Abnormal activation of signaling pathways related to angiogenesis, inflammation and oxidative stress has been implicated in the pathophysiology of retinopathy of prematurity (ROP), a leading cause of blindness in preterm infants.

Therapies for ROP include laser ablation of avascular retina and anti-vascular endothelial growth factor agents. However, “these therapies have side effects, and even with adequate treatment, visual acuity can be impaired. Targeted therapies with a lower side effect profile are needed,” says Gavin W. Roddy, M.D., Ph.D., with Ophthalmology at Mayo Clinic in Rochester, Minnesota.

Stanniocalcin-1 (STC-1) is a multifunctional protein that is upregulated by cellular stresses. STC-1 is cytoprotective in neurons, photoreceptors and retinal ganglion cells; it also reduces intraocular pressure, oxidative stress and inflammation.

Dr. Roddy and fellow researchers Lauren A. Dalvin, M.D., and Michael P. Fautsch, Ph.D., also with Ophthalmology at Mayo Clinic’s campus in Rochester, Minnesota, hypothesized that STC-1 might be a stress response protein capable of decreasing inflammatory and oxidative stress underlying ROP.

The research team evaluated STC-1 gene and protein expression in the Sprague-Dawley rat oxygen-induced retinopathy (OIR) model that is most similar to human ROP. After inducing OIR in wild-type and Stc-1(-/-) mice, the researchers isolated retinas and evaluated them for avascular and neovascular areas on retinal flat mounts.

Researchers performed a quantitative real-time polymerase chain reaction (PCR) to quantify gene expression and assayed the vascular endothelial growth factor (VEGF) by enzyme-linked immunosorbent assay (ELISA) in media obtained from induced pluripotent stem cell-derived retinal pigment epithelial (iPS-RPE) human cells after treatment with recombinant STC-1. Key findings from the study include:

• To determine whether STC-1 was upregulated in OIR, researchers quantified STC-1 in retina of rats subjected to OIR compared with room air controls. Compared with controls, OIR rat pups showed significantly increased expression of STC-1 mRNA at P17 (P < 0.01) and P20 (P = 0.02). This finding corresponded with the significant increase in STC-1 protein levels at P18 by Western blot, which was confirmed by densitometry.

• To determine whether STC-1 upregulation was pathological or protective, the researchers subjected Stc-1(-/-) and wild-type mice to OIR. Stc-1(-/-) mice subjected to OIR had increased avascular (15.2 ± 2.5% versus 20.2 ± 2.4%, P = 0.02) and neovascular (8.8 ± 3.7% versus 14.3 ± 2.7%, P < 0.05) areas at P17 compared with wild-type mice.

• To examine differential gene expression between wild-type and Stc-1(-/-) OIR mice, the research team selected known upregulated genes in the mouse OIR model at P17 as markers of disease induction. “Of particular note, VEGF-A was upregulated in Stc-1(-/-) versus wild-type controls (P = 0.03),” says Dr. Fautsch.

• To determine whether STC-1 had a direct effect on VEGF production, researchers...
selected iPS-RPE cells that produce VEGF in standard culture conditions. Treatment with STC-1 significantly reduced VEGF concentration in iPS-RPE conditioned media at 24 hours (P = 0.01).

The role of STC-1 in the OIR stress response
“We have shown that STC-1 is induced by OIR at the gene and protein level, adding OIR to the list of cellular stresses that induce STC-1 expression,” says Dr. Roddy. “Our data suggest STC-1 is a regulator of OIR severity in part due to its effect on VEGF production. Reduction in both avascular and neovascular retina in the presence of STC-1 could be related to a combined effect of neuroprotective and anti-VEGF properties.

“This study, which was published in Current Eye Research in 2020, adds ROP to the list of disease models with worse outcomes in the absence of STC-1,” says Dr. Dalvin. “Further studies are needed, however, to evaluate the therapeutic potential of STC-1 in ROP and other retinal vascular disorders.”

For more information

Gene Expression, Missplicing in Corneal Endothelium Differs in Patients With TCF4 TNR Expansion
Cytosine, thymine and guanine (CTG) trinucleotide repeat (TNR) expansion in an intron of the TCF4 gene is the most common genetic variant associated with Fuchs’ endothelial corneal dystrophy (FECD). Several pathogenic mechanisms directly attributable to TNR expansion and common to other TNR diseases have been identified, yet a specific set of mechanisms responsible for pathological progression of FECD is unknown.

To understand events leading from TCF4 TNR expansion to disease phenotype, a team comprising Eric D. Wieben, Ph.D., with Biochemistry and Molecular Biology, Keith H. Baratz, M.D., with Ophthalmology, and Michael P. Fautsch, Ph.D., with the Ophthalmology Research Unit at Mayo Clinic in Rochester, Minnesota, characterized splicing, gene expression and exon sequence changes in a rare cohort of patients with TNR expansions but no phenotypic FECD (RE+/FECD-). Their study was published in Investigative Ophthalmology & Visual Science in 2019.

“One of the challenges of studying TNR expansion diseases is the difficulty in directly associating the varied mechanisms of action from progression to disease, says Dr. Wieben.”At Mayo Clinic, we’ve developed a large FECD and non-FECD tissue bank where we have characterized samples for TNR expansion within the corneal endothelium. In our database, we identified five patients ranging in age from 68-88 years whose TCF4 genes contain pathological expansions of CTG repeats, but the patients did not have FECD. This enabled us to characterize gene expression in this unique group of samples.”

Comparison of RE+/FECD- data to data from patients with FECD and a TNR expansion (RE+/FECD+) revealed significant differences in splicing and gene expression profiles between the two groups. “We found that three genes — MBNL1, KIF13A and AKAP13 — that were previously identified as misspliced in RE+/FECD+ patients were normally spliced in RE+/FECD- samples,” says Dr. Fautsch. “Additionally, gene expression analysis of RE+/FECD- patients identified several important signaling pathways that have been implicated in FECD.”

Qualitative splicing differences
Previous studies on corneal endothelial tissue obtained from RE+/FECD+ patients have identified a set of 24 genes whose missplicing patterns are a signature of the disease. To determine if these missplicing events were also found in RE+/FECD- samples, researchers performed RNA sequencing and compared the transcriptome of RE+/FECD- samples to previously reported data sets of both RE+/FECD+ and non-FECD patients who did not have a TNR expansion (RE-/FECD-). Analysis of the missplicing events in samples from RE+/FECD- patients showed traits of both RE+/FECD+ and RE-/FECD-.

Analysis of missplicing in RE+/FECD- samples identified four genes whose percent spliced-in (PSI) values were within one standard deviation from the mean of RE+/FECD+ values. For the remaining 17 genes, the PSI values for at least one RE+/FECD- sample fell between those recorded for RE+/FECD+ and the RE-/FECD- samples.

Researchers also examined the impact of the TNR expansion on splicing in the TCF4 gene. “We had previously reported that the intron just upstream of the TNR expansion in TCF4 is preferentially retained in RE+/FECD+ samples, but absent in samples that lacked a TNR expansion,” says Dr. Baratz. “Examining this region in the RE+/FECD- samples revealed that this intron is also retained, suggesting that while retention
of this intron might be a reliable marker for identifying the presence of a TNR expansion, it is not a reliable marker for FECD status.”

**Effect of TNR expansion on gene expression**

Researchers compared gene expression profiles between RE+/FECD+ and RE+/FECD- patients to identify genes that link TNR expansion to FECD pathophysiology. A total of 810 genes with at least a twofold higher expression in RE+/FECD+ compared with RE+/FECD- samples were identified, including SLC4A11, a gene that has previously been implicated in the pathogenesis of FECD.

Researchers also identified 1,372 genes that have more than a twofold lower expression in RE+/FECD+ samples when compared with RE+/FECD-. Notes Dr. Fautsch: “Analysis of this gene set revealed significant reduction for intracellular signaling pathways, including decreased expression of TGFβ-like receptor signaling, transforming growth factor-beta (TGFβ) superfamily members, and genes involved in TGFβ2 expression and activation. It also identified a number of downregulated molecules in the RE+/FECD+ group whose expression is associated with cell senescence.”

**Genetic variants in RE+/FECD-**

Exome sequencing performed on leukocyte DNA obtained from the RE+/FECD- patients did not identify any genetic variants common to all five samples, but did identify 94 genes that had uncommon variants in at least two of the RE+/FECD- samples. Comparison of the exome sequencing results with those obtained by RNA sequencing showed that 64 of the 94 genes were expressed in the corneal endothelium.

Dr. Fautsch concludes: “Identification of novel splicing patterns and differential gene expression in RE+/FECD- samples provides new insights and more relevant gene targets that may be protective against FECD disease in vulnerable patients with TCF4 TNR expansions. Insights gained may serve as a platform to develop productive approaches to therapy across the spectrum of TNR diseases.”

**For more information**


---

**Intravitreal Anti-VEGF Pharmacotherapy Safe for Treatment of Diabetic Macular Edema**

Ocular complications rank among the most debilitating health consequences for adults with diabetes mellitus. One complication, diabetic macular edema (DME), is a leading cause of vision loss in working age adults. DME has historically been treated with macular laser photocoagulation and local corticosteroid injection. In recent years, however, intravitreal anti-vascular endothelial growth factor (VEGF) pharmacotherapy has emerged as a new standard of care for the treatment of patients with decreased vision secondary to center-involving DME.

“We have strong clinical trial evidence supporting the efficacy of pharmacological management with intravitreal anti-VEGF agents,” says Andrew J. Barkmeier, M.D., with Ophthalmology at Mayo Clinic in Rochester, Minnesota. “Meta-analysis of these trials, however, remains underpowered to evaluate for potentially significant differences in systemic serious adverse event rates after treatment.”

To address the evidence gap, Dr. Barkmeier and fellow researchers retrospectively assessed the relative rates of systemic serious adverse events (SAE) after DME treatment with intravitreal anti-VEGF pharmacotherapy, macular laser photocoagulation, and injection of corticosteroid medications, using a large national administrative claims database of commercially insured adults. Results were published in *Ophthalmology* in 2019.

**VEGF as therapeutic target**

“VEGF is a critical growth factor in angiogenesis and acts on vascular endothelia, abnormally increasing retinal vascular permeability. This makes it an attractive therapeutic target,” says Dr. Barkmeier. “However, intravenous anti-VEGF therapy has been associated with serious systemic complications and increased mortality in patients with cancer. Given that association, there is concern that intravitreal injection of anti-VEGF medications may also pose a systemic risk.”

From the OptumLabs database, researchers identified patients who were privately insured or enrolled with Medicare Advantage, age 18 years or older, and treated with anti-VEGF for DME between Jan. 1, 2006, and Dec. 31, 2015 — as well as control patients receiving macular laser or intravitreal corticosteroid injections for DME. Patients had a minimum of one year of medical coverage before initial treatment.

Of the patients receiving treatment for DME during the study period, 23,348 met criteria for
inclusion in the analysis. Of that cohort:

- 9,219 patients were initially treated with intravitreal anti-VEGF pharmacotherapy
- 13,365 patients were initially treated with macular laser photocoagulation
- 764 patients received intravitreal corticosteroid as initial DME management

Overall, patients in the study received 24,685 anti-VEGF injections, 20,574 macular laser photocoagulation procedures and 981 intravitreal corticosteroid injections within six months of their initial treatment for DME, or before being censored due to receiving an alternative treatment.

“Baseline characteristics between the groups were similar, although a smaller proportion of patients receiving initial macular laser photocoagulation were age 65 years or older, compared with the anti-VEGF or corticosteroid groups,” says Dr. Barkmeier. “Anti-VEGF pharmacotherapy was more frequently used as initial DME treatment in the latter years of the study.”

In the anti-VEGF and macular laser groups, respectively:

- 5.5% and 4.3% of patients had a history of a myocardial infarction
- 12.9% and 11.3% had prior cerebrovascular disease
- 23.0% and 16.4% had moderate or severe renal disease

**Comparison of systemic risk**

Primary systemic SAE outcome measures identified by the research team included myocardial infarction, cerebrovascular disease, major bleeding and all-cause hospitalization occurring within six months of initial DME treatment. Researchers then compared the rate of these outcomes after anti-VEGF pharmacotherapy versus the rate after macular laser photocoagulation or intravitreal corticosteroid treatment.

Researchers found no difference in the risk of cerebrovascular disease (HR 0.96 [95% CI, 0.65-1.41], p = 0.83), major bleeding (HR 1.23 [95% CI, 0.76-1.99], p = 0.41), or myocardial infarction (HR 1.03 [95% CI, 0.73-1.44], p = 0.88) between patients receiving anti-VEGF pharmacotherapy and patients treated with macular laser photocoagulation. Similarly, there were no differences in the risk of primary systemic SAE outcomes compared to patients receiving intravitreal corticosteroid pharmacotherapy.

Subgroup analyses of patients at potentially elevated systemic risk revealed similar findings. Patients who received anti-VEGF pharmacotherapy did, however, have an increased rate of all-cause hospital admission compared with those receiving initial macular laser photocoagulation (HR 1.17 [95% CI, 1.05-1.30], p = 0.01).

Overall, this study offers further evidence of a relatively safe systemic risk profile for intravitreal anti-VEGF pharmacotherapy.

“We identified no increased risk of stroke, major bleeding or myocardial infarction after initiation of intravitreal anti-VEGF treatment for DME,” says Dr. Barkmeier. “Although the potential difference in all-cause hospitalization may merit further investigation, it is increasingly evident that these medications are well-tolerated systemically when delivered as intravitreal pharmacotherapy for sight-threatening retinal disease, including in the real-world treatment of DME.”

For more information


---

**Education Opportunities**

Visit [https://ce.mayo.edu/ophthalmology](https://ce.mayo.edu/ophthalmology), call 800-323-2688 (toll-free) or email cme@mayo.edu.

**Mayo Clinic Neuro-Ophthalmology and Beyond: A Multi-Disciplinary Clinical Case-Based Approach 2020**