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Endothelial PAS Domain-Containing Protein 1 (*EPAS1*) Gene Polymorphisms and Response to Anti-VEGF Therapy in the Comparison of AMD Treatments Trials (CATT)

The efficacy of the intravitreal anti-vascular endothelial growth factor (anti-VEGF) agents bevacizumab and ranibizumab has revolutionized the treatment of neovascular age-related macular degeneration. Results from the Comparison of AMD Treatments Trials (CATT), the Alternative Treatments to Inhibit VEGF in Patients with Age-Related Choroidal Neovascularisation (IVAN) trial, and other multicenter randomized clinical trials that compared bevacizumab and ranibizumab indicate that both drugs provide dramatic and lasting visual improvements for patients. However, results from these trials also make clear that there is individual variation in the initial response to therapy and in the durability of the clinical effect. Genetic assessment of participants in these trials provides an ideal opportunity to investigate pharmacogenetic associations using rigorously defined phenotypic data.

A recent report from the IVAN Study Group evaluated 509 participants across 494 single nucleotide polymorphisms (SNPs) for evidence of a genetic association with response to anti-VEGF therapy as measured by change in total retinal thickness (TRT) in 1 year.[1] Eyes in the highest quartile of change in TRT (n = 126) were designated as responders whereas those in the lowest quartile (n = 128) were designated as nonresponders. The strongest association observed was for rs9679290 in the endothelial PAS domain-containing protein 1 (*EPAS1*) gene (unadjusted $P = 0.002$); however, the association was not significant after Bonferroni correction for multiple comparisons ($P = 0.84$). Interestingly, 4 of the top 10 strongest associations from the IVAN study were in this gene, although none of them were significant after Bonferroni correction.

The *EPAS1* gene represents a plausible gene for influencing anti-VEGF treatment response. It is a transcription factor expressed predominantly in highly vascularized tissues and likely regulates vascularization.[2] Mice that are *Epas1*$^{-/-}$ demonstrate severe retinopathy at a young age, including photoreceptor loss, retinal thinning, and abnormal retinal vasculature.[3]

In an effort to replicate the pharmacogenetic association between *EPAS1* SNPs and response to anti-VEGF therapy, we evaluated the top 4 *EPAS1* SNPs from the IVAN study (rs6726454, rs7589621, rs9679290, and rs12712973) in 831 CATT participants.[4] Similar to IVAN, we classified participants as responders or nonresponders to anti-VEGF therapy based on TRT as determined by optical coherence tomography. We calculated the change in TRT from baseline at the latest time point for which optical coherence tomography data were available through 1 year (4, 8, 12, 24, or 52 weeks). Eyes with changes in TRT greater than or equal to the 75th percentile were classified as responders, and those with changes less than or equal to the 25th percentile were classified as nonresponders.

We classified 211 participants as responders and 210 classified as nonresponders. The distribution of change in TRT in CATT was remarkably similar to that seen in IVAN (Fig 1). The genotypic frequencies of all 4 SNPs in CATT were also similar to that seen in IVAN (Table 1; available at www.aaojournal.org). In the CATT patient cohort, no significant association was observed for any of the genotypes at the 4 *EPAS1* SNPs. Similar to the IVAN result, the strongest association was at rs9679290 ($P = 0.21$); however, the odds ratio was in the opposite direction (0.84 for CATT, 1.87 for IVAN). The other 3 *EPAS1* SNPs (rs6726454, rs12712973, rs7589621) were also not associated with response to therapy in CATT, also with odds ratios in the opposite direction as seen in IVAN.

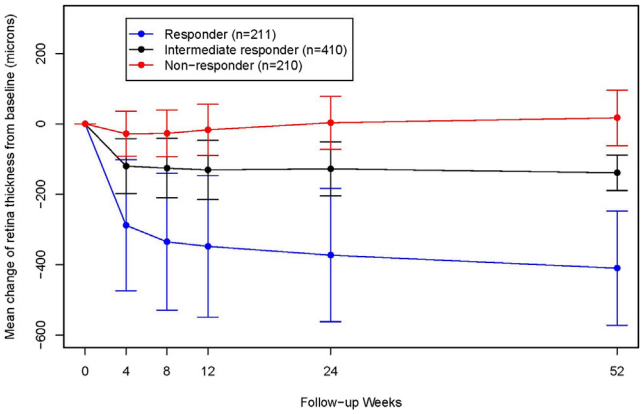

Figure 1. Mean value ± standard deviation change of total retinal thickness (microns) from baseline in the Comparison of AMD Treatments Trials (CATT).

The CATT data do not support a pharmacogenetic association between the 4 SNPs tested in *EPAS1* and response to anti-VEGF therapy in patients with neovascular age-related macular degeneration.

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References

1. Lotery AJ, Gibson J, Cree AJ, et al. Pharmacogenetic associations with vascular endothelial growth factor inhibition in

participants with neovascular age-related macular degeneration in the IVAN study. Ophthalmology 2013;120:2637–43.
2. Tian H, McKnight SL, Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. Genes Dev 1997;11:72–82.
3. Ding K, Scortegagna M, Seaman R, et al. Retinal disease in mice lacking hypoxia-inducible transcription factor-2alpha. Invest Ophthalmol Vis Sci 2005;46:1010–6.
4. Hagstrom SA, Ying G-S, Pauer GJT, et al. Pharmacogenetics for genes associated with age-related macular degeneration in the Comparison of AMD Treatments Trials (CATT). Ophthalmology 2013;120:593–9.

Adult Orbital Xanthogranuloma Successfully Treated with Rituximab

Xanthogranulomatous disease (XD) of the ocular adnexa is rare and of unknown etiology. Four subtypes are recognized: Adult-onset xanthogranuloma (AOX), adult-onset asthma and periocular xanthogranuloma (AAPOX; similar to AOX plus late-onset asthma, often nasal and paranasal sinus disease, and reactive lymphadenopathy), necrobiotic xanthogranuloma (with necrobiosis and a strong association with multiple myeloma, monoclonal gammopathy and lymphoma), and Erdheim-Chester disease (which affects the posterior orbit, has systemic manifestations, and a high mortality).[1,2]

Treatment of XD has included surgery, radiation, corticosteroids, and other immunosuppressive and chemotherapeutic agents.[1] Recent reports have focused on the beneficial effects of methotrexate (MTX).[3,4] However, these treatments have at best improved symptoms and signs. Erdheim-Chester disease has recently been found to have the BRAF V600E mutation, with successful therapy with vemurafenib, a BRAF enzyme inhibitor.[5] We have reported the results of treatment of 2 cases of AOX and 1 case of AAPOX with the anti-CD20 antibody rituximab.

Appropriate Human Research and Ethics Committee approval was obtained for this study.

We reviewed the records of 3 consecutive cases of XD treated with rituximab after modest improvement with corticosteroids and MTX.

Case 1

A 58-year-old woman presented in 2002 with yellowish patches and swelling in the upper lids for 5 years, and more recent onset of right ptosis. Surgical debulking and right ptosis repair had been performed 1 year earlier. There was no history of asthma.

She had a 2-mm right ptosis and 1-mm left ptosis, with reduced right levator excursion of 8 mm and 15 mm on the left. Both upper lids had bright yellow plaques, and generalized fullness, worse on the right (Fig 1A, available at www.aaojournal.org). There was moderate bilateral dry eye. Eye movements were normal. Computed tomography showed an infiltrative process in the preseptal tissues and anterior orbits.

Further surgical debulking of the right upper eyelid and anterior orbit was performed. Tissue showed features consistent with AOX. She declined further treatment. By 2005, the disease had worsened. Treatment with MTX and oral prednisolone led to a moderate improvement in her symptoms and signs. The MTX was ceased in mid 2008, and low doses of oral prednisolone maintained. By September 2009, she had increased swelling and 3 mm of right ptosis and reduced levator excursion of 5 mm, and significantly dry eyes.

The pathology was reviewed and showed 80% of lymphocytes were CD-20$^+$ B cells associated with small numbers of T cells (CD3$^+$, predominantly CD4$^+$ with a few CD8$^+$). Rituximab 375 mg/m^2 intravenously weekly for 4 consecutive weeks was commenced in February 2010, followed by 3 monthly infusions, continued to May 2012 without side effects. She was initially on small doses of oral prednisolone (reducing from 5 to 1 mg), which were ceased in late 2010.

Within 2 months of commencing rituximab the ptosis recovered almost completely, eyelid swelling and discoloration, and symptoms and signs of dry eye resolved (Fig 1B). Twenty months after ceasing all treatment, she has maintained a complete clinical response.

Case 2

A 29-year-old man presented in October 2006 with an 18-month history of swelling and yellowish discoloration of both upper eyelids and a history of psoriasis and vitiligo. There was no history of asthma. Clinical and radiologic features were consistent with AOX (Fig 2A, available at www.aaojournal.org) and biopsies of the right upper eyelid, lacrimal gland and anterior orbital fat confirmed the diagnosis.

Oral MTX and a weaning course of oral prednisolone led to a modest improvement, but in late 2008 his liver enzymes became abnormal and it was discontinued. A trial of azathioprine and prednisolone was ineffective.

Rituximab 375 mg/m^2 was then administered intravenously weekly for 4 consecutive weeks, followed by 2-monthly maintenance infusions. There was marked improvement and within 3 months, proptosis had reduced by 2 to 3 mm, the eyelids were markedly less swollen, less red, and the yellow skin plaques were reduced (Fig 2B, available at www.aaojournal.org). After 12 months of 2-monthly infusions, the frequency was reduced to every 3 months, maintained until December 2012. The therapeutic response has continued, with no side effects from the rituximab.

Case 3

A 54-year-old man presented in 2010 with several years' worsening upper eyelid swelling and ptosis and asthma from the age of 52. He had intermittent symptoms of nasal obstruction. Clinical and radiologic features were consistent with a diagnosis of AAPOX (Fig 3A, available at www.aaojournal.org) and biopsies obtained from the left upper eyelid skin, orbicularis muscle, and anterior orbital tissues confirmed this.

He was commenced on MTX (10 mg/wk, increasing to 25 mg over 3 months) and oral prednisolone (initially 25 mg). There was a moderate clinical and radiologic improvement. He was then commenced on rituximab in May 2011. He received 375 mg/m^2 intravenously weekly for 4 consecutive weeks, followed by 3-monthly maintenance infusions through May 2013. There was further significant clinical and radiologic improvement (Fig 3B). Improvement continued until cessation of treatment in May 2013, with a sustained response since, and no side effects from the rituximab.

This is the first report of treating AOX or AAPOX with the monoclonal anti-CD20 antibody rituximab. The response has been dramatic and sustained, and the therapeutic effect has been better than the combination of MTX and corticosteroids in these 3 patients.

In the first patient in this series, 80% of the B cells were CD20$^+$ and prominent B-cell populations in the other 2 cases.

Table 1. Association of *EPAS1* Genotype and Morphologic Response in CATT (n = 421)

	Good Response Group (%)	Poor Response Group (%)	Odds Ratio (95% CI)	Linear Trend *P* Value
rs9679290				
CC	40 (19.0)	51 (24.3)		
CG	108 (51.2)	103 (49.0)		
GG	63 (29.9)	56 (26.7)		
CATT frequency (%) of allele C	188 (44.6)	205 (48.8)	0.84 (0.64, 1.11)	0.21
IVAN frequency (%) of allele C	(52)	(36)	1.87	
rs6726454				
GG	49 (23.2)	56 (26.7)		
AG	102 (48.3)	105 (50.0)		
AA	60 (28.4)	49 (23.3)		
CATT frequency (%) of allele G	200 (47.4)	217 (51.7)	0.84 (0.65, 1.11)	0.22
IVAN frequency (%) of allele G	(56)	(42)	1.80	
rs12712973				
AA	51 (24.2)	43 (20.5)		
AC	108 (51.2)	109 (51.9)		
CC	52 (24.6)	58 (27.6)		
CATT frequency (%) of allele A	210 (49.8)	195 (46.4)	1.15 (0.87, 1.51)	0.33
IVAN frequency (%) of allele A	(41)	(54)	0.59	
rs7589621				
AA	12 (5.7)	14 (6.7)		
AG	85 (40.3)	92 (43.8)		
GG	114 (54.0)	104 (49.5)		
CATT frequency (%) of allele A	109 (25.8)	120 (28.6)	0.86 (0.63, 1.18)	0.36
IVAN frequency (%) of allele A	(29)	(20)	1.70	

CATT = Comparison of AMD Treatments Trials; CI = confidence interval; IVAN = Alternative Treatments to Inhibit VEGF in Patients with Age-Related Choroidal Neovascularisation.