showed that the monocytes of wet AMD patients had significantly lower expression levels of the complement regulators CD46 and CD59 [73]. Taken together, the findings suggest that the levels of circulating complement proteins increase with aging. At the same time, patients with AMD tend to develop a greater degree of complement activation, due to an uncontrolled balance between activation and regulation. The site of the activation that leads to a rise in systemic levels of these markers is not yet clear. It is unlikely that local retinal complement activation will lead to a significant rise in such a large body compartment as the blood, indicating that there must also be other sites of activation in AMD

patients. It has been suggested that AMD and atherosclerosis share pathological pathways, and that atherosclerotic plaques might cause the higher levels of complement activation products observed in AMD patients [35].

As mentioned above, the potential activation level of the complement system depends on the balance between complement proteins and their regulators. Most of the complement proteins are present in excess in the circulation and, as such, are harmless. In the presence of an activator, the system becomes activated and this is subsequently dampened by various regulators.

Complement Factor D

One of the essential proteins governing the feedback cycle of the alternative pathway is FD [26]. FD is one of the proteins shown to have an elevated level in AMD patients [62, 69, 72]. It is a serine protease with a molecular weight of approximately 25 kDa, and its gene is located on chromosome 19 p13.3. It has a very low plasma concentration range of 1–2 µg/ml and forms the rate-limiting step of the activation of the so-called alternative pathway [26]. Small changes in blood FD level will thus have marked effects on the activation of the alternative pathway and can be seen as a powerful way to prevent overactivation of this pathway. Circulating FD levels can be limiting by up to a 9- to 10-fold increase of the normal concentration [72].

FD is the enzyme that forms the alternative C3 convertase C3bBb after cleaving the FB component of the C3bB complex. FD is secreted by adipocytes and is also known as adipsin [74]. In adipocytes, FD is responsible for the generation of C3a-desArg (acylation-stimulating protein), which, in turn, regulates triglyceride synthesis [75]. Once secreted by adipocytes, it is rapidly excreted by the kidneys. The median FD plasma level was shown to be approximately 30% higher in AMD patients than in age-matched controls [69]. In view of the central role of FD in the C3 feedback loop and its raised levels in AMD, it has been suggested that it is one of the complement proteins that could be therapeutically targeted in AMD patients [76]. We have recently shown that lutein supplementation was able to markedly decrease the levels of plasma FD and the activation products C3d, C5a and sC5b-9 in patients with early AMD [77, 78]. Earlier studies reporting FD levels in the plasma from AMD patients did not mention if their subjects were taking supplements. If they did indeed take lutein supplements, the difference in FD level between AMD patients and controls

could well have been even greater. Of interest is the fact that adipose tissue is the major storage site for lutein [79, 80], and we have hypothesized that lutein might affect the expression of FD by these cells [77]. Earlier in vitro observations of adipocyte cell lines showed that the FD mRNA half-life was markedly shortened by retinoic acid [80].

Gene variants have been identified for FD and an earlier 2-stage study investigating an FD gene polymorphism in AMD showed an OR of 1.11 ($p = 0.032$), whereby the association was mainly confined to female patients [72]. The ORs and p values were not very impressive, and further studies are needed to prove the exact role of FD gene polymorphisms in AMD.

Taken together, the facts described above have prompted the use of FD control in the treatment of AMD. This subject will be discussed in the last paragraph of this review.

AMD: A Disease Caused by a Hyperactive Complement System

If complement plays such an essential role in AMD development, one might expect individuals with low circulating complement levels to have a lower incidence of AMD. This has, to our knowledge, not yet been addressed, and such studies could be hampered by the fact that these patients may also have a shorter life span. A well-defined disease with known low levels of complement includes systemic lupus erythematosus. Other examples include patients with known genetic complement deficiencies [81]. Of interest is the observation that patients with a kidney disease known as ‘dense deposit disease’, also caused by a hyperactive complement system, show a marked accumulation of macular drusen at a much earlier time point in their lives than is generally seen in AMD [19, 20].

There are some limitations and questions concerning the timing associated with the role played by complement in AMD. Drusen are the early hallmark of AMD, and it was thought that complement plays an important role during early events, whereby the formation of C5b-9 (MAC) leads to the death of RPE cells and results in the progression of early AMD to later forms such as wet and geographic AMD. Once the damage has been done, one would tend to think that the role of complement in further damage could be limited. The fact that clinical trials show that FD blockade still has an effect in late AMD [76] indicates that the damaging effect of complement is ongoing.

The link between zinc and antioxidant supplements and the complement system is also intriguing. Epidemiological studies have shown that a higher intake of zinc and lutein delays the progression of late AMD [76, 82]. Not only lutein affects complement [77, 78]. A Dutch study recently showed that zinc supplementation leads to decreased systemic complement activation in AMD [83]. These data were confirmed by in vitro findings showing that alternative pathway complement activation was decreased in a dose-dependent fashion by the addition of zinc sulfate [83]. Long-term intervention studies are needed to show whether zinc and lutein supplementation may affect the development of AMD at an earlier stage. Studies where a local intravitreal anti-FD treatment is combined with systemic FD control should also be undertaken, to further develop the exciting early findings of local FD control in geographic atrophy [76].

Further evidence for a role of the complement system can be obtained by evaluating prediction models. One model, in which 11 complement genetic polymorphisms and 6 complement proteins were included, showed that AMD could be predicted with an accuracy of 74% [62]. Further modeling, including other noncomplement genes and risk factors such as diet, smoking and BMI, may provide tools for the early diagnosis and possible prevention of AMD.

The role of lipoprotein deposits in Bruch's membrane as an important trigger of events leading to AMD suggests that this should also be a target of future interventions. Local enzymatic cleaning of the membrane or early surgical replacement could be considered in cases where prediction models indicate a rapid progression.

Complement as a Therapeutic Target in AMD

In view of the pivotal role of the complement system in the development of AMD, several clinicians have opted to start trials to investigate the effect of complement inhibitors on this disease [76]. Animal studies using the laser-induced CNV model in mice have already shown that CNV could markedly be inhibited by blocking the complement system (for an extensive review, see Bora et al. [17]).

Recent clinical trials have used humanized monoclonal antibodies against C3, C5 and FD [76]. Preliminary findings suggest that blocking FD may offer the best approach and we will therefore focus on this factor.

The first observation showing that regulation of FD might offer therapeutic possibilities for retinal diseases

came from an experimental mouse model showing that photoreceptors were protected from light-induced damage in FD gene knockout animals [84]. Genentech subsequently developed a humanized IgG murine anti-FD antibody (FCFD4514S) and showed that it could block the formation of the alternative pathway, C3 convertase [85]. The FD antibody was named lampalizumab. A phase 1 trial, in which patients with geographic atrophy had lampalizumab injected intravitreally, showed this drug to be safe and well-tolerated and there were no adverse events [86]. This was followed by the so-called MAHALO study, a phase 2 study which found that intravitreally injected lampalizumab inhibited disease progression in patients with geographic atrophy. A 20% reduction rate in atrophic area was observed at 18 months in patients with advanced dry AMD [76]. These studies are extremely exciting and are being succeeded by 2 phase 3 trials currently recruiting geographic atrophy patients, in which the safety and efficacy of a 10-mg intravitreal dose of lampalizumab will be compared with sham injections (clinicaltrials.gov).

Conclusions

Abundant evidence from physiological as well as genetic studies has led to a breakthrough in our understanding of the pathogenesis of AMD. Current understanding is that AMD is a disease caused by a hyperactive complement system that acts, at both a systemic and local level, over a period of decades in a permissive region of the posterior segment of the eye in genetically predisposed individuals. It can therefore now be classified as a 'complementopathy'.

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