The eye is an immunoprivileged site that can harbor viruses for years. Chronic infection of the positive-sense single-stranded rubella virus (RV) is a cause of the Fuchs heterochromic iridocyclitis (FHI) phenotype. Prior reports have noted that intraocular fluid obtained from many cases of FHI demonstrate the presence of antibodies to RV, yet reverse transcription–polymerase chain reaction (RT-PCR) has frequently failed to demonstrate the presence of RV RNA. This discrepancy has been suggested to reflect a limited period during which the virus may be detected or persist in the eye.

Unbiased metagenomic deep sequencing (MDS) is a high-throughput sequencing approach that can identify all genomes present in a clinical sample. Previous studies have demonstrated that MDS of intraocular fluid can detect fungi, parasites, and DNA and RNA viruses in patients with intraocular inflammation. We present a case series of patients with rubella-associated uveitis diagnosed with MDS and assess the utility of MDS in identifying these infections.

### Methods

This case series included 6 patients referred to the Francis I. Proctor Foundation, University of California, San Francisco (UCSF) for evaluation of recurrent or chronic hypertensive nongranulomatous anterior uveitis or hypertensive intermediate uveitis with concern for vitreal lymphoma (eTable in the Supplement). Ethical clearance was obtained from the institutional review board at UCSF, and the study adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients, and all data were deidentified.
Five patients (83%) were immigrants to the United States, whereas 1 (17%) was born in the United States before the institution of routine RV vaccination. Two patients exhibited anterior uveitis, whereas 4 exhibited anterior-intermediate inflammation. Two patients had no prior topical corticosteroid exposure. Five patients had a history of ocular hypertension, with 4 having diffuse stellate keratic precipitates, 4 having diffuse stellate keratic precipitates. Four eyes from 3 patients exhibited both iris atrophy and iris transillumination defects, 2 patients exhibited iris heterochromia, and 1 patient had no iris defects compared with the contralateral unaffected eye. All patients maintained visual acuity, ranging from 20/20 to 20/60. Two patients had fluorescein angiography performed without vascular leakage or staining of the disc (eFigure in the Supplement).

Three patients received confocal microscopic imaging in our clinic. We found that the affected eyes exhibited spotlike holes, increased intercellular spaces, and infiltration of endothelial cells (Figure 1). In addition, all affected eyes exhibited features of polymorphism and polymegathism compared with the contralateral eye (Figure 1D). Two patients also exhibited stellate keratic precipitates on confocal microscopy, and 1 patient exhibited spotlike holes and endothelial infiltration in the unaffected eye. Similar findings have previously been described for herpes simplex virus-associated endothelial involvement.11

All 6 patients had anterior chamber paracenteses performed at the Proctor Foundation, where fluid was submitted for targeted herpes simplex virus 1 and 2, varicella-zoster virus, and cytomegalovirus PCRs, with residual fluid subjected to MDS.8

Results

All samples from the 6 white male patients (age range, 36-61 years) tested negative for herpes simplex virus 1 and 2, varicella-zoster virus, and cytomegalovirus by PCR, but all tested positive for RV RNA by MDS. Figure 2 shows the heterogeneous capture of the RV genome regions among samples, reflecting the unbiased nature of the assay. The MDS coverage of the RV genome for patient 6 is described elsewhere.8 Genes in the nonstructural coding sequence were detected for all 6 samples, whereas only 3 samples tested positive for genes in the structural coding sequence. Orthogonal testing using RT-PCR for the RV E1 gene was performed on 2 samples. Only the sample that yielded the highest number of reads on MDS tested positive on RT-PCR with a low cycling threshold of 38, demonstrating the potential utility of MDS in suspected cases of FHI.8

Discussion

The World Health Organization declared RV elimination in the Americas in 2015 as the result of effective vaccination policies.12

Because humans are the only host for RV, the prevalence of FHI in the United States was reduced substantially after the introduction of RV vaccination.13 Rubella infection, however, remains a threat throughout other parts of the world. Five of 6 patients in this study emigrated from regions where the vaccination policies were not strictly enforced. From a clinical standpoint, asking patients who live outside the United States about their immunization status or checking RV IgG levels is of little help in the workup for FHI because all immigrants are required to have the mumps-measles-rubella vaccine before entry. Furthermore, few patients will remember if they were exposed because only 2 of

Key Points

Question What are the ocular findings of patients with the rubella viral genome detected with metagenomic deep sequencing?

Findings In this case series study, 6 patients with detectable rubella virus RNA in the intraocular compartment exhibited typical and atypical characteristics of Fuchs heterochromic iridocyclitis. Confocal imaging of the cornea revealed endothelial cell alterations in the affected eyes.

Meanings These findings suggest that patients with persistent intraocular rubella virus infection can present with heterogeneous clinical findings, including endothelial cell damage.

Figure 1. Confocal Images From the Patient Cohort

- A: Affected right eye of patient 3
- B: Unaffected left eye of patient 3
- C: Unaffected right eye of patient 5
- D: Affected left eye of patient 5

Confocal images of the affected right eye (A) and unaffected left eye (B) of patient 3 showing infiltration of endothelial cells with endothelial infiltration (blue arrowhead) and spotlike holes (yellow arrowhead). Confocal images of unaffected right eye (C) and affected left eye (D) of patient 5 showing polymegathism and polymorphism as well as infiltration of endothelial cells (blue arrowhead).

- A
- B
- C
- D

Key Points

Question What are the ocular findings of patients with the rubella viral genome detected with metagenomic deep sequencing?

Findings In this case series study, 6 patients with detectable rubella virus RNA in the intraocular compartment exhibited typical and atypical characteristics of Fuchs heterochromic iridocyclitis. Confocal imaging of the cornea revealed endothelial cell alterations in the affected eyes.

Meanings These findings suggest that patients with persistent intraocular rubella virus infection can present with heterogeneous clinical findings, including endothelial cell damage.
The patients in our study recalled having German measles as children. Although the theoretical risk of transmission exists because the patient’s RV strain in the eye can be genetically different than that of the vaccine strain,\(^8\) in the absence of intraocular surgery or globe trauma, the functional risk is likely minimal. In the 3 patients who underwent RT-PCR testing for RV RNA in the tears, nasopharynx, and urine, all samples tested negative for viral genome, indicating that the virus was exclusively localized to the eye.

Molecular diagnostics for intraocular rubella infections are not routinely available in the United States. The Goldmann-Witmer coefficient assay (requiring both intraocular fluid and a serum sample) and the RT-PCR for RV, which are available in Europe, can be limited in scope.\(^3,7,14\) The RV-directed RT-PCR usually targets a small region (approximately 185-739 base pairs [bp]) of the \(E1\) gene, which is a small fraction of the 9762 bp of the entire RV genome.\(^8,15\) However, MDS is an unbiased approach that has the potential to detect any genome region of a particular pathogen in a clinical sample (Figure 2). Furthermore, the unbiased nature of the assay allows for the detection of both common and rare pathogens in minute amounts of intraocular fluid (approximately 20-50 μL) without requiring a priori knowledge, as is the case with pathogen-directed PCR.\(^8\) In contrast to prior work that used RT-PCR,\(^3\) this study found that regardless of age, the RV genome can be detected in patients with FHI using MDS. Our findings also demonstrated that anterior chamber paracentesis for RV testing by MDS may be sufficient even if the inflammation is localized mainly in the vitreous cavity of phakic patients.

A major limitation of this study is that the population studied is from a referred group of patients with previously undifferentiated anterior and intermediate uveitis. In such cases, other causes, including syphilis and tuberculosis, must be ruled out. All of the patients had negative treponemal antibody and interferon gamma release assay test results and normal findings on chest radiography.

Conclusions

In summary, these findings from MDS suggest that inflammation in patients with FHI (in the classic FHI phenotype or anterior-intermediate uveitis) is stimulated by the persistent presence of RV in the eye and that these affected eyes exhibit evidence of corneal endothelial cell damage previously not appreciated without confocal imaging. As other studies\(^14,16\) have demonstrated, patients with RV uveitis can maintain usable visual acuity.
Diagnosis in Western medicine is based on grouped classification of symptoms, signs, and test results to determine treatment and prognosis. Some conditions are known to have a specific underlying cause, such as infection or genetic mutation, but many labeled diseases are simply naming conventions for a set of correlated signs and symptoms without a known underlying cause.

Moving from syndromic description to etiologic diagnosis is a holy grail in the practice of medicine. Understanding disease pathogenesis and etiology is the basis for development of treatments or cure. In the modern era of uveitis, we have accepted that approximately 50% of uveitis is idiopathic. When uveitis is associated with a presumed noninfectious systemic condition, such as sarcoidosis, Behçet disease, or tubulointerstitial nephritis, the temptation may be to claim to have found a cause, but we must concede that these systemic diseases are also idiopathic conditions. Progress in understanding the role of microbes in the pathogenesis of human disease best exemplifies the successes of the transition from syndromic to etiologic diagnosis. For example, Marshall and Warren \(^1\) won the Nobel Prize in Physiology or Medicine in 2005 for their discovery of the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease, conditions previously attributed to stress or lifestyle.

Fuchs heterochromic iridocyclitis (FHI), also known as Fuchs uveitis syndrome, is a chronic, minimally symptomatic, typically unilateral anterior and intermediate uveitis marked by diffuse small or stellate white keratic precipitates, anterior iris atrophy or heterochromia, and association with cataract and glaucoma. In 2004, Quentin et al \(^2\) were the first of several investigators \(^3\) to report an etiopathologic link between FHI and rubella virus (RV), detecting elevated levels of rubella-specific antibodies in the aqueous samples of patients with FHI compared with control individuals and sometimes finding RV RNA by directed polymerase chain reaction. These studies suggest that perhaps the holy grail has been found for FHI.

**REFERENCES**

1. Varkey JB, Shantha JG, Crozier I, et al. Persistence of ebola virus in ocular fluid during convalescence. *N Engl J Med*. 2015;372(25):2423-2427. doi:10.1056/NEJMoai1503006
2. Fuchs E. Ueber komplikationen der heterochromie. *Z Augenheilkd*. 1906;15:191-212.
3. Quentin CD, Reiber H. Fuchs heterochromic cyclitis: rubella virus antibodies and genome in aqueous humor. *Am J Ophthalmol*. 2004;138(1): 46-54. doi:10.1016/j.ajo.2004.02.055
4. de Groot-Mijnes JD, de Visser L, Rothova A, Schueller M, van Loon AM, Weersink AJ. Rubella virus is associated with fuchs heterochromic iridocyclitis. *Am J Ophthalmol*. 2006;141(1):212-214. doi:10.1016/j.ajo.2005.07.078
5. Abernathy E, Pears RR, Chen MH, Icenogle J, Namdari H. Genomic characterization of a persistent rubella virus from a case of Fuchs’ uveitis syndrome in a 73 year old man. *J Clin Virol*. 2015;69:104-109. doi:10.1016/j.jcv.2015.06.084
6. ten Berge JC, van Daele PL, Rothova A. Rubella virus-associated anterior uveitis in a vaccinated patient: a case report. *Ocul Immunol Inflamm*. 2016;24(1):113-114. doi:10.3109/09273948.2014.925126
7. Liu Y, Takasagawa HL, Chen TC, Pasquale LR. Fuchs heterochromic iridocyclitis and the rubella virus. *Int Ophthalmol Clin*. 2011;51(4):1-12. doi:10.1080/09273948.2011.613655
8. Doan T, Wilson MR, Crawford ED, et al. Illuminating uveitis: metagenomic deep sequencing identifies common and rare pathogens. *Genome Med*. 2016;8(1):90. doi:10.1186/s13073-016-0344-6
9. Gonzales J, Doan T, Shantha JG, et al. Metagenomic deep sequencing of aqueous fluid detects intraocular lymphomas. *Br J Ophthalmol*. 2018;102(1):6-8. doi:10.1136/bjophthalmol-2017-311151
10. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191-2194. doi:10.1001/jama.2013.281053
11. Hillenstaar T, Weenen C, Wubbejs RJ, Remeijer L. Endothelial involvement in herpes simplex virus keratitis: an in vivo confocal microscopy study. *Ophthalmology*. 2009;116(11):2077-2086, e2071-2072.
12. Pan American Health Organization. Americas Region is Declared the World’s First to Eliminate Rubella. 2015. http://www.paho.org/US/index.php?option=com_content&view=article&id=135%3AAmericas-region-free-of-rubella&Itemid=130&lang=en. Accessed March 30, 2016.
13. Birbaum AD, Tessler HH, Schultz KL, et al. Epidemiologic relationship between Fuchs heterochromic iridocyclitis and the United States rubella vaccination program. *Am J Ophthalmol*. 2007;144(3):424-428. doi:10.1016/j.ajo.2007.05.026
14. de Visser L, Braakenburg A, Rothova A, de Boer JH. Rubella virus-associated uveitis: clinical manifestations and visual prognosis. *Am J Ophthalmol*. 2008;146(2):292-297. doi:10.1016/j.ajo.2008.04.011
15. Zhu Z, Xu W, Abernathy ES, et al. Comparison of four methods using throat swabs to confirm rubella virus infection. *J Clin Microbiol*. 2007;45(9): 2847-2852. doi:10.1128/JCM.00289-07
16. Wensing B, Relvas LM, Caspers LE, et al. Comparison of rubella virus- and herpes virus-associated anterior uveitis: clinical manifestations and visual prognosis. *Ophthalmology*. 2011;118(10):1905-1910. doi:10.1016/j.joso.2011.03.033