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Aqueous Fluid Transcriptome Profiling Differentiates Between Non-Neovascular and Neovascular AMD

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Purpose. Early and intermediate non-neovascular AMD (NN-AMD) has the potential to progress to either advanced NN-AMD with geographic atrophy, or to neovascular AMD (N-AMD) with CNV. This exploratory study performed an unbiased analysis of aqueous humor transcriptome in patients with early or intermediate NN-AMD vs. treatment-naïve N-AMD to determine the feasibility of using this method in future studies investigating pathways and triggers for conversion from one form to another.

Methods. Aqueous humor samples were obtained from 20 patients with early or intermediate NN-AMD and 20 patients with untreated N-AMD, graded on clinical examination and optical coherence tomography. Transcriptome profiles were generated using next-generation sequencing methods optimized for ocular samples. Top-ranked transcripts were compared between groups, and pathway enrichment analysis was performed.

Results. Seventy-eight differentially expressed transcripts were identified. Unsupervised clustering of differentially expressed transcripts was able to successfully differentiate between the two groups based on aqueous transcriptome alone. Pathway analysis highlighted changes in expression of genes associated with mitochondrial respiration, oxidative stress, ubiquitination, and neurogenesis between the two groups.

Conclusions. This pilot study compared the aqueous fluid transcriptome of patients with early or intermediate NN-AMD and untreated N-AMD. Differences in transcripts and transcriptome pathways identified in the aqueous of patients with early or intermediate NN-AMD compared with patients with N-AMD are consistent with those previously implicated in the pathogenesis of these distinct AMD subtypes. The findings from this exploratory study warrant further investigation using a larger, prospective study design, with the inclusion of a control group of eyes without AMD.

Keywords: age-related macular degeneration, senescence, aqueous transcriptome

AMD is a major cause of blindness in those aged more than 55 years worldwide.[1] Recent advances in the understanding of neovascular AMD (N-AMD) pathophysiology have allowed for the development of effective treatments. Since the advent of anti-VEGF therapy, it is estimated that the number of patients legally blind from N-AMD has decreased by 75%.[2]

The early and intermediate stages of AMD are characterized by the deposition of extracellular debris and lipids resulting in drusen, as well as migration of RPE cells, which can be seen as pigmentary changes on fundus examination.[3] Over years, the early stages of AMD can progress to advanced AMD, resulting in visual impairment. In advanced non-neovascular AMD (NN-AMD), or geographic atrophy (GA), there is progressive loss of photoreceptors, RPE cells, and choriocapillaris.[3] N-AMD develops owing to CNV, resulting in leakage of fluid into the retina or subretinal space.[3] Therapy aimed at decreasing the risk of progression from early or intermediate NN-AMD to N-AMD is currently lacking.

The etiology of AMD is multifactorial, with a complex interplay between aging, genetic susceptibility, and environmental risk factors. With aging, there is cumulative oxidative damage to the retina owing to the increased production and impaired elimination of reactive oxygen species.[4,5] The consequences of insufficient waste removal are exacerbated in postmitotic and quiescent cells, such as neurons and RPE cells.[6] Over time, the accumulation of oxidative stress and alterations in antioxidant response contribute to the impairment of RPE cell function and their ability to support and maintain photoreceptor function. Environmental factors associated with AMD, including smoking and cardiovascular risk factors, contribute to oxidative stress and impaired blood supply.[3] The heritability of N-AMD is estimated to be up to 50%.[7] Thirty-four AMD loci have been reported so far involving complement, lipid, metabolism, and extracellular

matrix pathways. Two major loci (CFH and ARMS2/HTRA1 variants) are specifically associated with progression to late AMD. There is also a link between high-risk genes and elevated oxidative stress and mitochondrial damage in the RPE.[5]

Transcriptome profiling has been used for the unbiased identification of gene coexpression and functional networks in the RPE–choroid and retina tissue of human donor eyes with varying AMD phenotypes and severity.[8] Prior studies have also investigated differentially expressed proteins in the aqueous of NN-AMD eyes compared with normal controls,[9,10] and N-AMD eyes compared with normal controls.[11,12] Transcriptome profiles of aqueous humor from treatment-naïve N-AMD eyes, and early or intermediate NN-AMD eyes have not previously been reported in the literature; identifying these is the aim of the current study. We hypothesize that the distinct pathological features of early or intermediate NN-AMD and N-AMD are reflected in the secreted aqueous humor transcriptome. Insight into the pathways associated with these disease states has the potential to inform future research directed at mitigating disease activity and progression from one form to another.

Methods

Patient Selection

This prospective study was approved by the University of California, San Francisco, Institutional Review Board and adhered to the tenets of the Declaration of Helsinki for research involving human subjects. Informed consent was obtained from all patients.

Adult patients who were diagnosed with AMD (on clinical examination and confirmed on OCT) at the University of California, San Francisco, between January 2016 and December 2021 were identified. Patients were excluded if they had been treated with intraocular steroids or anti-VEGF medication within the 12 months before sample collection, had a history of vitrectomy, or had a history of any other significant ocular disease. Patients were enrolled in the study if they were willing to participate and met the following criteria: (1) early or intermediate NN-AMD defined as those patients with drusen and no history of GA in the included eye, no history of N-AMD in either eye, and scheduled for routine cataract surgery; and (2) N-AMD defined as those patients with evidence of choroidal neovascular membrane with associated intraretinal or subretinal fluid, and/or subretinal heme, with any grade of AMD in the other eye, and scheduled to initiate intravitreal anti-VEGF injections. Where both eyes met the inclusion criteria, only the first operated eye in the NN-AMD group was included.

All enrolled patients underwent a standardized ophthalmic examination, including Snellen visual acuity, slit-lamp biomicroscopy, and dilated fundus examination. Ophthalmic examination findings were used to screen for eligibility based on the inclusion criteria for each group listed above. Macula imaging by spectral-domain optical coherence tomography (Heidelberg Engineering, Heidelberg, Germany) was used to confirm the presence of drusen without GA, intraretinal fluid, or subretinal fluid in NN-AMD eyes, or the presence of intraretinal fluid, subretinal fluid, or subretinal hemorrhage in N-AMD eyes. Clinical data including demographics and prior medical and ocular history were extracted from the electronic patient record.

Sample Collection

Aqueous humor was collected via anterior chamber paracentesis for all patients. Paracentesis was performed using a 30G needle before injection of any intraocular agents. Approximately 100 µL of aqueous humor was collected. Paracentesis was performed at the time of cataract surgery for patients in the early or intermediate NN-AMD group, and immediately before intravitreal anti-VEGF injection in the N-AMD group. Aqueous samples were then labeled with a predetermined identification number. The samples were transported on dry ice and stored at −80°C until sample processing.

RNA Isolation and Sequencing

Laboratory personnel were masked to the identity of the samples. Samples were prepared for metagenomic deep sequencing, as previously described.[13] Briefly, total RNA was extracted from 100 µL aqueous fluid samples using the Zymo quick-RNA microprep kit (Zymo Research, Irvine, CA) per the manufacturer's instructions. Sequencing libraries were prepared using the NEBNext RNA Ultra II kit (New England Biolabs, Ipswich, MA) and then amplified with 21 cycles. Libraries were sequenced on the NovaSeq 6000 using two technical replicates.

Bioinformatics

A blinded data analysis was performed. Reads were quality filtered with PriceSeqfilter (v1.2, using parameters –rqf 85 0.98 –rnf 90). Reads were aligned to the GRCh38 human genome assembly using hisat2 (version 2.1.0).[14] Transcript abundance was determined using the default parameters in stringtie2 (version 1.3.4d) and the ENSEMBL GRCh38.87 for transcriptome annotation. Read counts for genes were obtained with stringtie's "prepDE.py" script. Genes were then filtered to include only protein-coding genes that were expressed in at least 20% of the patients. Gene count data were analyzed with DESeq2 (version 1.28.1) to test for differences between the early or intermediate NN-AMD and N-AMD groups.[15] Genes with a false discovery rate of less than 0.05 (determined using Topconfects version 1.4.0) and with at least a two-fold change were considered notable.[16] Topconfects is a method of ranking by confidence bounds on the log fold change, providing false discovery rate and false coverage statement rate control.[17] This process is known to generate a rank list that emphasizes different biological processes compared to the top-ranked genes by P value.[17] G:profiler web-based functional gene profiling pipeline was used to detect enriched pathways. Bonferroni correction was used to correct for multiple comparisons.

Results

Clinical Characteristics

Forty eyes from 40 patients with AMD were included in this study. Twenty eyes had early or intermediate NN-AMD and 20 eyes had N-AMD. The clinical characteristics of patients in each group are shown in Table 1. There was no significant difference in age, sex, or inclusion of the right eye vs. the left eye between groups. Both groups had significantly more females than males (65% females in each group). Nine of 20 patients in each group were on Age-Related Eye Disease Study vitamins. Only one patient in the N-AMD

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Table 1. Baseline Characteristics

	NN-AMD (n = 20)	N-AMD (n = 20)	P Value
Age (years)	79.6 ± 10.3	79.9 ± 8.5	0.916
Sex, male/female	7/13	7/13	0.999
Eye, OD/OS	9/11	10/10	0.751
Presence of GA	1 (5)	0 (0)	
Prior intravitreal anti-VEGF	0	1 (5)	
Taking AREDS vitamins	9 (45)	9 (45)	

AREDS, Age-Related Eye Disease Study.
Values are mean ± SD, number, or number (%).

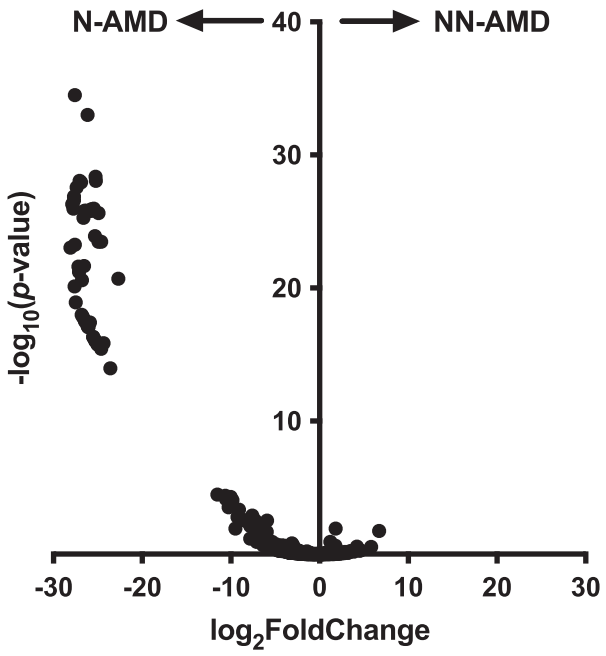

Figure 1. Volcano plot of differentially expressed genes in N-AMD and NN-AMD groups.

group had previously had a single intravitreal anti-VEGF injection (which was performed >12 months before sample collection). The remainder of the patients were treatment naïve.

Transcriptome Profile

After data filtering, 766 genes were ranked by Topconfects. Seventy-eight genes were found to be differentially expressed in eyes with early or intermediate NN-AMD compared with eyes with N-AMD (Supplementary Table S1). There was reduced expression of seventy-six of these genes and increased expression of two genes in N-AMD eyes (Fig. 1).

The top 10 ranked transcripts by Topconfects (Table 2) were associated with distinct biological pathways, namely, mitochondrial respiration (NDUFA10, NDUFS1, and adenylate kinase 4), apoptosis and ubiquitination (MEG3, GUCY1A2, and LMO7), calcium signaling (CACNG8), oxidative stress (SOD2), and epigenetic transcriptional regulation (SMARCE1). Unsupervised clustering was able to successfully differentiate between the two groups by aqueous transcriptome alone (Fig. 2).

Pathway Enrichment Analysis

We performed pathway enrichment analyses using the g:Profiler platform. This platform uses data from multiple sources of functional evidence, including Gene Ontology terms, biological pathways, regulatory motifs of transcription factors and microRNAs, human disease annotations, and protein–protein interactions.

Analyses using Gene Ontology terms, the REAC database, and transcription factors revealed significant pathways relevant to the etiology of retinal degeneration and NN-AMD, including neurogenesis, lipid metabolism, cellular stress, ubiquitination, generation of reactive oxygen species, and mitochondrial respiration (Table 3). Phenotype ontology analysis found a significant association between the early or intermediate NN-AMD transcriptome profile and ocular diseases and phenotypes, including Leber optic atrophy ($P_{adj} = 1.79 \times 10^{-4}$), centrocecal scotoma ($P_{adj} = 5.23 \times 10^{-4}$), and retinal artery tortuosity ($P_{adj} = 3.06 \times 10^{-3}$).

Discussion

AMD results in a damaged and dysfunctional photoreceptor, RPE, Bruch's membrane, and choriocapillaris complex. Early or intermediate NN-AMD has the potential to progress to either advanced NN-AMD with GA or to N-AMD with CNV, and each form is known for its distinct pathological features and treatment. This study performed an unbiased analysis

Table 2. Top 10 Ranked Transcripts by Topconfects

Ensemble ID|Gene	Gene Name	log2 Fold Change	SE	P Value	Adjusted P Value
ENSG00000130414|NDUFA10	NADH:ubiquinone oxidoreductase subunit A10	26.59	2.14	1.52E-35	1.17E-32
ENSG00000214548|MEG3	Maternally expressed 3	27.15	2.50	1.75E-27	1.67E-25
ENSG00000142408|CACNG8	Calcium voltage-gated channel auxiliary subunit gamma 8	26.94	2.45	4.02E-28	5.13E-26
ENSG00000162433|AK4	Adenylate kinase 4	26.95	2.47	8.16E-28	8.93E-26
ENSG00000023228|NDUFS1	NADH:ubiquinone oxidoreductase subunit S1	27.03	2.51	4.10E-27	3.49E-25
ENSG00000152402|GUCY1A2	Guanylate cyclase 1 soluble subunit alpha 2	26.54	2.39	1.27E-28	1.95E-26
ENSG00000187775|DNAH17	Dynein axonemal heavy chain 17	26.33	2.34	2.03E-29	3.90E-27
ENSG00000136153|LMO7	LIM domain 7	25.37	2.08	2.61E-34	9.98E-32
ENSG00000112096|SOD2	Superoxide dismutase 2	27.38	2.71	4.74E-24	1.45E-22
ENSG00000073584|SMARCE1	SWI/SNF related, matrix-associated, actin-dependent regulator of chromatin E1	26.99	2.64	1.46E-24	5.07E-23

AK4, adenylate kinase 4.

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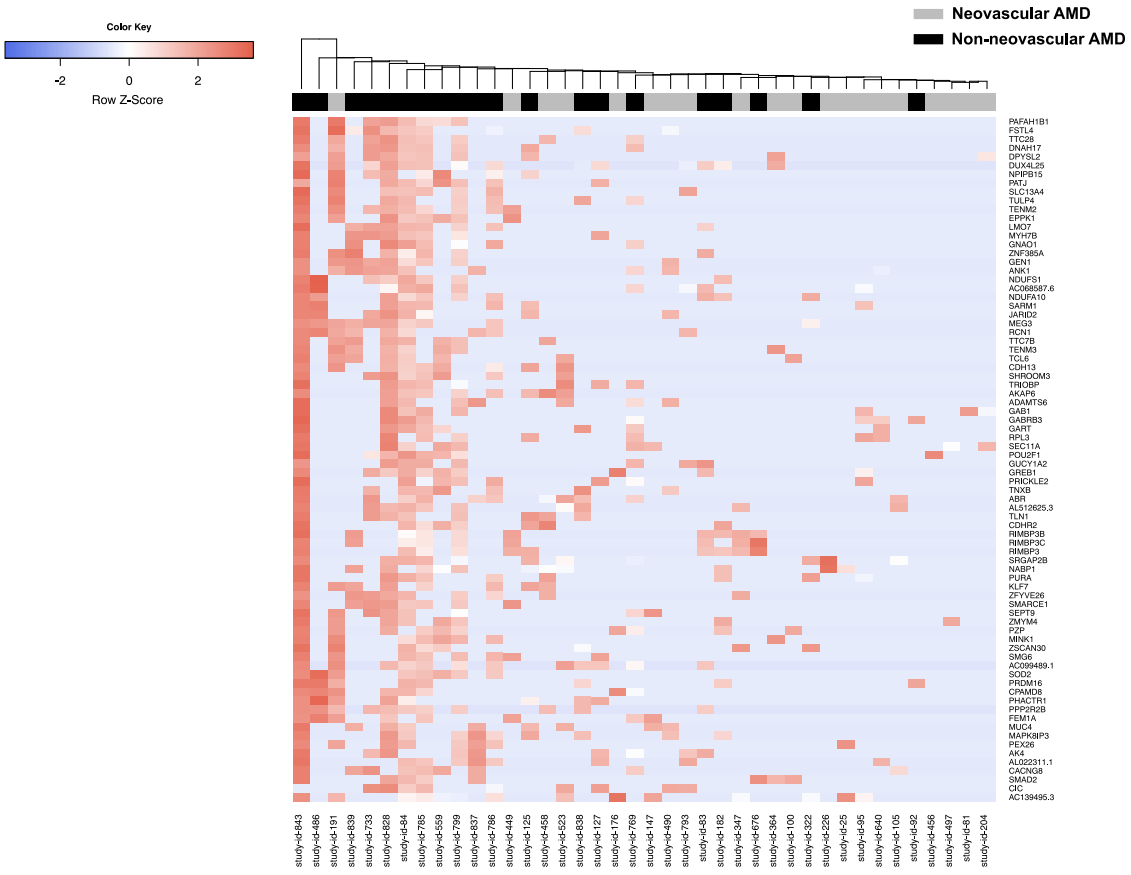

Figure 2. Unsupervised clustering of 78 differentially expressed transcripts.

of the aqueous humor transcriptome in early or intermediate NN-AMD vs. treatment-naïve N-AMD. Using an unbiased high-throughput sequencing pipeline optimized for aqueous samples, unsupervised clustering was able to successfully differentiate between the two groups based on aqueous transcriptome alone. Seventy-eight differentially expressed transcripts were identified. Pathway analysis highlighted relative underexpression of genes associated with mitochondrial respiration, oxidative stress, ubiquitination, neurogenesis, and neural remodeling in the N-AMD group compared with the early or intermediate NN-AMD group. Alterations in these pathways have been implicated previously in the pathogenesis of AMD.[4,18,19] Interestingly, upregulation of the same cluster of pathways are characteristic of cellular senescence and the senescence-associated secretory phenotype (SASP), which is thought to be a signature of NN-AMD and may be a key factor in the progression from NN-AMD to N-AMD.[20]

AMD is well-known to cause changes to the composition, structure, and function of the mitochondrial matrix.[4,21] Specifically, mitochondrial energy production, lipid metabolism, mitochondrial calcium homeostasis, and activation of apoptosis pathways are affected, and this topic has been reviewed extensively elsewhere.[4,5] Although many of these changes also occur in the normal aging retina, they are accelerated in eyes with NN-AMD.[3,5,21] Pathway analysis in the current study highlighted both mitochondrial respiration and cellular stress as prominent pathways in the early or intermediate NN-AMD group compared with the N-AMD group. NDUFA10 and NDUFS1 transcripts code for subunits of complex I of the mitochondrial electron transport chain and were 2 of the top 10 differentially expressed transcripts we identified. Complex I forms an integral part of mitochondrial respiration to generate adenosine triphosphate, and, like the *NDUFS1* gene, is upregulated in conditions of cellular stress.[22,23] Adenylate kinase 4, another top-ranked transcript in our study, increases under hypoxic conditions and is involved in regulating mitochondrial adenosine triphosphate production.[24] Under pathological conditions, complexes I and III also play an important role in reactive oxygen species production, which further implicates these top-ranked transcripts in the pathogenesis of AMD.[4]

Upregulation of endogenous antioxidants including manganese superoxide dismutase occurs in response to excessive oxidative stress in RPE cells.[25–28] SOD2, a member of the manganese superoxide dismutase family, neutralizes toxic superoxide to clear reactive oxygen species. This response has been shown to increase in the macula of early AMD eyes compared with both control eyes and advanced N-AMD eyes.[28] Further, in early AMD eyes, the response is greatest in morphologically normal RPE cells compared with dysmorphic RPE cells, suggesting that healthy RPE cells are required to produce a compensatory antioxidant effect.[28] We found a significant downregulation of SOD2 as well as antioxidant pathways in the aqueous of N-AMD eyes compared with early or intermediate NN-AMD eyes, which supports the findings reported in these prior studies.

Oxidative stress in RPE cells promotes cellular protein, nucleic acid, and organelle (including mitochondrial) damage. There are two major pathways available to

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Table 3. Significantly Enriched Biological Pathways in Eyes With Early and Intermediate NN-AMD

Pathway	P_{adj}	Source
Retinal remodeling and neurogenesis		
Neuron projection	2.53×10^{-15}	GO:CC
Axon	3.95×10^{-11}	GO:CC
Neuron development	1.16×10^{-9}	GO:BP
Somatodendritic compartment	1.28×10^{-9}	GO:CC
Neurogenesis	3.00×10^{-9}	GO:BP
Neuron projection development	3.08×10^{-9}	GO:BP
Dendrite	3.19×10^{-9}	GO:CC
Dendrite tree	3.59×10^{-9}	GO:CC
Neuron differentiation	1.56×10^{-8}	GO:BP
Generation of neurons	8.64×10^{-8}	GO:BP
Postsynapse	3.40×10^{-7}	GO:CC
Synapse	2.40×10^{-6}	GO:CC
Dendritic spine	2.47×10^{-6}	GO:CC
Neuron spine	3.41×10^{-6}	GO:CC
Central nervous system development	1.15×10^{-4}	GO:BP
Main axon	2.82×10^{-4}	GO:CC
Lipid metabolism		
CREB3	1.47×10^{-8}	TF
Cellular stress		
CREB3	1.47×10^{-8}	TF
XBP1	3.43×10^{-6}	TF
Ubiquitination		
Thiol-dependent deubiquitinase	1.20×10^{-4}	GO:MF
Deubiquitinase activity	1.52×10^{-4}	GO:MF
Omega peptidase activity	5.78×10^{-4}	GO:MF
Protein deubiquitination	7.361×10^{-3}	GO:BP
XBP1	3.43×10^{-6}	TF
Antioxidants/ROS generation		
NADH dehydrogenase (ubiquinone) activity	3.14×10^{-3}	GO:MF
NADH dehydrogenase (quinone) activity	3.84×10^{-3}	GO:MF
Inactivation of CDC42 and RAC1	5.00×10^{-3}	REAC
NAD(P)H dehydrogenase (quinone) activity	5.65×10^{-3}	GO:MF
Mitochondrial respiration		
Respiratory electron transport chain	4.19×10^{-3}	GO:BP
Aerobic electron transport chain	4.39×10^{-3}	GO:BP
Cellular respiration	4.91×10^{-3}	GO:BP
Oxidative phosphorylation	7.86×10^{-3}	GO:BP
Regulation of GTPase activity	9.11×10^{-3}	GO:BP

GO, Gene Ontology.

remove damaged proteins and organelles, namely, the ubiquitin–proteasome and the lysosomal–autophagy pathways.[5] Damaged mitochondria activate mitophagy via depolarization of the inner membrane, which allows for ubiquitination and proteasomal degradation, whereas intracellular molecules are often eliminated by lysosomal degradation.[5,19,29,30] The removal of waste products via these pathways becomes increasingly impaired in the RPE during the progression of AMD. Eventual failure of these pathways presents as increased levels of ubiquitin in the RPE of advanced AMD donor eyes,[5] and by examining an RPE-specific autophagy knockout model that shows characteristic cellular degeneration similar to that seen in AMD.[29] Pathway enrichment analysis of the aqueous transcriptome in the current study found a relative reduction in ubiquitination pathways in the N-AMD group, which may reflect pathway failure in end-stage disease. In addition, 2 of the top 10 ranked transcripts (MEG3 and LMO7) are thought to promote apoptosis and ubiquitination in the retina. MEG3 is a long noncoding RNA that has been identified previously as an upregulated transcript in light-induced models of retinal degeneration and plays a role in inducing mitochondrial-mediated apoptosis pathways.[31] LMO7 is known to be important in retinal development[32] and is upregulated after vascular injury, where it acts to mediate ubiquitination and proteasomal degradation and modulate postinjury fibrotic responses.[33] Enhancement of the ubiquitin-proteasomal system improves photoreceptor survival and delays vision loss in mouse models of blindness caused by a misfolded protein, suggesting that this pathway may be a promising treatment target for NN-AMD.[34]

There is increasing evidence to suggest that the combination of impaired mitochondrial function, phagocytic ability, and antioxidant response induces a senescent state in RPE cells and contributes to the progression of AMD from intermediate to end stage phenotypes.[26,35] Oxidative stress is a key factor that can trigger the activation of the p53/p21 signaling pathway, causing RPE cell-cycle arrest and thereby induce a senescent state.[20,36] Senescent cells secrete a milieu of proinflammatory molecules and growth factors known as the SASP, which can alter the phenotype of the SASP cell, as well as neighboring cells in a paracrine fashion.[20,35,37,38] Interestingly, the characteristic features of senescent RPE cells are reflected in the aqueous transcriptome of the early or intermediate NN-AMD group in our study. Specifically, the upregulation of SOD2 and SMARCE1 in RPE cells are key markers of senescent cells.[18,26,36] We found a significant relative reduction in expression of SOD2 and SMARCE1 in the N-AMD group compared with the early or intermediate NN-AMD group, suggesting that the peak activity of senescent cells may be occurring before the development of N-AMD. SMARCE1 makes up a subunit of the SWI/SNF chromatin remodeling complex, and chromatin accessibility is specifically altered in the RPE from an early stage of NN-AMD.[39] Through chromatin remodeling SMARCE1 also plays a role in epigenetic transcriptional regulation, which may contribute to the transcriptionally inactive state that we identified in N-AMD eyes vs. early or intermediate NN-AMD eyes in the current study.

Retinal remodeling is a process common to many forms of photoreceptor degeneration.[40] It occurs in the context of progressive alterations in cellular metabolism, stress responses, apoptosis, and protein aggregation and continues as cellular senescence ensues.[40,41] The features of retinal remodeling include photoreceptor degeneration, glial responses, neural reprogramming, and widespread rewiring, and there is an increase in the activity of these pathways before the eventual cell death seen in end-stage disease.[40] We found a relative decrease in pathways associated with retinal remodeling in the N-AMD group compared with the early or intermediate NN-AMD group in our study, including those involved in neuron projections and synapses. Neuronal differentiation pathways were also decreased in the same group, which may be related to a decrease in photoreceptor-induced Muller cell gliosis in N-AMD eyes, a remodeling phenomenon known to occur in NN-AMD.[42] Muller glia can proliferate under pathological conditions in vivo, and this target may be promising for future treatments aimed at promoting retinal regeneration in NN-AMD.[43]

The sequence of events that promotes the conversion from NN-AMD to N-AMD is not well-elucidated. There is good evidence that microvascular changes affecting the choriocapillaris occur in aging and are exacerbated in eyes with NN-AMD, suggesting that relative ischemia to the macula plays a role.[44] There is supporting evidence that the greatest VEGF levels occur during the intermediate stage of NN-AMD (compared with early NN-AMD or during

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active CNV).[45] Hypoxic regions of retina show low rates of apoptosis owing to widespread senescent cells,[37] which secrete high levels of VEGF and simultaneously downregulate complement factor H expression.[46] In addition, transcriptome signatures show that metabolic changes (citric acid cycle, respiratory electron transport, and NADH dehydrogenase [quinone] activity) are associated specifically with the intermediate stage of senescence.[47] These same pathways were highly upregulated in the early or intermediate NN-AMD group compared with the N-AMD group in our study, suggesting that this group of patients may have high levels of senescent cells in the intermediate stage. Restoration of blood flow to an ischemic retina as a result of CNV has been hypothesized to change the secretory phenotype acutely and may result in the downregulation of SASP.[48] This theory could explain the decreased expression of secreted gene transcripts related to cellular senescence in our N-AMD group.

Our study was performed using a well-characterized group of patients with AMD to explore the feasibility of using aqueous transcriptome signatures in distinct AMD phenotypes. Prior studies have indicated that the transcriptome profile in the aqueous and vitreous are well-correlated in retinal diseases.[49,50] In the context of the current literature, it is evident that transcriptome profiles of human ocular tissue highlight different relevant pathways in retina vs. the RPE and choroid.[8,51] These differences may be exacerbated when comparing tissue transcript profiles with those found in aqueous or vitreous humor, making it difficult to compare our results with prior studies directly. It is also possible that the aqueous transcriptome predominantly reflects secreted transcripts which represent a subset of the tissue-specific cellular activity.

The metagenomic deep sequencing pipeline used in the current study is a key strength of our methodology. This pipeline has been optimized previously for ocular samples with low levels of RNA and includes two technical replicates for sequencing of each participant, decreasing the risk of false-positive findings. Despite this finding, polymerase chain reaction validation of significant transcripts is necessary to further validate the significant transcripts that we identified and was not performed in this study. Another limitation of this study includes the small sample size. This exploratory study is the first step in demonstrating the feasibility of detecting relevant transcripts in the aqueous of NN-AMD and N-AMD groups. Further characterization of the aqueous transcriptome in AMD eyes using a longitudinal design, and with the addition of a control group, would be necessary to determine the pathways implicated in disease progression. This methodology also has the potential to assess changes after the institution of novel treatments in future studies.

Conclusions

This pilot study was able to detect distinct differences in transcriptome signatures in the aqueous of patients with early or intermediate NN-AMD compared with patients with N-AMD. It is plausible that the restoration of blood supply with the development of CNV has an acute effect on the secretory profile and transcriptional activity of damaged and healthy RPE cells. The functional networks identified in the NN-AMD group are also characteristic of cellular senescence and the SASP, which may be key in the progression from NN-AMD to N-AMD. Exploration of this alteration in transcriptome using a larger, prospective study design, with the inclusion of a control group of non-AMD eyes, may help to further explore the drivers for CNV development.

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