Supplementary Online Content

Tian T, Chen C, Zhang X, Zhang Q, Zhao P. Clinical and genetic features of familial exudative vitreoretinopathy with only-unilateral abnormalities in a Chinese cohort. *JAMA Ophthalmol.* Published online June 6, 2019. doi:10.1001/jamaophthalmol.2019.1493

eMethods. Criteria, Evaluation, Testing, Analysis, Validation

eFigure. Pedigrees, Chromatograms, and Clinical Presentations of Representative Patients With Entirely Unilateral Familial Exudative Vitreoretinopathy

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Criteria, Evaluation, Testing, Analysis, Validation

The Diagnostic Criteria and Exclusion Criteria

Diagnostic criteria included the following: (1) have at least one of characteristic fundus findings of FEVR in the affected eye as previously described by Trese MT; [9] (2) with normal fellow eye demonstrated by a complete ophthalmologic evaluation and wild-field fluorescein angiography; (3) carrying mutation(s) in at least one of the following FEVR-related genes: *NDP*, *FZD4*, *LRP5*, *TSPAN12*, and *ZNF408*; (4) with a gestational age of more than 38 weeks and without systemic abnormalities. Of note, patients were excluded if they were bilaterally affected or a diagnosis of FEVR could not be confirmed by genetic testing. The patients with a negative family history were not excluded in this study.

The Ophthalmologic Evaluation

The complete ophthalmologic evaluation included visual acuity measurement (if available), anterior segment examination, ultrasound examination, indirect ophthalmoscopy with a 28D lens, fundus examination using Retcam (Clarity Medical Systems, Pleasanton, CA) or Optos 200Tx (Optos, Inc., Marlborough, MA, USA) imaging device, and wide-field fluorescein angiography to the ora serrate using Retcam under anesthesia or Spectralis HRA2 (Heidelberg Engineering GmbH, Germany) on the basis of patients' age. Additionally, wide-field fluorescein angiography was also routinely performed in patients' direct family members, primarily the parents and siblings (if any) who could tolerate fluorescein sodium using Spectralis HRA2 (Heidelberg Engineering GmbH, Germany) examination in the clinic when available. Optos imaging was performed in family members who could not tolerate fluorescein sodium. Data collected from charts included gender, gestational age at birth, birth weight, age at presentation, affected eye, family history, referring diagnosis, visual acuity if available, and fundus presentations of the affected eye.

Genetic Testing

Targeted gene capture and next-generation sequencing (NGS)

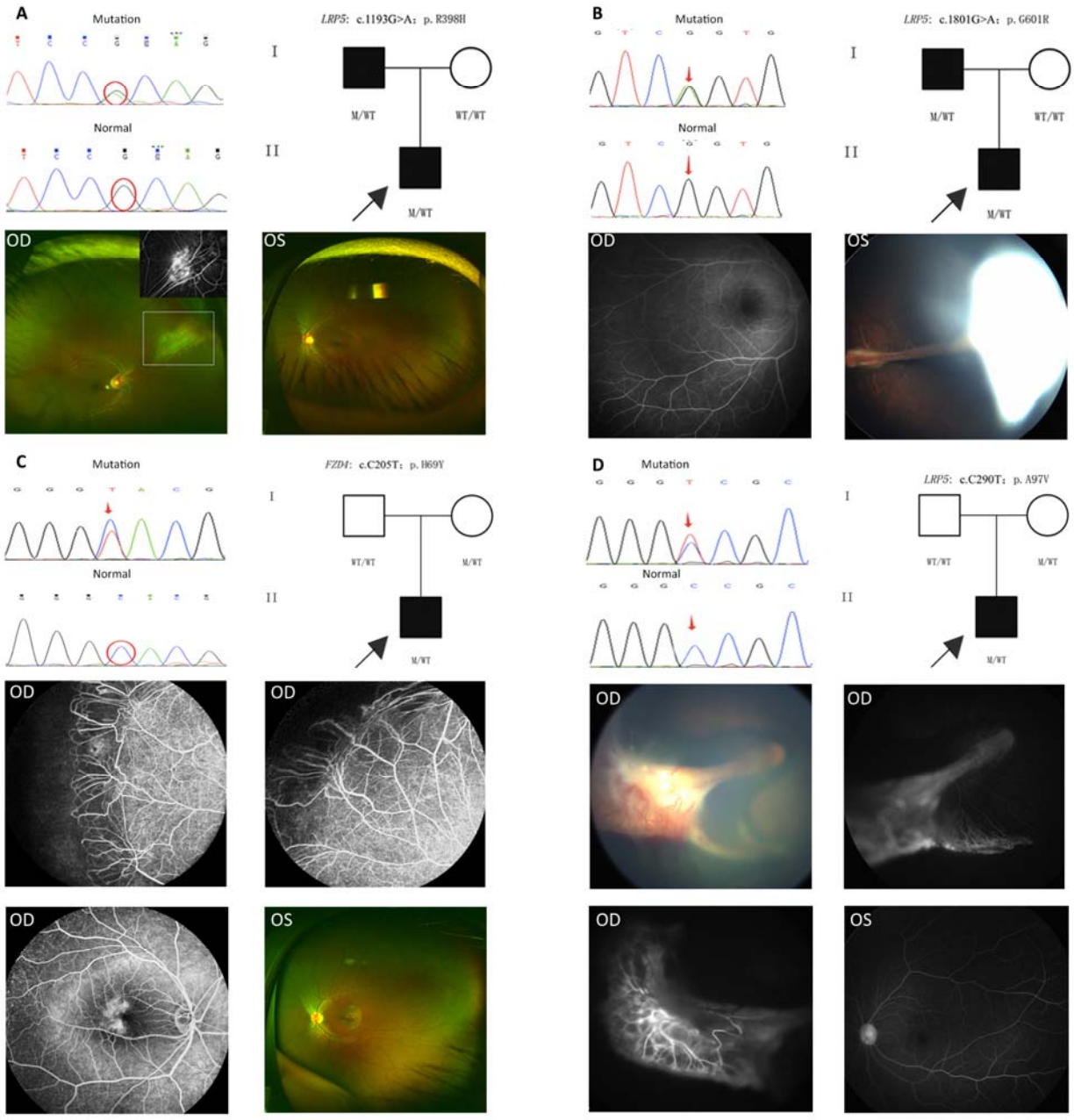
Targeted gene capture and sequencing was performed by MyGenostics (MyGenostics, MD, USA). Briefly, peripheral blood was drawn from each proband and their direct family members, and the genomic DNA was extracted and fragmented. Illumina adapters were added to the fragments, and the samples were size-selected for the 350 - 400 bp products. This pool of DNA fragments was amplified using PCR and allowed to hybridize with DNA capture probes that were specifically designed for the targeted genes. The captured DNA fragments were eluted, amplified again and subjected to NGS using an Illumina HiSeq 2000 (Illumina, Inc., San Diego, CA, USA). A custom Genetic Pediatric Retinal Diseases Panel based on targeted exome capture technology was used and covered the following twenty-one genes: *ABCB6, GDF6, LRP5, RS1, SOX2, TENM3, VSX2, FZD4, IKBKG, NDP, SALL2, STRA6, TSPAN12, YAP1, GDF3, KIF11, PAX6, SHH, TBX1, TUBA8*, and *ZNF408*.

Data Analysis

The sequenced reads were mapped to the UCSC hg19 (http://genome.ucsc.edu) human reference genome using the Burrows Wheeler Aligner (BWA) (http://bio-bwa.sourceforge.net/bwa.shtml). Variants were detected with GATK and further annotated using the 1000 Genomes database, ESP6500, dbSNP and the company's own in-house database of 800 samples. The pathogenicity of each variant was assessed with the following databases: PolyPhen-2 (http://genetics.bwh. harvard.edu/pph2/), Sorting Intolerant From Tolerant (http://sift.jcvi.org /www/SIFT_enst_submit.html), Mutation Taster (http://www.mutationtaster.org/), and GERP+ + (http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html).

PCR and Sanger Sequencing Validation

The resulting amplicons were analyzed with Sanger sequencing to validate the potential pathogenic variants. PCR primers were designed using Primer3. The average amplicon size was 400 bp. The DNA was sequenced on the ABI 3130XL platform and subsequently analyzed using Mutation Surveyor.



eFigure. Pedigrees, Chromatograms, and Clinical Presentations of Representative Patients With Entirely Unilateral Familial Exudative Vitreoretinopathy

In the pedigrees, M sign represents a mutation; WT, a normal allele. In the chromatograms, the variation is marked with red cycle or arrow. OD and OS represent right and left eye, respectively. A, Fundus photo and fluorescein angiography of case 8 show nasal dragging disc and intraretinal exudation (box). The

vascularization of the left eye is normal. B, Fundus photo of case 1 shows a falciform retinal fold dragging from disc to the temporal aspects of lens with aberrant retinal vascular pattern in the left eye. No abnormal vascularization of the right eye is observed in fluorescein angiograms. C, Fundus fluorescein angiography image of case 15 shows aberrant peripheral vascular and leakage at the macular in the right eye. The vascularization of left eye is normal. D, Color and fluorescein angiograms of case 11 show retinal fold extending from disc to the inferotemoral aspects of lens in the right eye. Abundant neovascularization is observed on the mass behind the lens. The vascularization of left eye is normal.