umab, but 2 months later a second, more anterior stage 3 complex developed. Although the occurrence of multiple ridges is well documented, it is a rare event compared with other patterns of ROP regression. To our knowledge, this is the first angiographic documentation of circumferential anastomosis at the bevacizumab-induced regression site with radial vessels progressing anteriorly to form a second stage 3 complex. The time course seems to indicate quiescence due to bevacizumab followed by reactivation from its waning effect. Although this case provides further evidence of the efficacy of bevacizumab as a treatment option for patients with ROP when laser treatment is not feasible, it also emphasizes that the angiogenic stimulus potentiating the sight-threatening complications of ROP may recur or persist after a single injection of an anti-VEGF agent. This case also provides further support that intravitreal bevacizumab does not necessarily inhibit subsequent retinal vascular development.1

Quan V. Hoang, MD, PhD
Daniel F. Kiernan, MD
Felix Y. Chau, MD
Michael J. Shapiro, MD
Michael P. Blair, MD

Support for TGFB1 as a Susceptibility Gene for High Myopia in Individuals of Chinese Descent

Zha et al1 reported a haplotype-tagging single-nucleotide polymorphism (SNP) (rs4803455) within the transforming growth factor β1 (TGFB1 [OMIM 190180]) gene and observed evidence of association between it and severe myopia (spherical equivalent [SE] ≤−8.0 diopters [D]) using a case-control sample of Chinese individuals (N=600; P<.001). They also provided evidence of replication for a previously reported neighboring SNP (rs1800470) in moderate linkage disequilibrium with rs4803455.2 For both SNPs, the minor allele was at decreased risk for high myopia and their effect was strongest within a model of recessive inheritance. Exhaustive analysis by Zha and colleagues showed the best correlate for high myopia susceptibility within TGFB1 to be rs4803455. We thus sought to assess this association within Chinese children in the Singapore Cohort Study for Risk Factors in Myopia.

Methods. The Singapore Cohort Study for Risk Factors in Myopia cohort has been previously described.3,4 The students enrolled in the study are examined annually, and serial eye measurements are taken using standardized protocols. These include cycloplegic refraction and axial length measurement of the eyeball. To remove ethnicity as a potential source of population heterogeneity, we only included children of Chinese descent in this genotyping exercise (n=978). Phenotypic classification of the children into those with severe myopia, those with nonsevere myopia, and nonmyopic controls was made at visit 4 when the children were aged 10 to 12 years. The SE was defined as sphere plus half-negative cylinder. High myopia was defined as an SE of −5.0 D or less; mild to moderate myopia was defined as an SE between −5.0 D and −0.5 D; and nonmyopic controls included those with an SE greater than −0.5 D. The axial length of the globe was measured by contact ultrasound A-scan biometry as previously described.4,5 The SNP rs4803455 was analyzed in an opportunistic but hypothesis-driven manner as the data were available from an ongoing genome-wide association study using the Illumina HumanHap 550 Beadchips (Illumina, Inc, San Diego, California; http://www.illumina.com). Rigorous quality-control steps were performed, including genotype success rate, missingness, population stratification, departure from Hardy-Weinberg equilibrium in controls, monomorphism, excess heterozygosity, cryptic relatedness, and sex discrepancy. Data analysis was performed using SPSS version 17 statistical software (SPSS Inc, Chicago, Illinois). Pairwise linkage disequilibrium between markers was computed based on the squared Pearson correlation coefficient ($r^2$) using the overall data set. We used the linkage disequilibrium information to select a set of 4000 independent autosomal markers ($r^2<0.16$) with approximately equal intermarker distance (approximately 670 kilobases [kb]) across the genome. This set of markers was used to examine sample relationships with the Graphical Representation of Relationships program (Cen-
Results. We observed a marginally significant overall genotypic association at \textit{TGFB1} rs4803455 when children with any myopia vs nonmyopic children were compared (n=348 controls, 630 cases; \( P = .01 \)) (Table). When analyzed spe-

ter for Statistical Genetics, University of Michigan, Ann Arbor; http://www.sph.umich.edu/csg/abecasis/GRR/) and to examine population structure with \textsc{Structure} software (Department of Statistics, University of Oxford, Oxford, England). Additional evaluative testing of population structure was performed with the \textsc{Eigenstrat} method using all markers with no Hardy-Weinberg equilibrium deviation and with greater than 1\% minor allele frequency. For related samples identified by \textsc{Graphical Representation of Relationships}, we retained those with a higher call rate. Meta-analysis of all available data was performed using inverse-variance weighting as previously described.7

| \textbf{Genotype or Association} | \multicolumn{2}{c}{\textbf{Zha et al.} \textsuperscript{7} 2009} | \multicolumn{2}{c}{\textbf{SCORM Study}} |
|----------------------------------|----------------|----------------|----------------|----------------|
|                                  | Controls | High Myopia | Controls | All Myopia | High Myopia |
| \textbf{Genotype, No.}           |          |             |          |            |             |
| G/G                              | 115      | 142         | 155      | 246        | 38          |
| G/T                              | 141      | 140         | 141      | 315        | 61          |
| T/T                              | 44       | 18          | 52       | 69         | 8           |
| \textbf{Total}                   | 300      | 300         | 348      | 630        | 107         |
| \textbf{Association test}       |          |             |          |            |             |
| \textbf{Genotypic} \( P \)       | .001     |              | .01      | .007       |             |
| \textbf{Recessive} \( P \)       | 4.9 \times 10^{-4} |          | .07      | .046       |             |
| \textbf{OR (95\% CI)}\textsuperscript{a} | 0.37 (0.20-0.68) |          | 0.70 (0.47-1.05) | 0.46 (0.19-1.05) |

\textbf{Abbreviations}: CI, confidence interval; OR, odds ratio; SCORM, Singapore Cohort Study for Risk Factors in Myopia.

\textsuperscript{a}Odds ratio for the recessive model.

Figure. Haploview version 3.2 plot showing a pairwise linkage disequilibrium map for single-nucleotide polymorphisms within a 100–kilobase (kb) flanking region centered on \textit{TGFB1} rs4803455 in the Singapore Cohort Study for Risk Factors in Myopia cohort. The \( r^2 \) algorithm was used. Single-nucleotide polymorphisms are identified by their dbSNP rs numbers, and their relative positions are marked by vertical lines. Numbers within the diamonds indicate the \( r^2 \) value expressed as a percentile. Increasing degrees of \( r^2 \) values are denoted by squares of a darker shade.
specifically for high myopia, the association became more pronounced (n = 348 controls, 107 cases; P = .007). Children who were recessive for the minor T allele were markedly underrepresented among the high myopia cases (7.5%) compared with nonmyopic controls (14.9%) (P = .046) (Table). The relationship between recessivity of the minor T allele and myopia-related quantitative traits in the entire Singapore Cohort Study for Risk Factors in Myopia cohort (n = 978) was then assessed. Children who were recessive for the minor T allele had less myopic SEs (mean, −1.54 D) and shorter axial lengths (mean, 23.94 mm) compared with wild-type children (mean SE, −1.95 D; mean axial length, 24.15 mm) (P = .04 and P = .05, respectively).

When assessed in the context of the entire genome-wide association study, 2618 SNPs had single-point P values that exceeded the significance of TGFB1 rs4803455 (lowest P = 7.9 × 10⁻⁶ at chromosome 11). Full correction for multiple testing in light of more than 400 000 independent tests rendered the TGFB1 rs4803455 observation insignificant.

However, as our study was hypothesis-based with regard to TGFB1, we proceeded to assess TGFB1 rs4803455 in the context of the nearby flanking SNPs that were also genotyped. A linkage disequilibrium plot (Figure) using 32 SNPs genotyped within the 100-kb flanking region localized rs4803455 to a small block that included 3 other neighboring SNPs (rs2241715, rs2241714, and rs1046909). All 3 showed marginal evidence of association (P = .06, P = .04, and P = .02, respectively), which did not exceed that observed with rs4803455 (P = .007). No other SNP within the 100-kb flanking region upstream and downstream of TGFB1 rs4803455 showed any evidence of association, thus localizing the signal to TGFB1.

Comment. Zha et al.¹ identified a new marker within TGFB1 that proved to be more informative in predicting the risk of high myopia in individuals of Chinese descent over and above the previously reported rs1800476.² We provide evidence in another Chinese population in support of the observations by Zha and colleagues. We show new data linking TGFB1 rs4803455 and myopia-related quantitative traits. When our data are interpreted in the context of earlier results,¹ ² association of TGFB1 rs4803455 was reproduced in both the broad genotypic model and specific recessive models, thus showing further consistency with previous studies. Even if this is not the functional variant(s) involved, the TGFB1 SNP rs4803455 is very likely the closest correlate. We are mindful of 2 previous reports that show no association between TGFB1 genetic variation and high myopia.³ ⁴ Although rs4803455 was not genotyped in these 2 reports, Wang et al.⁵ did genotype rs1800470, which was moderately correlated with rs4803455 (r² = 0.56), and failed to observe a significant association (recessive: odds ratio = 0.83; 95% confidence interval, 0.56-1.23). Despite not being significant, the disease odds ratio observed by Wang and colleagues is in keeping with that observed by the 2 previous studies.¹ ² and meta-analysis of all available studies.¹ ² ³ on the rs1800470 variant revealed suggestive evidence of an association with reduced susceptibility to high myopia (P = 3.0 × 10⁻⁵). Meta-analysis of all available data (by Zha and colleagues and our study) on rs4803455 resulted in a slightly stronger effect estimate (P = 9.88 × 10⁻⁵).

We acknowledge that the association observed with TGFB1 rs4803455 is not significant after being subjected to more than 400 000 independent tests and that the meta-analysis does not yet yield a genome-wide significant association. However, given the consistent evidence for the role of TGFB1 and high myopia,¹ ² and considering this in light of new evidence by Zha and colleagues and our study, the overall evidence thus far suggests support for an association between TGFB1 and high myopia. Additional samples should be genotyped for the SNPs that have been implicated in this locus to increase power to detect a significant effect of this locus.

Chiea C. Khor, MD, PhD
Qiao Fan, MSc
Liang Goh, PhD
Donald Tan, FRCP (Hons)
 Terri L. Young, MD
Yi-Ju Li, PhD
Mark Seielstad, PhD
Denise L. M. Goh, MD
Seang Mei Saw, PhD

Author Affiliations: Division of Infectious Diseases, Genome Institute of Singapore (Drs Khor and Seielstad), Division of Genetic Medicine, Singapore Institute for Clinical Sciences, Agency for Science, Technology, and Research (Drs Khor and D. L. M. Goh), Department of Epidemiology and Public Health, Faculty of Medicine (Ms Fan and Dr Saw) and Department of Ophthalmology, Yong Loo Lin School of Medicine (Dr Tan), National University of Singapore, Duke–National University of Singapore Graduate Medical School (Drs L. Goh and Young), Singapore Eye Research Institute (Dr Tan), and Department of Paediatrics, Children's Medical Institute, National University Health System and National University of Singapore and National University of Singapore–Genome Institute of Singapore Center for Molecular Epidemiology (Dr D. L. M. Goh), Singapore; and Center for Human Genetics, Duke University, Durham, North Carolina (Drs Young and Li).

Correspondence: Dr Khor, Division of Infectious Diseases, Genome Institute of Singapore, 60 Biopolis St, Genome, Singapore 138672 (khorcc@gis.a-star.edu.sg).

Financial Disclosure: None reported.

Funding/Support: This work was supported by grant 06/12/19/466 from the Biomedical Research Council, Agency for Science, Technology, and Research (a statutory board of the Singapore government) (Dr Saw). Dr Khor is a scholar of the Agency for Science, Technology, and Research.

Additional Contributions: We are grateful to all of the participants involved in the Singapore Cohort Study for Risk Factors in Myopia study cohort.

6. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D.
Orbital Smooth Muscle Tumor Associated With Epstein-Barr Virus in a Human Immunodeficiency Virus–Positive Patient

Immunodeficiency has been recognized as a risk factor for the development of orbital and adnexal tumors such as Kaposi sarcoma and non-Hodgkin lymphoma. Smooth muscle tumor (SMT) associated with Epstein-Barr virus (EBV) (SMT-EBV) is a rare entity that may be encountered in human immunodeficiency virus (HIV)–infected patients and organ transplant recipients. We describe an HIV-positive man who was diagnosed with an orbital SMT-EBV after presenting with a progressive retrobulbar mass.

Report of a Case. A 43-year-old HIV-positive man (CD4 lymphocyte count, 175/mL) was referred to the orbit service with progressive left proptosis and vision loss. On magnetic resonance imaging, there was a fusiform orbital mass surrounding the posterior portion of the optic nerve (Figure 1); the diameter of the mass had enlarged from 14 mm to 25 mm over a 9-month period on serial scans. The patient had visual acuity of no light perception, 2 mm of proptosis, and left optic disc edema. During orbital biopsy, the deep intracanal mass was noted to be a firm, whitish tumor. Permanent sections revealed the mass to be a proliferation of bland spindle cells with low mitotic activity (Figure 2A) and a Ki-67 labeling index of 18%. Immunohistochemistry results were negative for S-100 protein, desmin, epithelial membrane antigen, and CD34, but stains were positive for smooth muscle actin (Figure 2B). In situ hybridization study results were strongly positive for EBV (Figure 2C). The final pathologic diagnosis was SMT-EBV.

Comment. To our knowledge, this is the first case of a primary orbital SMT-EBV reported in the literature. Our patient had documented HIV but no previously identified SMTs elsewhere in the body. Although a rare entity, cases of SMT-EBV have been reported in HIV-positive patients, organ transplant recipients, and other immune-compromised individuals.1-4 Typically SMT-EBV is a multifocal disease, with tumors arising in the abdominal cavity, adrenal glands, liver, and epidura of the brain and spinal cord.1-4 A case series of 19 patients with SMT-EBV reported by Suankratay et al1 listed 1 patient with an orbital mass, although details were limited and the patient also had tumors in the spinal cord, vocal cord, and adrenal glands. There is no reliable treatment currently available for SMT-EBV; chemotherapy and radiotherapy are thought to be ineffective, and recurrences following surgical excision are common.2 A fusiform orbital mass in an HIV-positive patient that demonstrates progressive enlargement may be due to a rare entity: SMT-EBV.

Jonathan W. Kim, MD
Diana K. Lee, BA
Martin Fishman, MD

Figure 1. Sagittal T1-weighted magnetic resonance image showing a fusiform mass surrounding the optic nerve in the posterior orbit.

Figure 2. Histopathology of the biopsy specimen (original magnification ×128). A, Spindle and oval tumor cells with scant cytoplasm, few mitoses, and moderate pleomorphism (hematoxylin-eosin). B, Smooth muscle actin stain showing diffuse, positive staining. C, In situ hybridization study showing diffuse staining for Epstein-Barr virus by tumor cells.