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C-reactive protein and complement factor H in aged human eyes and eyes with age-related macular degeneration (AMD)

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Abstract

Background—There is increasing evidence that inflammation and immune-mediated processes (complement activation) play an important role in age-related macular degeneration (AMD) pathogenesis. A genetic variation in the complement factor H (CFH) gene and plasma levels of C-reactive protein (CRP), a systemic marker of subclinical inflammation, have been consistently shown to be associated with an increased risk for AMD. In the present study, we examined the immunolocalization of CRP and CFH in aged control human donor eyes (n=10; mean age 79 yrs) and eyes with AMD (n=18; mean age 83 yrs).

Methods—Alkaline phosphatase immunohistochemistry was performed using polyclonal antibodies against CRP and CFH on cryopreserved tissue sections from disc/macular blocks. Three independent masked observers scored the reaction product (0-8).

Results—In aged control eyes, the retinal pigment epithelium/Bruch's membrane/choriocapillaris (RPE/BrM/CC) complex including intercapillary septa (ICS) had the most prominent immunostaining for CRP and CFH. CRP was significantly higher than controls in BrM/CC/ICS and choroidal stroma in early and wet AMD eyes ($p<0.05$). In contrast, CFH was significantly lower in BrM/CC/ICS complex of AMD choroids than in controls ($p<0.05$). Interestingly, CRP and CFH were significantly reduced in BrM/CC/ICS complex in atrophic area of macula in geographic atrophy (GA) ($p<0.05$). Drusen and basal laminar deposits were intensely positive for CRP and CFH.

Conclusion—These immunohistochemical findings show that changes in distribution and relative levels of CRP and CFH were evident in early and late AMD eyes. This suggests that high levels of CRP and insufficient CFH at the retina/choroid interface may lead to uncontrolled complement activation with associated cell and tissue damage. This study supports the hypothesis that inflammation and immune-mediated mechanisms are involved in the pathogenesis of AMD.

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Keywords

age-related macular degeneration; C-reactive protein; complement factor H; retina/choroid interface; immunohistochemistry

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss among the elderly population worldwide. [1] Recent studies provide increasing evidence that inflammation and immune-mediated processes play an important role in AMD pathogenesis. Immunohistochemical studies demonstrate that ocular drusen, in sub-RPE space, and the capillary pillars of the choroid contain complement components including C3 complement fragments, C5, and the membrane attack complex C5b-9 [2-4] and other molecules that mediate local inflammation. [5-7]

C-reactive protein (CRP) is an acute phase reactant, synthesized predominantly in the liver and adipocytes [8], and is considered to be a nonspecific serum biomarker for subclinical inflammation. Elevated CRP is considered as a risk for heart disease, [9] type 2 diabetes [10] and AMD, [11] but the mechanism underlying the link between CRP and these diseases is not fully understood. CRP immunoreactivity has also been identified in ocular drusen and other sub-RPE deposits. [6-7] Several biological activities have been attributed to CRP, such as complement activation, [12] macrophage activation, [13] and platelet activation. [14]

Complement factor H (CFH) is a major inhibitor of the alternate complement pathway and indirectly inhibits the lectin pathway. CFH mainly functions to control the alternate complement activation in plasma, host cells and tissue, and sites of tissue inflammation. It binds to cells and circulates freely. CFH performs three actions in complement regulation: inhibiting the cleavage of C3 to C3a and C3b; binds C3b on host cells preventing their destruction; and removes C3bBb convertase. [15] Recent evidence suggests that CFH may play a significant role in pathogenesis of AMD. A common polymorphism in the gene of CFH (Y402H) has been reported as a novel risk factor for AMD. [16-19] Furthermore, recent compelling evidence indicates that the AMD-associated complement factor H variant CFH-Y402H in the SCR7 domain of CFH confers reduced binding of CFH to CRP, resulting in high levels of unbound CRP in the choroid, and could thus be permissive for chronic inflammation. [20]

Despite the mounting evidence that inflammation is important in AMD, there have been only a few studies describing CFH localization in choroid. [20-21] In vivo expression and accumulation of CRP in human choroid with AMD still remains to be elucidated. The purpose of this study was to examine for the first time the localization of CRP and CFH in the same aged control human retinas and choroids and to determine if the localization or relative levels in the submacular area were changed in AMD eyes.

MATERIALS AND METHODS

Donor Eyes

Human donor eyes were obtained with the help of Janet Sunness, M.D., and Carol Applegate (Greater Baltimore Medical Center, Baltimore, MD, USA) and from the National Disease Research Interchange (NDRI; Philadelphia, PA, USA) within 10-35 hours of death. Eyes from eighteen subjects with AMD (age range 74-104 years; mean age 83.8 years) and ten aged control donors (age range 73-86 years; mean age 79.5 years) with no evidence of macular disease were studied. All donors were Caucasian. Table 1 summarizes the characteristics of each subject. The diagnosis of AMD was made by reviewing ocular medical history on the eye bank transmittal sheets and the postmortem gross examination of posterior eyecup, using transmitted and reflected illumination with a dissecting microscope (Stemi 2000; Carl Zeiss Meditec, Inc, Thornwood, NY, USA). During gross examination, AMD eyes were classified according to the severity of disease: early AMD (n=8; soft indistinct drusen with or without pigmentary changes, or soft distinct drusen with pigmentary changes) and late (end-stage) AMD. The latter was sub-classified into dry or nonexudative AMD with geographic atrophy (GA; n=6), which is characterized by thick basal deposits and distinct areas of RPE loss, or wet, exudative AMD with choroidal neovascularization (CNV; n=4). Genomic DNA information of AMD subjects was not available. The protocol of the study adhered to the tenets of the Declaration of Helsinki regarding research involving human tissue and was approved by the Johns Hopkins JCCI.

Tissue Preparation

After the anterior segment of the eye was removed, the posterior eyecup was grossly examined and fundus digital images were captured. The posterior eyecup was then fixed in 2% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) with 5% sucrose at room temperature for 1 hour. The tissue was cut into calottes of vitreous/retina/choroid complex and cryopreserved as previously described. [22] Serial 8- μ m sections were cut from the disc/macular blocks (extending from nasal peripapillary to two disc diameters temporal to macula), dried, and stored at -80°C .

Immunohistochemistry

Tissue sections (8 sections/eye) were processed for immunohistochemistry as described in detail previously. [23] Sections were incubated at 4°C overnight with rabbit monoclonal antibody directed against CRP diluted 1:80,000 (1568-1, Epitomics, Inc, Burlingame, CA, USA), 1:10,000 goat anti-human CFH antibody (AF4779, R&D Systems, Inc, Minneapolis, MN, USA), or mouse anti-human CD-34 (1:800; Signet Laboratory, Dedham, MA, USA) antibody in adjacent sections to label blood vessels. As negative controls, either the primary antibodies were omitted or a non-immune IgG was used at the same protein concentration as the primary antibody. The pigment was bleached from RPE and choroidal melanocytes as described previously. [23] Histopathology was confirmed on adjacent sections of the disc/macula with the periodic acid Schiff's (PAS) and hematoxylin staining. Some sections were stained with hematoxylin and eosin for visualizing retinal morphology.

Three independent masked observers, using a previously described eight point grading system [23], graded the relative intensity of the immunoreactivity for both antibodies in retinal and choroidal structures. Immunoreactivity for CRP was not uniform but rather heterogenous, so the graders scored a particular structure like intercapillary septa throughout the whole tissue section. The intensity of labeling was graded qualitatively as: 8, uniformly intense immunoreactivity; 7, patchy and intense; 6, uniform and moderate; 5, patchy and moderate; 4, uniform and weak; 3, patchy and weak; 2, uniform and very weak; 1, patchy and very weak; and 0, comparable to non-immune IgG control section.

Statistical analysis

The combined mean score ($\pm$ SEM) from the all graders was calculated for each retinal and choroidal structure. The *p* values were determined by comparing mean scores from the aged control eyes with scores from eyes with either early, wet, or dry AMD using the Student's unpaired *t*-test and assuming unequal variance and two tails. Two groups were always compared. The *p* value <0.05 was considered significant. Statistical analysis was done with InStat software (version 2.0, GraphPad Software, San Diego, CA, USA).

RESULTS

Immunolocalization of CRP and CFH in aged control human macular retina and eyes with AMD

In aged control retina, moderate CRP and CFH immunoreactivity was detected almost exclusively in the endothelial cells (ECs) and the wall (probably smooth muscle cells; SMC) of the large retinal blood vessels (figs 1D and 1G). The ECs of retinal blood vessels were intensely labeled for CD34 (fig 1A). The neural retina was negative for both CRP and CFH.

In early and wet AMD retinas, the immunoreactivity for CRP was more intense in retinal vessels than in the aged controls (figs 1E and 1F). In GA retinas, however, the CRP immunoreactivity was weak in retinal vessels compared to the aged control retinas (data not shown). Mean immunoreactivity scores for the retinal vessels of the aged control and AMD eyes are shown in figure 2. Scores for CRP were significantly higher in retinal arteries, veins, and capillaries in wet AMD (*p* 0.02) compared to the aged control retinas (fig 2A). Inversely, the immunoreactivity score for CRP was significantly lower in retinal arteries and veins in GA (*p* 0.007) compared to the aged control retinas (fig 2C). In contrast, the intensity and pattern of CFH immunoreactivity was similar in early and late AMD retinas as compared to aged controls. There was no significant difference in immunoreactivity scores for CFH in retinal vessels between aged control and both wet and dry AMD retinas (figs 2B and 2D). CRP and CFH were expressed prominently in intraluminal serum and the perivascular space of some large blood vessels in wet AMD retinas (figs 1F and 1I).

Immunolocalization of CRP and CFH in aged control human submacular choroid and eyes with AMD

In aged control choroids, PAS and hematoxylin staining showed no deposits or drusen or other pathologic evidence of AMD (fig 3A). The choroidal vessels, including choriocapillaris (CC), were intensely labeled for CD34 and appeared normal

morphologically with broad lumens (fig 3D). Basal laminar deposits and drusen were often observed in early AMD choroids (fig 3B). The CC lumens appeared irregular and constricted in early and wet AMD eyes (fig 3E-F), and attenuated in AMD eyes with GA (fig 4D).

In aged control choroid, CRP immunoreactivity was prominently localized in and around the CC and intercapillary septa (ICS) and, to a lesser extent, in large choroidal blood vessels and individual cells in choroidal stroma (fig 3G). Weak CRP immunoreactivity was observed in RPE and Bruch's membrane (BrM). Immunostaining for CFH was predominantly present in BrM-CC complex including the ICS, to less extent in large choroidal blood vessels and choroidal stroma (fig 3J). No immunoreactivity for CFH was detected in RPE cells. There was no staining when primary antibodies were eliminated or non-immune IgG with matched protein concentration to the primary antibody (figs 3M-O) was used except in hard drusen (fig 3N).

In early and wet AMD choroids, CRP immunostaining was more intense and prominent in BrM-CC complex including the ICS and choroidal vessels (figs 3H-I) compared to the aged controls (fig 3G). In contrast, immunostaining for CFH was significantly reduced in BrM-CC complex including the ICS in wet AMD subjects (fig 3L). In eyes with early AMD, CRP and CFH immunoreactivity were present in drusen and around the choroidal capillaries (figs 3H and 3K). Both antibodies generally labeled drusen homogeneously (figs 3H and 3K). We also examined subretinal CNV and CNV within disciform scars for CRP and CFH in wet AMD subjects. CFH immunoreactivity was weak to negative in both forms of CNV, whereas the CNV and scar had moderate CRP staining (data not shown).

In GA subjects, we compared adjacent non-atrophic and atrophic regions of macula. The pattern of CRP and CFH immunostaining in non-atrophic areas was similar to aged control choroids (figs 4E and 4G). However, in the atrophic area, CRP was significantly reduced in BrM-CC complex including the ICS (fig 4F) and choroidal vessels compared to the non-atrophic area. CFH was also greatly reduced in BrM-CC complex including the ICS in the atrophic area (fig 4H).

Mean immunoreactivity scores for the choroidal structures of the aged control versus AMD eyes are shown in figure 5. Scores revealed that CRP immunoreactivity was significantly higher in BrM, CC, ICS and large choroidal arteries and veins in early AMD ($p = 0.0037$) and in wet AMD ($p = 0.05$) eyes compared to the aged controls (fig 5A). Immunoreactivity scores for CRP were significantly lower in BrM ($p = 0.03$) and CC ($p = 0.04$) in atrophic areas of GA compared to aged control (fig 5C). However, the score for CRP was higher in choroidal structures in non-atrophic area than the atrophic area (fig 5C). In contrast, immunoreactivity scores for CFH were significantly lower in choroidal structures in early AMD ($p = 0.014$) and wet AMD choroids (BrM, CC, ICS, arteries, veins, and stroma) ($p < 0.001$, fig 5B) as well as in non-atrophic ($p = 0.0013$) and atrophic areas of GA ($p = 0.03$, fig 5D) compared to the aged controls.

DISCUSSION

In this study, we demonstrate the expression pattern of the CRP and CFH in macular retina and submacular BrM–choroid complex in aged control human eyes. The immunoreactivity of CRP was significantly increased in retinal vessels and in BrM–choroid complex in submacular choroid in early and wet AMD eyes, whereas CRP was significantly decreased in choroid with RPE atrophy in GA. In contrast, CFH was significantly decreased in BrM–choroid complex of early and both wet and dry AMD. These findings show that there is a significant inverse correlation between the CRP and CFH levels in eyes with advanced AMD.

CRP is a multifunctional, nonspecific marker of systemic inflammation. It has pro-inflammatory and pro-thrombotic roles in the pathogenesis of atherosclerosis. [24] It can activate the classic complement pathway through its interaction with C1q, which enhances the inflammatory response and phagocytosis, up-regulates the expression of adhesion molecules, increases cytokine release, and inhibits the expression of endothelial nitric oxide synthase in endothelial cells. [24] The pro-inflammatory role of CRP has been demonstrated to be closely associated with the induced secretion of the proinflammatory cytokines IL-8 and MCP-1. CRP is a known constituent of drusen [6,7] and the accumulation of drusen and sub-RPE deposits in early AMD are regarded as an indication of chronic local inflammation at the RPE-choroid interface. [5,7] Thus, the elevated levels of CRP in eyes with early AMD may be interpreted as evidence for local inflammation and cellular injury in the RPE-choroid. Elevated CRP in CC has many implications. When CRP binds the Fcγ receptors, NF-κB activation occurs [25,26] which could result in the endothelial cell death that we have observed in AMD [27]. NF-κB activation causes upregulation of ICAM-1, which would result in increased firm adhesion of some white blood cells. Mullins has observed increased ICAM-1 in macula of normal subjects. [28] Also CRP can reduce eNOS expression, [29] which we have observed in CC AMD subjects. [30]

One of the major and most rigorously defined biological functions of CRP in humans is activation of the complement system via the classical pathway. Complement activation is thought to be involved in the pathogenesis of AMD, in part because certain gene polymorphisms in CFH, an important regulator of the alternative complement activation pathway, are high risk factors for AMD. CFH is involved exclusively in the alternative pathway (AP) of complement activation. CFH is a potent fluid state negative regulator of the AP, synthesized predominately in the liver and released into the blood, from where it is transported to other tissues. CFH has been detected immunohistochemically in the choroid, and at low levels in the RPE cell and interphotoreceptor matrix, but appeared to be concentrated in dense patches in Bruch's membrane. [31] RT-PCR studies have shown that the RPE may synthesize CFH locally. [16] It was proposed that a local complement regulation system exists at the retinal/choroidal interface and dysfunction of this regulatory system may contribute to the pathogenesis of AMD. [21]

We compared the immunoreactivity levels of CRP and CFH in atrophic and nonatrophic areas of choroid in AMD with GA. In the atrophic area, both proteins were significantly decreased in BrM/CC complex compared to non-atrophic area. These results were not

surprising. Previously we have demonstrated a 50% mean reduction in vascular area in regions with RPE atrophy compared with regions in GA eyes with RPE and with aged control eyes without maculopathy. [27] The surviving capillaries that remained in the regions of RPE atrophy were extremely constricted and were half the diameter of capillaries in areas with RPE present. We also noted a loss of fenestrations in the endothelium of these constricted capillaries, suggesting functional changes. [27] Fenestrations, pore-like structures in endothelial cells, are thought to be important for the CC to facilitate the rapid exchange of nutrients and waste with the overlying RPE. It is reasonable to conclude that decreased levels of CRP and CFH in GA where the RPE has atrophied was a result of capillary loss, and lost fenestrations in surviving capillaries [27] and, therefore, reduced extravasation of serum compounds.

In AMD, it is highly likely that the age-related accumulation of drusen and basal deposits on the retinal side of Bruch's membrane acts as a local inflammatory stimulus in an anatomical compartment to which the immune system has limited access. Because CRP can up-regulate the expression of proinflammatory molecules, significant CRP deposition within the BrM/choroid complex may trigger and exacerbate an inflammatory response. Reduced CFH, which regulates the complement cascade, may further enhance this inflammatory process. Our results suggest that high levels of CRP and insufficient CFH at the retina/choroid interface may lead to uncontrolled complement activation with associated cell and tissue damage. This study supports the hypothesis that inflammation and immune-mediated mechanisms are involved in the pathogenesis of AMD.

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Figure 1.

Retinal sections from an aged control, early, and wet AMD eyes are immunolabeled for CD34 (B,F,J), CRP (C,G,K) and CFH (D,H,L). The morphology of the retina is shown in the hematoxylin and eosin stained sections in A, E, and I. Endothelial cells of large retinal vessel (large arrow) and capillaries are intensely labeled for CD34 (B,F,J). Immunoreactivity for CRP (C) and CFH (D) is weak and associated with retinal vessel in aged control eye. Note that with severity of AMD, immunoreactivity for CRP (G,K) and CFH (H,L) is significantly increased. CFH and CRP in wet AMD are prominent within lumens (K,L) and in perivascular spaces around some large blood vessels (K). (scale bar=50 μ m in A-D; 30 μ m in E-L)

Figure 2.

Mean immunoreactivity scores $\pm$ SEM for CRP and CFH in retinal vessels of aged control (black), early AMD (white), and late AMD (gray) eyes. The scores for CRP were significantly higher in retinal vessels in wet AMD (arteries $p = 0.0203$; veins $p = 0.0026$; and capillaries $p = 0.0009$) (A) compared to aged controls and significantly lower in retinal artery ($p = 0.0026$) and vein ($p = 0.0065$) in atrophic and non-atrophic area in GA compared to aged controls (C). In contrast, there was no significant difference in scores for CFH in retinal vessels between wet AMD and aged controls (B) as well as between atrophic or non-atrophic areas in GA (D) and the aged controls. (* $p < 0.05$ compared to control; unpaired Student's t -Test analysis)

Figure 3.

CRP and CFH immunoreactivity in choroid from aged control, early, and late wet AMD eyes. Periodic acid Schiff's (PAS) and hematoxylin staining shows morphological features of the choroid from aged control (A), drusen (asterisk) in early AMD (B) and CNV (large arrow) anterior to RPE (open arrowhead) in wet AMD. The choriocapillaris (CC; small arrow) and large choroidal vessels are intensely labeled for CD34 and appear morphologically normal with broad lumens in aged control (D), whereas CC lumens appears irregular and constricted in early (E) and wet AMD (F). In aged control choroid, CRP (G) and CFH (J) are prominently localized to the CC, ICS and BrM (open arrowhead) and, to a lesser extent, in large choroidal vessels and stroma. CRP immunoreactivity is significantly increased in early (H) and late AMD (I) choroids compared to the aged control. CFH in early AMD (K) is comparable to aged control, whereas significantly decreased in wet AMD (L). Drusen are intensely positive for CRP and CFH (H and K). Note that in wet AMD the

CNV (large arrow) area has more CRP and less CFH (I and L). Non-immune rabbit IgG yields a very weak to negative reaction product except in drusen (M, N, and O). (scale bar=20µm)

Figure 4.

CRP and CFH immunoreactivity in AMD eye with GA. Note that the non-atrophic area (A) of choroid has RPE (arrowhead) and PAS-positive thickened BrM (double arrows) and no RPE in the atrophic area (B). CD34 localization demonstrates that the CC (arrow) is limited in the atrophic area compared to the non-atrophic area (C and D). In non-atrophic area, CRP is very intense in CC and blood vessels (asterisks) and diffuse throughout stroma (E). CFH is prominent in BrM, CC and ICS in nonatrophic areas (G). In the atrophic area, both antibodies yield reduced reaction product in BrM, CC and ICS (F and H). (scale bar=20µm)

Figure 5.

Mean immunoreactivity scores $\pm$ SEM for CRP and CFH in choroidal structures of aged control (black), early AMD (white), and late AMD (gray) eyes. The scores for CRP were significantly higher in early AMD compared to controls: BrM $p=0.0037$, CC $p<0.0001$, ICS $p<0.0001$, arteries $p=0.002$, and veins $p<0.001$ (A). Scores were also significantly higher in wet AMD compared to controls: BrM $p=0.05$, CC $p=0.027$, and ICS $p=0.024$ (A). Scores were significantly lower for CRP in atrophic area in GA (C) compared to aged control and higher in non-atrophic regions compared to controls. In contrast, scores for CFH were significantly lower in choroidal structures in early and wet AMD (B). In early AMD, BrM ($p=0.001$), CC ($p=0.014$), and ICS ($p<0.0001$) had significantly less CFH than control subjects. In wet AMD, BrM, CC, ICS, arteries, veins, and stroma ($p<0.0001$, $p<0.0001$, $p<0.0001$, $p=0.002$, $p<0.001$ respectively) had significantly less CFH than control subjects. Significant differences in CFH immunoreactivity were found in both non-atrophic (BrM, $p<0.0001$; CC, $p=0.0013$; and ICS, $p<0.0001$) and atrophic areas of GA (BrM, $p=0.03$; CC, $p<0.0001$; ICS, $p<0.0001$; artery, $p=0.002$) compared to the aged controls. (* $p<0.05$ by unpaired Student's t -Test analysis between AMD and aged controls)

Table 1

Characteristics of human subjects

Subjects	Time (hours)		Age/race/sex	Primary cause of death	Medical history
	DET	PMT			
Aged					
1	3.5	29	73/C/F	Colon cancer	Unknown
2	2.5	33	75/C/F	Heart disease	Pulmonary HTN, CVA, CAD
3	3	24	75/C/M	Bronchitis secondary to Lung cancer	Smoker (1~1.5) ppd; colon cancer
4	1	26	77/C/M	COPD	CAD, CHF, HTN, Hyperlipidemia, osteoarthritis
5	2.5	28	80/C/M	COPD	Abdominal aortic aneurysm
6	7	28	80/C/M	Intracranial hemorrhage	HTN, angioplasty, arthritis
7	3	15	82/C/M	Metastasis Brain cancer	CAD
8	3	16	83/C/M	Cardiac respiratory arrest	MI, CABG
9	4	31	84/C/M	Cardiac arrhythmia	Pneumonia, HTN, CHF, CAD, CABG
10	3	32	86/C/M	CVA	COPD, HTN, osteoarthritis
Early AMD					
11	4	33	74/C/M	Prostate cancer	COPD
12	2	38	76/C/F	Brain death	CVA, CHF, COPD, hypoxia ischemia
13	4	28	77/C/F	Gastrointestinal Bleed	COPD, casual smoker
14	6	26	79/C/F	Lymphoma	Sepsis
15	3	33	79/C/M	Pneumonia	HTN, COPD, CAD, Alzheimer's, prostate cancer
16	7	28	82/C/M	Pneumonia	CHF, Transient Ischemic Attack, CABG
17	3	12	83/C/M	Prostate cancer	DM, HTN, arthritis
18	1.5	21	95/C/F	Pneumonia	HTN, CAD, osteoarthritis
Wet AMD (Late)					
19	7	30	75/C/M	Aspiration pneumonia	CAD, CVA
20	4	17	80/C/F	Colon cancer	End-stage colon cancer
21	7	28	89/C/F	Pancreatic cancer	N/A
22	4	20	93/C/F	Multi system failure	DM, HTN, Transient Ischemic Attack
AMD (GA)					
23	5	26	78/C/F	CAD	IDDM, HTN, COPD, CHF, MI, Rheumatoid arthritis
24	7.5	26	79/C/M	COPD	CHF, CVA, CAD, NIDDM, arthritis
25	3	36	84/C/M	Cardiac failure	CHF
26	6	9	88/C/M	CHF	CAD, HTN, hyperlipidemia, smoker
27	3.5	-	95/C/M	Cardiomyopathy	CAD, CHF, MI
28	4.5	11	104/C/M	COPD	

DET, death to enucleation time; PMT, postmortem time (death to fixation); C, Caucasian; AMD, age-related macular degeneration; CVA, cerebrovascular accident; DM, diabetes mellitus; HTN, hypertension; COPD, chronic obstructive pulmonary disorder; GA, geographic atrophy; MI, myocardial infarction; CAD, coronary artery disease; CHF, congestive heart failure; CABG, coronary artery bypass grafting

Note: All AMD subjects except #18 were clinically diagnosed with AMD.

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