MAYO CLINIC

OphthalmologyUpdate

Ophthalmology News From Mayo Clinic Vol. 4, No. 3, 2014

INSIDE THIS ISSUE

Trinucleotide Repeat in *TCF4* Responsible for Fuchs' Endothelial Corneal Dystrophy

Variation in the transcription factor 4 (*TCF4*) gene is known to be a major contributor to Fuchs' endothelial corneal dystrophy (FECD). Researchers at Mayo Clinic's campus in Rochester, Minnesota, however, have identified the specific genetic defect in the *TCF4* gene that appears to be responsible for FECD.

"The defect is a trinucleotide repeat — an uncommon type of genetic defect that, up until now, was only seen in neurological and neuromuscular degenerations such as Huntington's disease and myotonic dystrophy," says Keith H. Baratz, M.D., with the Department of Ophthalmology at Mayo Clinic. "Our data demonstrate a strong association between expansion of a noncoding trinucleotide repeat in the *TCF4* gene and FECD."

Dr. Baratz's team tested for an association between an intronic thymine-guanine-cytosine (TGC) trinucleotide repeat in *TCF4* and FECD by determining repeat length in 66 affected participants with severe FECD and 63 control subjects with normal corneas (Figure) in a three-stage discovery, replication and validation study.

For the study, polymerase chain reaction primers flanking the repeat were used to amplify leukocyte-derived genomic DNA. Repeat length was determined by direct sequencing, short tandem repeat (STR) assay. Genomic Southern blots were used to evaluate samples for which only a single allele was identified by STR analysis.

Outcomes for data compiled from the three arms of the study include:

- A TGC repeat length > 50 was present in 52 of 66 (79 percent) of FECD cases and in only two of 63 (3 percent) of normal control cases ($p < 0.001$).
- Among FECD cases, 13 subjects (20 percent) had < 40 repeats and one (2 percent) had an intermediate repeat length.
- The repeat length was greater than 1,000 in four FECD cases and no control cases.
- The sensitivity and specificity of > 50 TGC repeats identifying FECD in this patient cohort was 79 percent and 96 percent, respectively.

"Our data indicate that the expansion of the TGC repeat within the *TCF4* gene is found in a very high proportion of patients with FECD, which suggests the likelihood that FECD is a trinucleotide repeat expansion disorder in the majority of cases," says Dr. Baratz. "The TGC trinucleotide repeat expansion in *TCF4* is strongly associated with FECD. A repeat length > 50 is highly specific for the disease. This association suggests that trinucleotide expansion may be a predictor of disease risk."

Incidence of expanded repeat

In the study, 6 of the 52 patients with FECD and none of the control cases had two expanded alleles > 50 TGC repeats. There were no distinguishing clinical features, such as disease severity or age of onset, among the FECD cases homozygous for repeat expansion.

"It is not clear from the data whether the

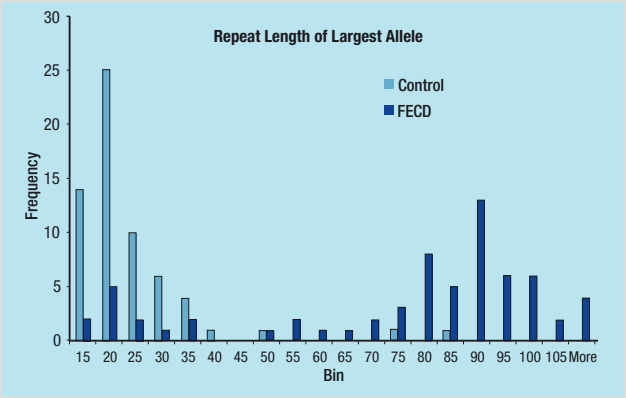

Figure. Frequency histogram of the TGC repeat length of the longest allele in all 129 samples. The length of the longest repeat in each sample is shown for patients with FECD (dark blue bars) and normal control subjects (light blue bars).


Keith H. Baratz, M.D.

occurrence of expanded alleles in unaffected individuals reflects reduced penetrance, delayed disease onset or the possibility of linkage disequilibrium between another causative allele and the expanded repeat," says Dr. Baratz. "If the repeat expansion is causative for FECD, we hypothesize that the effect is to alter the expression of the gene in some way rather than to simply inactivate it."

The frequency of FECD, ease of assessing the phenotype and availability of diseased tissue from corneal transplant patients indicate that further investigation may yield information generalizable to other rare but devastating trinucleotide repeat disorders. "The interesting aspect, from a genetic standpoint, is that trinucleotide repeat diseases have various mechanisms through which they cause disease," says Dr. Baratz.

This research was presented in part at the May 2012 Association for Research in Vision and Ophthalmology annual meeting and published in *PLOS ONE* in November 2012.

For more information

Wieben ED, et al. A common trinucleotide repeat expansion within the transcription factor 4 (*TCF4*, E2-2) gene predicts Fuchs corneal dystrophy. *PLOS ONE*. 2012;7:e49083.

Expanded trinucleotide repeat in *TCF4*, E2-2 may be a functional cause of Fuchs corneal dystrophy. *Ophthalmology Update*. 2012;2:1.

Research confirms association between *TCF4* gene and Fuchs corneal dystrophy. *Ophthalmology Update*. 2011;1:4.

Disease-Causing Mutations Exhibit Disparate Effects on the Localization of Bestrophin-1


Alan D. Marmorstein, Ph.D.

Mutations in *BEST1* are a common cause of inherited retinal degeneration in humans. The gene encodes bestrophin-1 (Best1), a homo-oligomeric, integral membrane protein localized to the basolateral plasma membrane of the retinal pigment epithelium (RPE). More than 200 distinct mutations have been reported to cause five distinct retinal degenerative diseases, including:

- Best vitelliform macular dystrophy (BVMD)
- Adult vitelliform macular dystrophy (AVMD)
- Autosomal recessive bestrophinopathy (ARB)
- Autosomal dominant vitreoretinochoroidopathy (ADVIRC)
- Retinitis pigmentosa (RP)

These diseases, collectively called the bestrophinopathies, are distinguished by type of inheritance, effects on the electro-oculogram and electroretinogram, age of disease onset, and location and extent of retinal lesions. The mechanisms that underlie the bestrophinopathies and determine why mutations cause one disease over another are not yet known.

Localization and oligomerization

To gain insights into these diseases, Alan D. Marmorstein, Ph.D., and a research team at Mayo Clinic's campus in Rochester, Minnesota, conducted a localization and oligomerization screen for 28 Best1 mutants associated with AVMD, ARB, ADVIRC and RP. Results of their study were published in *Experimental Eye Research* in 2014.

Researchers expressed the mutants, fused to yellow fluorescent protein, in polarized Madden-Darby canine kidney monolayers. The team screened the mutants for defects in localization and oligomerization using confocal microscopy and immunofluorescence, live-cell fluorescence resonance energy transfer (FRET) and reciprocal coimmunoprecipitation experiments.

"All 28 mutants exhibited comparable FRET efficiencies to and coimmunoprecipitated with wild-type (WT) Best1, indicating unimpaired oligomerization," says Dr. Marmorstein. "RP- and ADVIRC-associated mutants were properly localized to the basolateral plasma membrane of cells, while two AVMD and most ARB mutants were mislocalized. When coexpressed, all mislocalized mutants caused mislocalization of WT Best1 to intracellular compartments."

The results indicate that:

- Mislocalization of Best1 is not an absolute feature of any individual bestrophinopathy, since it occurs in BVMD, AVMD and ARB
- Mislocalization of Best1 is not a cause of disease, since some ARB mutants that do not also cause dominant disease cause mislocalization of *Best1*
- Absence of Best1 activity from the plasma membrane is tolerated

The research team also found that ARB truncation mutants L174Qfs*57 and R200X can form oligomers with WT Best1, indicating that the first approximately 174 amino acids of Best1 are sufficient for oligomerization to occur.

"Ultimately, this research indicates that AVMD, ARB, ADVIRC and RP are associated with disparate effects on Best1 localization, but

not oligomerization," says Dr. Marmorstein. "Further research is indicated. This research did not explore specific patterns of localization, which could further distinguish individual mutations and potentially differentiate mislocalization in different diseases. The study also did not assess the functional consequences of mislocalized mutants in RPE cells. For now, we postulate that unique changes in calcium homeostasis, phagocytosis, binding partners or other factors may underlie the differential pathogenesis of one disease over another."

For more information

Johnson AA, et al. Disease-causing mutations associated with four bestrophinopathies exhibit disparate effects on the localization, but not the oligomerization, of bestrophin-1. *Experimental Eye Research*. 2014;121:74.

Researchers seek stem cell donors for Best disease clinical trial

Dr. Marmorstein's laboratory is currently using stem cells in their work to find a cure for the bestrophinopathies. Best1 is expressed only in retinal pigment epithelial cells in the eye. The most common bestrophinopathy, Best disease, is dominantly inherited. "Stem cells from a patient with a bestrophinopathy have the same disease as the donor," says Dr. Marmorstein, "and stem cells derived from a patient's skin can be turned into retinal pigment epithelial cells in the laboratory."

Dr. Marmorstein and collaborators Jose S. Pulido, M.D., and Raymond Iezzi Jr., M.D., at Mayo Clinic, are currently recruiting patients with bestrophinopathies to provide a skin biopsy for these studies. "We envision using patients' own stem cell-derived retinal pigment epithelial cells to determine the best course of treatment for their specific diseases," says Dr. Marmorstein. "In some cases this approach may involve gene therapy. In others, the stem cell-derived retinal pigment epithelial cells may be repaired in the laboratory and transplanted back into the patient's eye, preventing loss of vision."

For more information, visit *www.mayo.edu/research/clinical-trials/cts-20095640* or contact Dr. Marmorstein at *marmorstein.alan@mayo.edu*.

Use of Antidepressant Medications and the Incidence of Cataract Surgery

Cataract surgery rates in the United States have more than doubled over the last 20 years — a rate faster than can be explained by aging demographics alone. The rate of increase has accelerated even more for women.

A population-based Canadian study published in *Ophthalmology* in 2010 suggested an association between the use of selective serotonin reuptake inhibitors (SSRIs) and the diagnosis of cataract. An association between serotonin and cataract formation is plausible, as serotonin receptors have been identified in the crystalline lens, and increased serotonin levels have been shown to cause lens opacities in animal models.

A research team led by Jay C. Erie, M.D., with the Department of Ophthalmology at Mayo Clinic's campus in Rochester, Minnesota, recently conducted a population-based, case-control study to investigate an association between SSRI use and incident cases of first-eye cataract surgery within a defined American population. Study results were published in the *American Journal of Ophthalmology* in 2014.

Selective serotonin reuptake inhibitors are the most commonly prescribed antidepressants in the U.S. Estimates indicate that 1 in 4 women over the age 50, and 10 percent of all U.S. residents, are prescribed antidepressants, primarily SSRIs and serotonin norepinephrine reuptake inhibitors (SNRIs).

"Given the increasing use of antidepressant medications in the last decade, an association between SSRI use and cataract surgery rates would be useful information," says Dr. Erie. "This study is an excellent fit for the Rochester Epidemiology Project database, which links and archives the medical records, medical diagnoses, prescriptions, surgical interventions and demographic information of virtually all people residing in Olmsted County, Minnesota."

Jay C. Erie, M.D.

Case-control analysis

Retrospectively identified cases included 6,024 county residents age 50 years or older who underwent first-eye cataract surgery between Jan. 1, 2004, and Dec. 31, 2011. Controls included 6,024 residents who never had cataract surgery and were matched to cases by age, sex and date of surgery.

Logistic regression models were used to compute odds ratios for differences in SSRI use between cases and controls, and were adjusted for age, sex and potential confounding variables, including diabetes and steroid use. Analysis showed:

- After adjusting for age and sex, the use of SSRIs for more than one year was significantly associated with incident cataract surgery (odds ratio [OR] 1.36, 95 percent confidence interval [CI] 1.23-1.51; $P < 0.001$).
- The association between SSRI use and incident cataract surgery was significant for men (OR 1.34, 95 percent CI 1.12-1.61) and women (OR 1.37, 95 percent CI 1.22-1.55) and remained significant after adjusting for cataract formation risk factors, including diabetes mellitus and steroid use ($P < 0.001$).
- Use of selective SNRIs also was significantly

Mayo Clinic
Ophthalmology Update

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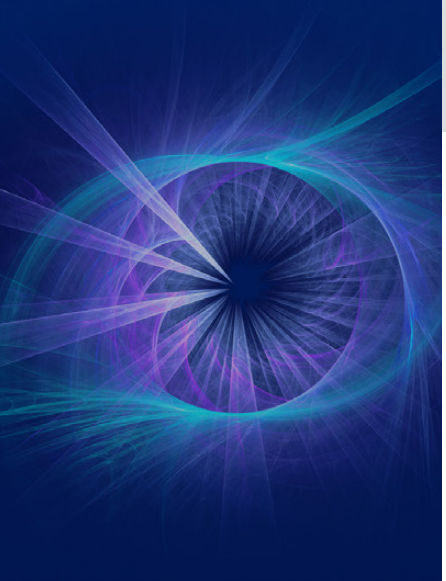

associated with incident cataract surgery (OR 1.37, 95 percent CI 1.11-1.70).

Further studies are warranted to confirm and understand these findings. "Using these resources, we found that cataract surgery in people age 50 years or older was significantly associated with an increased use of SSRIs," says Dr. Erie. "The risk was highest with the use of citalopram. The associations were similar in women and men. Our findings confirm the earlier Canadian study."

The research team noted some limitations in data interpretation:

- The study assessed cataract surgery rather than actual cataract formation.
- Drug formularies and prescribing patterns vary across health care practices and may influence the choice of specific medication within the SSRI and SNRI drug groups.
- Smoking status and exposure to secondhand smoke could not be controlled.

"The association between SSRI use and cataract surgery does not prove causation and should not be taken as reason to avoid or discontinue SSRI therapy. However, the possibility that the observed recent increases in SSRI use is contributing to accelerated growth of incident cataract surgery in our population, especially for women, cannot be excluded," says Dr. Erie.

For more information

Etminan M, et al. Selective serotonin reuptake inhibitors and the risk of cataracts. *Ophthalmology*. 2010;117:1251.

Erie JC, et al. Selective Serotonin Reuptake Inhibitor Use and Increased Risk of Cataract Surgery: A Population-Based, Case-Control Study. *American Journal of Ophthalmology*. 2014;158:192.

Learn more about the Rochester Epidemiology Project at *www.Mayo.edu/research/centers-programs/rochester-epidemiology-project/overview*.

Mayo Medical School Receives RPB Grant

Research to Prevent Blindness (RPB) has awarded a $115,000 grant to the Department of Ophthalmology at Mayo Medical School to support its research into the causes, treatment and prevention of blinding diseases. "We are honored to receive this grant from RPB," says Sanjay V. Patel, M.D., department chair. "The funding supports and reinforces our commitment to research that contributes to a greater understanding of blinding diseases."

Michael Brodsky, M.D., Named Knights Templar Professor

Michael C. Brodsky, M.D., with the Department of Ophthalmology at Mayo Clinic's campus in Rochester, Minneosta, has been named Knights Templar Eye Foundation Inc. Professor of Ophthalmology Research.

Michael C. Brodsky, M.D.

Education Opportunities

For more information or to register for courses, visit *www.Mayo.edu/cme/ophthalmology*, call 800-323-2688 (toll-free) or *email cme@mayo.edu*.

Current Management of Age-Related Macular Degeneration & Diabetic Macular Edema, Oct. 18, 2014, in Chicago

Current Concepts in Primary Eye Care, Oct. 30, 2014, in Rochester, Minn.

Retina Update and Case Conference, Oct. 31-Nov. 1, 2014, in Rochester, Minn.

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