Researchers at Mayo Clinic report the first instance of RNA toxicity in patients with a non-neurological or neuromuscular disease — Fuchs’ endothelial corneal dystrophy (FECD).

FECD is a common inherited, degenerative disease that affects the internal endothelial cell monolayer of the cornea. Advanced FECD, treatable only by corneal transplantation, is characterized by guttae, endothelial cell loss and loss of vision due to stromal edema. It affects nearly 5 percent of middle-aged Caucasians in the United States and accounts for more than 14,000 corneal transplantations annually.

Trinucleotide repeat expansion in FECD

In 2010, a genome-wide association study by Keith H. Baratz, M.D., Ophthalmology, at Mayo Clinic’s campus in Rochester, Minnesota, and a multidisciplinary research team identified the single nucleotide polymorphism rs613872, located in an intron of the transcription factor 4 (TCF4) gene on chromosome 18, as a marker for FECD. Study results were published by Dr. Baratz and others in a 2010 issue of the *New England Journal of Medicine*.

This association has now been replicated repeatedly. Subsequent investigation by a research team that included Dr. Baratz and was led by Eric D. Wieben, Ph.D., Biochemistry and Molecular Biology, at Mayo Clinic in Rochester, Minnesota, found an even stronger linkage between a (CTG · CAG)n trinucleotide repeat expansion in a different intron of the TCF4 gene and FECD. Their findings were published in *PLOS One* in 2012.

“The strongest association among the genes and loci associated with FECD was with an intronic (CTG · CAG)n trinucleotide repeat expansion in the TCF4 gene, which was found in a majority of patients affected with the disease,” says Dr. Baratz. A repeat length longer than 150 nucleotides in leukocyte DNA is highly predictive of disease, so this trinucleotide repeat is a prime candidate for being pathogenic in an autosomal dominant, late-onset degenerative disease such as FECD.

The location of the repeat in an intron indicated that an alteration in protein coding was not the cause of the disease but raised the possibility that RNA toxicity might play a role in the pathogenesis of this disorder, as it does in several of the relatively rare neurodegenerative and neuromuscular repeat expansion diseases, such as myotonic dystrophy. They also concluded that the FECD patient population with the (CTG · CAG)n trinucleotide repeat expansion exceeded that of the combined number of patients in all other microsatellite expansion disorders, including Huntington’s disease, myotonic dystrophy types 1 and 2, fragile X syndrome, spinocerebellar ataxia, and C9ORF72-associated amyotrophic lateral sclerosis and frontotemporal dementia.

RNA toxicity and missplicing in FECD

A subsequent collaboration between the Mayo team and researchers at The Scripps Research Institute, La Jolla, California, confirmed evidence of RNA toxicity in FECD. “Corneal endothelial cells from patients with FECD harbored a poly(CUG)n RNA that could be visualized as RNA foci containing this condensed RNA and associated proteins,” says Dr. Baratz.

The poly(CUG)n RNA colocalized with and sequestered the mRNA splicing factor MBNL1,
Rituximab Offers No Benefit Over Placebo to Patients With Active or Moderate-to-Severe Graves’ Orbitopathy

Prevention of Graves’ orbitopathy (GO) remains an elusive goal. To date, there is no effective measure to prevent GO, a potentially sight-threatening disease. Some patients with mild disease benefit from selenium treatment, but other systemic therapies are generally not offered due to potential adverse effects. Intravenous corticosteroids, helpful for the inflammatory signs and symptoms of GO, are associated with significant adverse effects. Treatment requires repeated infusions over many weeks and nearly 30 percent of patients relapse.

Orbital decompression, a complex surgical procedure, is typically offered when the disease is severe, other treatment modalities have failed or inactive disease requires rehabilitative surgery. Therapeutic intervention is offered for patients with active disease, severe disease or both.

A literature review published by Mario Salvi and others in The Journal of Clinical Endocrinology & Metabolism in 2013 suggests that rituximab may benefit patients with GO. Rituximab is a humanized chimeric anti-CD20 monoclonal antibody that depletes both B lymphocytes in the intermediate stages of maturation and short-lived plasma cells.

Based on that review and similar reports, a multidisciplinary team that included James A. Garrity, M.D., Ophthalmology, at Mayo Clinic’s campus in Rochester, Minnesota, designed a double-masked, randomized controlled trial to study the efficacy of rituximab in patients with GO. “While rituximab might be of benefit, improvement in disease activity over time is also compatible with the natural history of GO,” says Dr. Garrity. The study was designed by Rebecca S. Bahn, M.D., an emeritus specialist in endocrinology at Mayo Clinic in Rochester, Minnesota, and Marius N. Stan, M.D., Endocrinology, at Mayo Clinic in Rochester, Minnesota, with that context in mind. Results appeared in The Journal of Clinical Endocrinology & Metabolism in 2014.

For more information


• Evidence of disease progression (through changes in disease activity, severity or both) during the previous two months or lack of improvement in the prior six months (assessed through patient questioning and by review of outside medical records from the referring ophthalmologist or endocrinologist, patient photographs, or both)
• Previous steroid treatment was acceptable if discontinued greater than or equal to four weeks before enrollment

“Each eligible patient was given the choice of trial participation, treatment with IV glucocorticoids, orbital decompression surgery if appropriate or close observation,” says Dr. Garrity, “and the potential for disease progression was discussed.”

Twenty-five patients with active moderate-to-severe GO opted to participate in the trial: 12 were enrolled in the placebo arm and 13 in the rituximab arm. Participants received either two infusions of rituximab (1,000 mg each) via IV or two saline infusions, given two weeks apart.

Patients were re-evaluated every two months for the first six months. Those who showed deterioration were removed from the trial and offered appropriate therapeutic options.

**Primary and secondary endpoints**

Baseline assessment of participants included medical history, physical examination by an endocrinologist, ocular evaluation by an ophthalmologist, orbital CT, thyroid-stimulating hormone, free thyroxine, thyrotropin receptor antibody levels and CD 19+ B cell count. The treatment groups were similar in all parameters at baseline.

“Our primary endpoint was a reduction in clinical activity score, assessed as a continuum and separately as improvement by greater than or equal to two points at 24 weeks,” says Dr. Garrity. “Twenty-one patients completed the study to the primary endpoint. We found no differences in the proportions of patients showing clinical activity score improvement at 24 weeks.”

Secondary endpoints included success and failure rates; proportions showing clinically significant improvement in proptosis, lid fissure width, diplopia score, lagophthalmos and disease severity; orbital fat, muscle volume or both; and quality of life as assessed by the SF-12 Health Survey.

Patients were re-evaluated at eight, 16, 24 and 52 weeks. There were no differences between groups in any of the secondary endpoints at either 24 or 52 weeks, but there were four adverse events in three of 12 patients receiving placebo and 11 adverse events in eight of 13 patients treated with rituximab. Five of six of the moderate or severe adverse events occurred in patients treated with rituximab, including two patients who developed an optic neuropathy.

“Our study indicates that rituximab offers no additional benefit over placebo to patients with active and moderate-to-severe GO,” says Dr. Garrity, “and carries non-negligible adverse effects.”

**For more information**


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**MYD88 L265P Mutation May Provide a New Marker for Diagnosis of Vitreoretinal Lymphoma**

A multidisciplinary team from the departments of Ophthalmology, Molecular Medicine, and Laboratory Medicine and Pathology at Mayo Clinic’s campus in Rochester, Minnesota, has studied three patients with known diffuse large B-cell lymphoma to determine whether an MYD88 L265P mutation was present in any case of definite vitreoretinal diffuse large B-cell lymphoma.

An L265P (T-C at position 38182641 at 3p22.2) mutation of MYD88, a protein associated with the innate immune system, is present in many cases of Waldenström’s macroglobulinemia. The mutation, which causes a constitutive activation of the MYD88 protein and triggers NF-kappaB signaling, has become a diagnostic criterion for Waldenström’s macroglobulinemia.

The same MYD88 L265P mutation occurs in about 15 percent of cases of systemic diffuse large B-cell lymphoma. The most common form of vitreoretinal lymphoma is the diffuse large B-cell form. “No one has shown whether this mutation occurs in vitreoretinal lymphoma, which is a rare form of central nervous system diffuse large B-cell lymphoma,” says Jose S. Pulido, M.D., Ophthalmology, at Mayo Clinic in Rochester, Minnesota. Results of the team’s research were published in *Retina* in April 2015.

**Mutation identified in 2 of 3 subjects**

The Mayo team found three patients with classic
clinical findings of vitreoretinal lymphoma and histologic confirmation — and enough tissue to evaluate for the mutation. The MYD88 mutation assay required a minimum input of 5 ng of DNA.

“Clinically and histologically, there did not seem to be differences in the three cases; all were severe,” says Dr. Pulido. “The ages were similar in all three cases, and all started as vitreoretinal lymphoma. All three had vitreous involvement. Two had subretinal involvement, one who was positive and one who was negative for the MYD88 mutation. All had diffuse large B-cell lymphoma noted histologically. Extraocular involvement occurred in all three cases, but systemic involvement was seen in the negative L265P case, and central nervous system involvement was seen in the positive L265P cases.”

The research team evaluated the formalin-fixed paraffin-embedded cells from the patients using a validated amplification-refractory mutation system polymerase chain reaction, which results in a normal product of 141 base pairs and a mutation amplicon of 72 base pairs (each plus or minus 5 base pair resolution by capillary electrophoresis), to determine the presence of the mutation.

The polymerase chain reaction product quality was determined from mean relative fluorescence unit values, requiring greater than a 0.1 relative fluorescence unit if the mutant product was detected and a relative fluorescence unit of 0.5 or greater for a negative (wild type) product.

Researchers validated the test on 54 samples that had either small B-cell lymphoma or Waldenström’s macroglobulinemia. A sensitivity of 1 percent mutant allele in a wild-type population could be detected. The test had a 92 percent clinical sensitivity and a 92 percent specificity.

Further studies required

“Our study confirms that MYD88 L265P constitutive activating mutations are present in at least some cases of the diffuse large B-cell lymphoma form of vitreoretinal lymphoma,” says Dr. Pulido. “The 74 ± 2 base pair product seen from the mutated MYD88 protein was noted in two of the three cases.” The finding is significant for two reasons:

• It helps in making the difficult diagnosis of vitreoretinal lymphoma by providing another marker that may help with diagnosis of the disease.
• There are now specific inhibitors of the MYD88 L265P mutation, as noted by Steven P. Treon, M.D., Ph.D., and others in Hematology/Oncology Clinics of North America in 2014.

“The limitations of this study are that we only used definite cases of vitreoretinal lymphoma,” says Dr. Pulido. “We cannot determine the efficacy of this test in people whose diagnosis from the histologic examination was negative. Further studies are required to determine the lower level of sensitivity in these cases.

“Also, we were only interested in seeing whether any of our cases would test positive, so further studies are required to determine the exact incidence of this mutation in cases of diffuse large B-cell form of vitreoretinal lymphoma.”

For more information

Pulido JS, et al. MYD88 L265P mutations are present in some cases of vitreoretinal lymphoma. Retina. 2015;35:624.