The Iron Carrier Transferrin Is Upregulated in Retinas from Patients with Age-Related Macular Degeneration

Itay Chowers,1 Robert Wong,2 Tzvete Dentchev,2 Ronald H. Farkas,5 Jared Iacovelli,2 Tushara L. Gunatilaka,3 Nancy E. Medeiros,4 J. Brett Presley,5 Peter A. Campochiaro,5,6 Christine A. Curcio,3,6,7 Joshua L. Dunaiief,2 and Donald J. Zack3,6,7,8

PURPOSE. Iron can cause oxidative stress, and elevated iron levels have been associated with several neurodegenerative diseases including age-related macular degeneration (AMD). Transferrin, an iron transport protein, is expressed at high levels in the retina. The purpose of this study was to assess transferrin involvement in AMD by determining the expression profile of transferrin in retinas with AMD compared with retinas without evidence of disease.

METHODS. Postmortem retinas were obtained from AMD and non-AMD eyes. Expression of transferrin was assessed in a microarray dataset from 33 retinas of unaffected donors and 12 retinas of patients with AMD (six with neovascular AMD and six with non-neovascular AMD). Quantitative real-time RT-PCR (QPCR) was used to confirm the microarray results. Transferrin protein expression was assessed by semiquantitative Western blot analysis and immunohistochemistry.

RESULTS. In comparison to unaffected retinas, mean transferrin mRNA levels, as measured by microarray analysis were elevated 3.5- and 2.1-fold in non-neovascular and neovascular AMD retinas, respectively. Semiquantitative Western blot analysis demonstrated a 2.1-fold increase in transferrin protein in AMD eyes. Immunohistochemistry showed more intense and widespread transferrin label in AMD maculas, particularly in large drusen, Müller cells, and photoreceptors.

CONCLUSIONS. These data demonstrate that transferrin expression is increased in the retinas of patients with AMD relative to those of healthy control patients of comparable age. Along with previous studies that have demonstrated elevated iron levels in AMD retinas, early onset drusen formation in a patient with retinal iron overload resulting from aceruloplasminemia, and retinal degeneration with some features of macular degeneration in the iron-overloaded retinas of ceruloplasmin/haphepinistin knockout mice, the present study suggests that altered iron homeostasis is associated with AMD. (Invest Ophthalmol Vis Sci. 2006;47:2135–2140) DOI:10.1167/iovs.05-1135

A ge-related macular degeneration (AMD) is characterized by progressive degeneration of the retina, retinal pigmented epithelium (RPE), and choroid. The concept that oxidative stress plays a central role in this degenerative process is attractive but still unproven. Conceivably, the oxygen-rich environment, combined with high polyunsaturated fatty acid content in photoreceptor outer segments, and exposure to light, predispose the RPE and outer retina to such damage.

Iron has been implicated in the pathogenesis of AMD. Although it is crucial for several metabolic pathways, it can also exert oxidative damage, especially when accumulated in excessive amounts. Iron-associated damage can occur through generation of hydroxyl radicals through the Fenton reaction in which iron in its ferrous (II) state reacts with hydrogen peroxide (H2O2). Iron-mediated damage has been implicated in several conditions affecting the central nervous system, including Alzheimer’s disease, Parkinson’s disease, glaucoma, and retinal degeneration. Retinas from patients with AMD were found to have increased levels of iron compared with retinas from patients of comparable age with no identifiable retinal disease. In addition, retinal degeneration develops in genetically modified mice that accumulate iron in the retina.

Transferrin, which binds and transfers iron within and across tissues, is crucial for maintenance of iron homeostasis. It is highly abundant in the retina and RPE. Human transferrin mRNA levels are more than six times higher in the retina than across tissues, is crucial for maintenance of iron homeostasis. It is highly abundant in the retina and RPE. Human transferrin mRNA levels are more than six times higher in the retina than in the liver and cerebral cortex. The unusually high expression of transferrin in the retina raises the question of whether it has a unique retinal function. Transferrin may protect the retina from the potentially toxic effects of unbound iron, because iron bound to transferrin does not cause oxidative stress. Transferrin may also have neurotrophic effects that are essential for normal retinal functioning.

The potential involvement of iron in the pathogenesis of AMD, combined with the important role of transferrin in iron metabolism and its high level of expression in the retina, suggests that transferrin may function as a component of the...
oxidative defense system of the retina. Furthermore, it suggests the hypothesis that transferrin levels may be modulated in the retinas of patients with AMD in an attempt to reduce oxidative damage. To test this hypothesis, we assessed the mRNA and protein levels for transferrin in retinas obtained after death from patients with AMD and from control patients of comparable age.

**METHODS**

**Microarray Analysis**

We analyzed a microarray data set that was obtained using retinal RNA isolated from eyes obtained after death from patients with AMD and appropriate control patients. The present study is part of a large ongoing project aimed at identifying changes in gene expression that occur in the retinas of patients with AMD.

Donor tissue processing, microarray platform, microarray procedure, and bioinformatic and statistical analysis methodologies have been described. Briefly, human donor eyes were obtained from the National Disease Research Interchange (NDRI, Philadelphia, PA) in accordance with institutional review board regulations and the provisions of the Declaration of Helsinki for research involving human tissue. Eyes were classified based on the medical history provided by the NDRI and on examination of the retina under a dissecting microscope after removal of the anterior segment. Donors without a history of retinal disease and with no grossly visible pathologic findings were classified as unaffected. Donors who had a diagnosis of AMD with typical findings (medium size drusen, pigmentary abnormalities, choroidal neovascularization, or a disciform scar) were classified as having AMD. Eyes with AMD were further classified into non-neovascular and neovascular AMD, based on the presence of findings associated with neovascular AMD such as subretinal blood, lipid, fluid, or fibrosis. Thirty-three eyes of 19 unaffected donors and 12 eyes of 6 donors with AMD were included in the data set. Donor age ranged between 29 and 90 years. Fifteen donors were women and 10 were men.

A human retina and RPE custom cDNA microarray, which includes 10,034 sequences, was used for gene expression profiling. Duplicate microarray experiments were performed in each eye. A common reference study design was used, followed by analysis with a custom two-step regression model combined with the Significance Analysis of Microarray (SAM) algorithm as previously described. Briefly, during the first regression step, we corrected for technical sources of variation (microarray slide batch, eye laterality, dye effect). Then, the normalized expression level difference associated with each of the biological factors that were included in the analysis (age, gender, age by gender interaction, non-neovascular AMD, and neovascular AMD) was measured in the second regression step. This statistical model facilitates comparison of the relative contribution of each of the biological factors on gene expression levels. In the present study, analysis was focused on microarray genes known to play a role in iron metabolism.

**Quantitative Real-Time RT-PCR**

For technical validation of microarray results we applied real-time quantitative RT-PCR (QPCR) on four unaffected retinas and four retinas affected by AMD that were randomly selected from the microarray sample set. One microgram of RNA from each sample was reverse transcribed (Superscript II; Invitrogen) and used as a template for QPCR reactions. Specific primers were then used to amplify glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward primer: 5'-GGGGGAGGCCCCAAGGGTCA-3', reverse primer 5'-CCCCAGGCTCA-AAGGTGGA-3') and transferrin (forward primer: 5'-CTTACACAA-TCTTGGCATCGA-3', reverse primer 5'-TTTGGCAGTTTCATCAATATA-GTCG-3'). Reactions were performed with a thermal cycler (LightCycler PCR system; Roche, Nutley, NJ). Five serial twofold dilutions of one of the samples were defined as standards in all the reactions. PCR products were quantified with the second derivate maximum values calculated by the system analysis software. Transferrin mRNA levels were normalized to GAPDH mRNA levels.

Transferrin was tested in duplicate PCR reactions, and the mean of the two reactions was used for calculating expression levels. Agarose gel electrophoresis was used to confirm that reaction products had the expected size (105 bp). The formation of a single product was also confirmed by observing the melting curve graph that was generated by the thermal cycler machine for each reaction tube.

**Western Blot Analysis and Histopathologic Evaluation**

Eyes obtained after death, distinct from the ones used for microarray and QPCR analysis, were obtained in pairs through the Alabama Eye Bank in accordance with the Declaration of Helsinki. Informed consent for donation and access to ophthalmic histories was obtained from eye donor next of kin. For histopathologic evaluation, the anterior segment of one eye of each pair was removed with a circumferential incision along the pars plana and the posterior segment was immersion fixed. Maculas with grossly visible drusen and RPE change were photographed with epi- and/or oblique transscleral illumination in stereo color, as has been described. For Western blot analysis, the fellow eye was incised circumferentially at the equator, penetrating the sclera, retina, and vitreous. The retina and vitreous body were removed together after excision of the retina at the optic nerve head. The retina was dissected, frozen in liquid nitrogen, and stored at −80°C in microfuge tubes. An RPE-choroid-sclera eye cup was pinned to a cellulose sponge well, frozen in liquid nitrogen, and stored at −80°C in biohazard bags for shipment.

For histopathology, tissue blocks were excised from the fovea, postfixed in 2% osmium, and embedded in resin (Polysbed 812; Polysciences, Warrington, PA), as has been described. Blocks were sectioned at 1 μm into the rod-free zone of the fovea. In samples in which the RPE separated from the retina, the RPE block was sectioned for the same distance as the retinal block, to identify RPE/Bruch’s membrane underlying the rod-free zone. At this location, toluidine blue-stained sections were evaluated as described. Color images of representative findings were obtained using a 40× planapochromat oil objective, numerical aperture of 1.4 (Optiphoto 2; Nikon, Melville, NY) and a charge-coupled device (CCD) camera (SpotRT; Diagnostic Instruments, Auburn Hills, MI). Silver–gold ultrathin sections at this location were poststained with uranyl acetate and lead citrate (2 minutes). Representative electron micrographs were taken at 7,500× and 20,000× with a transmission electron microscope (1200 EXII; JEOL USA, Peabody, MA). Negatives were scanned, printed, and composited as described.

Ascertainment of AMD in preserved eyes was based on history (reviewed by a medical retina specialist, NEM), gross appearance, and histopathology (both reviewed by an AMD histopathologist, CC). Ascertainment of AMD in frozen RPE-chorioid-sclera eye cups was based on independent gross evaluations by CC and an AMD pathologist/AMD clinician (JLD). These assessments agreed perfectly.

For Western blot analysis, homogenization buffer (5 mL of 10 mM K₂HPO₄, 150 mM NaCl, 200 mM sucrose, 1% Triton X-100, and 1 protease inhibitor cocktail tablet (Complete; Roche Diagnostics GmbH, Mannheim, Germany) was added to each frozen retina. Samples were then sonicated at 30% output for 30 pulses with a sonifier (Branson Model 250; WVR International, West Chester, PA) and centrifuged, and the supernatant was assayed for total protein concentration using the bicinchoninic acid (BCA) protein quantification kit (Pierce, Rockford, IL). Two micrograms of total protein from each retina was loaded into each lane of a 3% to 8% gel (NuPage; Invitrogen, Carlsbad, CA). Purified human transferrin (5 ng; Alpha Diagnostics International, San Antonio, TX) was added to one lane as a positive control and size marker. Protein was transferred to a nitrocellulose membrane (NuPage; Invitrogen). The membrane was cut in half, with the top half probed with a polyclonal anti-transferrin antibody (1:500; Cappel IG, Costa Mesa, CA) and the bottom half probed with anti-
GAPDH antibody (dilution 1:1000; Chemicon, Temecula, CA). Bound primary antibody was detected by incubation with an alkaline phosphatase-conjugated secondary antibody. Chemiluminescent bands were detected with enhanced chemiluminescence (ECL; GE Healthcare, Buckinghamshire, UK) and a phosphorescence image (Storm Phosphorimager; Molecular Dynamics, Sunnyvale, CA). Band intensities were quantified on computer (Image Quant analysis software; Molecular Dynamics). The intensities of the transferrin bands were normalized to GAPDH bands within each lane.

**Immunohistochemistry**

Eyes were obtained through the Foundation Fighting Blindness (Owings Mills, MD) eye donor program. Among the eyes with normal maculas, one was from a woman, and three were from men. The mean age was 73 years (range, 65–79) and the mean postmortem interval was 6.5 hours. Among the eyes with AMD, two were from women, one from a man, and the history with one eye did not specify sex. The mean age was 78 years (range, 71–89) and the mean postmortem interval was 7.5 hours. Eyes were processed for cryosectioning followed by fluorescence immunohistochemistry as described previously. The anti-transferrin antibody was the same as described for Western blot analysis and used at a dilution of 1:1000. Sections were processed in parallel, and imaging parameters were identical for all sections.

**RESULTS**

**Measurement of Transferrin mRNA Levels**

Transferrin mRNA levels were evaluated by microarray and QPCR analyses. Each of the 33 unaffected eyes and 12 eyes from patients with AMD was tested on two microarray slides. After correction for age and gender and their interaction, the mean transferrin mRNA level was 3.5- and 2.1-fold higher in non-neovascular AMD retinas and neovascular AMD retinas compared with unaffected retinas, respectively (Fig. 1). This difference was statistically significant (false discovery rate of 11%). The ANOVA that was applied enabled quantification of the age-associated alteration in transferrin mRNA level in comparison to alterations associated with AMD. There were fluctuations in transferrin mRNA levels with age that were indistinguishable from random variation (age associated change was 1.04-fold), but at the same FDR level, there was an increase of 1.4-fold in men compared with women (Fig. 1).

Other genes on the array known to be involved in iron metabolism included ferritin light and heavy chain and ceruloplasmin. Ceruloplasmin mRNA levels were 1.45-fold higher in AMD retinas than in unaffected retinas, whereas ferritin light and heavy chain mRNA levels were similar in AMD retinas and unaffected control subjects.

Transferrin mRNA levels were then measured with QPCR in four samples of retinal RNA from patients with AMD and four from control patients for technical validation of microarray results. Eyes included in this analysis were randomly selected from the pool of AMD and unaffected eyes used for microarray experiments. In this random subset of eyes the relative transferrin mRNA level was 1.7-fold higher in the retinas from the four patients with AMD compared to those from the control patients by both QPCR and microarray analysis (P = 0.04 and P = 0.05 for the QPCR and microarray results, respectively; t-test). For these eight samples there was a good correlation between mRNA level measured by microarray versus QPCR ($R^2 = 0.749$).

**Analysis of Transferrin Protein Expression**

To expand on the gene expression data that suggested that AMD retinas have an approximate twofold increase in transferrin at the RNA level, we next examined transferrin expression at the protein level. For these experiments, a new set of eyes was analyzed, and for each pair of eyes, one eye was used for Western blot analysis and the other was studied by histology, to classify the AMD phenotype. Comparison between the eyes seemed reasonable because, although there certainly can be variation between eyes, pathologic changes between fellow eyes tend to be correlated.

Eyes from eight donors (four men, four women; ages 74–98 years) were investigated. Median interval between death and processing was 6 hours (minimum, 4 hours, maximum, 16 hours). No patients had high myopia (> –4.0 D). Clinical and histopathologic characteristics of study eyes are summarized in Supplementary Table S1 (all supplementary material is available online only, at http://www.iovs.org/cgi/content/full/47/5/2135/DC1). Among the eyes used for Western blot analysis, four were grossly normal (mean age, 83 years) and four had AMD (mean age, 86 years; Supplementary Fig. S1). Among the fellow eyes evaluated for histopathology and ultrastructure, three were unremarkable, one had early AMD, two had late non-neovascular AMD (although CNV could not be definitively excluded without fluorescein angiography or exhaustive sectioning), and two had late neovascular AMD (Fig. 2 and Supplementary Figs. S2, S3).

**FIGURE 1.** Transferrin mRNA level differences across biological factors as detected by microarray analysis. Each bar represents the second step regression coefficient of difference in the transferrin mRNA level for the listed biological parameter, calculated from duplicate hybridizations with each RNA sample ($n = 45$ eyes). Analysis was performed as previously described.

Analysis of a representative normal eye revealed lipoprotein-like particles within Bruch’s membrane, remnants of coated membrane bounded bodies, and a banded material of 50 nm periodicity (Fig. 2A, Supplementary Figs. S1A, S2A; http://www.iovs.org/cgi/content/full/47/5/2135/DC1). An eye with late non-neovascular AMD exhibited thin basal deposits, rounded RPE cells sloughed into the subretinal space, and intra- and extravascular macrophages (Figs. 2B, 2C, and Supplementary Fig. S1C). Eyes with geographic atrophy exhibited drusen with calcific inclusions, a hyperpigmented junctional

---

**TRANSCRIPT**

**Retinal Transferrin in AMD**

May 2006, Vol. 47, No. 5

**Measurement of Transferrin mRNA Levels**

Transferrin mRNA levels were evaluated by microarray and QPCR analyses. Each of the 33 unaffected eyes and 12 eyes from patients with AMD was tested on two microarray slides. After correction for age and gender and their interaction, the mean transferrin mRNA level was 3.5- and 2.1-fold higher in non-neovascular AMD retinas and neovascular AMD retinas compared with unaffected retinas, respectively (Fig. 1). This difference was statistically significant (false discovery rate of 11%). The ANOVA that was applied enabled quantification of the age-associated alteration in transferrin mRNA level in comparison to alterations associated with AMD. There were fluctuations in transferrin mRNA levels with age that were indistinguishable from random variation (age associated change was 1.04-fold), but at the same FDR level, there was an increase of 1.4-fold in men compared with women (Fig. 1).

Other genes on the array known to be involved in iron metabolism included ferritin light and heavy chain and ceruloplasmin. Ceruloplasmin mRNA levels were 1.45-fold higher in AMD retinas than in unaffected retinas, whereas ferritin light and heavy chain mRNA levels were similar in AMD retinas and unaffected control subjects.

Transferrin mRNA levels were then measured with QPCR in four samples of retinal RNA from patients with AMD and four from control patients for technical validation of microarray results. Eyes included in this analysis were randomly selected from the pool of AMD and unaffected eyes used for microarray experiments. In this random subset of eyes the relative transferrin mRNA level was 1.7-fold higher in the retinas from the four patients with AMD compared to those from the control patients by both QPCR and microarray analysis ($P = 0.04$ and $P = 0.05$ for the QPCR and microarray results, respectively; $t$-test). For these eight samples there was a good correlation between mRNA level measured by microarray versus QPCR ($R^2 = 0.749$).

**Analysis of Transferrin Protein Expression**

To expand on the gene expression data that suggested that AMD retinas have an approximate twofold increase in transferrin at the RNA level, we next examined transferrin expression at the protein level. For these experiments, a new set of eyes was analyzed, and for each pair of eyes, one eye was used for Western blot analysis and the other was studied by histology, to classify the AMD phenotype. Comparison between the eyes seemed reasonable because, although there certainly can be variation between eyes, pathologic changes between fellow eyes tend to be correlated.

Eyes from eight donors (four men, four women; ages 74–98 years) were investigated. Median interval between death and processing was 6 hours (minimum, 4 hours, maximum, 16 hours). No patients had high myopia (> –4.0 D). Clinical and histopathologic characteristics of study eyes are summarized in Supplementary Table S1 (all supplementary material is available online only, at http://www.iovs.org/cgi/content/full/47/5/2135/DC1). Among the eyes used for Western blot analysis, four were grossly normal (mean age, 83 years) and four had AMD (mean age, 86 years; Supplementary Fig. S1). Among the fellow eyes evaluated for histopathology and ultrastructure, three were unremarkable, one had early AMD, two had late non-neovascular AMD (although CNV could not be definitively excluded without fluorescein angiography or exhaustive sectioning), and two had late neovascular AMD (Fig. 2 and Supplementary Figs. S2, S3).

**FIGURE 1.** Transferrin mRNA level differences across biological factors as detected by microarray analysis. Each bar represents the second step regression coefficient of difference in the transferrin mRNA level for the listed biological parameter, calculated from duplicate hybridizations with each RNA sample ($n = 45$ eyes). Analysis was performed as previously described. **Left to right:** Age, $x$-fold change in transferrin mRNA level over 10 years of age difference (donor age range, 29–90 years); Gender, male-female transferrin mRNA ratio ($n = 15$ women and 10 men); Age by Gender, $x$-fold transferrin mRNA level change over 10 years in women compared with men; Non-neovascular (NNV) AMD, $x$-fold expression difference between retinas with non-neovascular AMD ($n = 6$) and unaffected retinas ($n = 35$); and Neovascular (NV) AMD, $x$-fold difference in expression between retinas with neovascular AMD ($n = 6$) and unaffected retinas ($n = 35$). All values are in linear scale. Each value is normalized with respect to the other biological factors (e.g., coefficient associated with age is normalized to gender and AMD).
zone surrounding the atrophy, thick basal laminar deposits with type VI collagen,\textsuperscript{17} membranous debris with and without contents consistent with partly extracted solid lipid particles,\textsuperscript{17} and pooled neutral lipid (Fig. 2B and Supplementary Figs. S1B–E, S2B–C, S3C–D). Also observed were banded materials distinct from type VI collagen (perhaps single segment long spacing crystallite), breakdown products of various collagens\textsuperscript{21} (Supplementary Fig. S3A, arrows). Eyes with late neovascular AMD exhibited pre- and subretinal scars, extravasated macrophages, and basal deposits persisting after partial to complete loss of RPE, photoreceptors, and choriocapillaris (Figs. 2D–E and Supplementary Figs. S1D, S1E, S3B).

Transferrin protein in the retinas isolated from the fellow eyes was quantified by Western blot analysis (Fig. 3). A single band the same size as the purified human transferrin control was detected in each of the human retina samples (Fig. 3). The transferrin bands from all the non-neovascular and neovascular AMD retinas were more intense than those from the normal retinas. After normalization of the transferrin band intensity to the housekeeping enzyme GAPDH in each sample, the mean transferrin band intensity for the AMD retinas was 2.1 times greater than the mean transferrin band intensity for the normal retinas ($P < 0.05$).

Immunohistochemical Localization of Transferrin Expression

To determine whether localization of transferrin is altered in retinas with AMD, normal and AMD macular sections were immunostained with an anti-transferrin antibody. In the normal retinas, transferrin label was present, along the internal limiting membrane (ILM; Fig. 4A, arrow), consistent with our previous finding of transferrin localization in Müller cell end feet.\textsuperscript{4} In a retina from a donor with non-neovascular AMD (drusen), transferrin was present in large drusen (Fig. 4C, arrow). In a macula with geographic atrophy (Fig. 4G), in addition to the ILM label, transferrin label was present in the INL, in the outer plexiform layer (OPL) and in the vicinity of the remaining photoreceptors. The RPE cells are absent in this area. In two neovascular AMD maculas (Figs. 4K, 4O), transferrin label was present in many retinal layers. A comparison of the normal maculas (Fig. 4, first column) with the AMD maculas (Fig. 4, third column) showed prominent transferrin label in the AMD in a pattern consistent with Müller cells and generally more widespread and intense.

![FIGURE 2. AMD histopathology in eyes contralateral to those used for transferrin detection. (A, B, C) Detachment of the neurosensory retina from the RPE is an artifact. (A) Eye with unremarkable RPE, Bruch’s membrane, and choroid (Ch) (eye 2003121L; 90-year-old woman). M, macrophage; m, mast cell. (B) Eye with late non-neovascular AMD (2003128L; 87-year-old woman) had thick basal laminar and linear deposits (lam, lin) and druse (d) with calcified inclusions (magenta). (C) Eye with late non-neovascular AMD (2003136L; 74-year-old man) had thin basal laminar and linear deposits, rounded RPE cell sloughed into the sub-RPE space, and intra- and extravascular macrophages (arrowheads). (D) Eye with late non-neovascular AMD (2004016R; 85-year-old woman) had calcified Bruch’s membrane (BrM, bracketing arrowheads), cells in the sub-RPE space (arrow), basal lamina deposit (lam), hypertrophic RPE, photoreceptor (PR) hemirosette, and epiretinal membrane (Epi). (E) Eye with late non-neovascular AMD (2004023L; 98-year-old man) had pre-retinal and subretinal scars (Sc) and persisting basal laminar deposit (lam). Photoreceptors, RPE, and choriocapillaris were completely atrophied. One-micrometer-thick sections; toluidine-O-blue stain. Bar, 20 m.](https://tvst.arvojournals.org/article-pdf/47/5/2138/3111647/2138_chowers_et_al_2006 iovs_47_5_2138.pdf)

![FIGURE 3. Western blot analysis of transferrin in normal and AMD neurosensory retinas. Two micrograms of total protein extract from four normal neurosensory retinas (Normal) and four AMD retinas (AMD; contralateral to eyes in Fig. 2) were analyzed. The AMD subtype is as indicated: drusen only (drusen), exudative AMD (Ex), geographic atrophy (GA). Purified human transferrin (labeled TF Control) was used as a size marker. Top: portion of the membrane, labeled with anti-transferrin antibody; bottom: portion of the membrane labeled with anti-GAPDH.](https://tvst.arvojournals.org/article-pdf/47/5/2138/3111647/2138_chowers_et_al_2006 iovs_47_5_2138.pdf)
DISCUSSION

We have compared transferrin levels among normal retinas and retinas from patients with AMD. We observed transferrin mRNA and protein levels that were consistently elevated in both neovascular AMD and non-neovascular AMD compared with unaffected retinas. These findings were reproducible across three independent sample sets. Immunohistochemistry demonstrated that increased staining for transferrin is found throughout the inner and outer retinal layers in retinas from patients with AMD.

Transferrin expression has been documented in normal human, monkey, rodent, and avian retinas. In fact, transferrin mRNA is more abundant in the retina than in tissues such as cortex and liver, and higher transferrin levels have been reported in the periphery of the retina than in the macular area.\(^4,10,22\) Our data are consistent with those in previous reports in which both transferrin mRNA and protein were localized to the inner and outer retinal layers.\(^5,9,23,24\) This expression pattern suggests the possibility that Müller cells may be a site of transferrin synthesis. Furthermore, the increased levels of transferrin expression observed in the AMD retinas may be at least partially a result of Müller cell activation. Such activation, manifested by increase expression of GFAP, has been reported by two groups.\(^25-27\)

Transferrin and iron have been associated with several diseases affecting the central nervous system and the retina, among them Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, glaucoma, and aceruloplasminemia.\(^3,4,7\) Iron has also been implicated in AMD. Increased iron levels were reported in the RPE and sub-RPE deposits of retinas of patients with AMD compared with control retinas.\(^2\) Furthermore, ceruloplasmin- and hephestin-deficient mice, which accumulate iron in their retinas, manifest RPE and photoreceptor degeneration, RPE hypertrophy, and subretinal neovascularization, resulting in a phenotype that resembles some salient features of AMD.\(^8\) Conceivably, iron, a known generator of free radicals, causes retinal damage in this mouse model and in human AMD through the generation of oxidative damage. The retina is highly susceptible to oxidative damage, which has been implicated in the pathogenesis of AMD.\(^1\) Therefore, free-radical generation from elevated retinal levels of iron may play a role in the pathogenesis of AMD.

The importance of increased transferrin levels in the context of iron-mediated damage in AMD is unclear. Based on the known function of transferrin in iron binding and transport and on its potential neurotrophic function,\(^12,13\) it is possible that retinal transferrin expression increases in AMD secondary to the degenerative process. According to this hypothesis, the transferrin level may increase as part of a mechanism to protect the retina from iron-associated oxidative damage, similar to the increased levels of the ferroxidase ceruloplasmin observed immediately after photo-oxidative stress in a mouse model.\(^29\)
Elucidating the role of iron in general and of transferrin in particular in the pathogenesis of AMD may have practical implications. Iron metabolism can serve as a target for novel therapeutic interventions in non-neovascular AMD. Such therapies would be designed to reduce iron-associated damage, thereby slowing the degenerative process, and, hopefully, delaying the development of choroidal neovascularization with its devastating visual consequences. Although such treatment is not available yet, similar therapeutic approaches are currently being developed for Parkinson’s disease and Alzheimer’s disease.29–31

Acknowledgments

The authors thank the Alabama Eye Bank for timely retrieval of donor eyes, donor families for their generosity, eye care providers for ophthalmic histories, and Hardik Kapadia for technical assistance.

References