

Pathogen profiling of inflammatory eye diseases with metagenomic deep sequencing in a tertiary referral center

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Research Article

Keywords: metagenomic deep sequencing, uveitis, posterior uveitis, infectious uveitis, endophthalmitis, panuveitis

Posted Date: October 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11107341/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background

Intraocular inflammation is a leading cause of blindness globally, but the precise etiology may be unknown in many cases due to challenges with traditional diagnostic modalities. Metagenomic deep sequencing (MDS) is an unbiased method for pathogen detection and may provide advantages over conventional diagnostic methods.

Methods

Patients with possible infectious eye diseases were enrolled at the Emory Eye Center from January 2018 to March 2021 for MDS analysis of intraocular fluid samples in addition to standard-of-care conventional testing, including polymerase chain reaction and bacterial and fungal cultures. Samples were sent to the Proctor Foundation at the University of California, San Francisco for analysis. Pathogens were identified through a rapid computational pipeline that analyzed non-host sequences. Data were collected on patient demographics, medical history, ophthalmic exam, and conventional diagnostic results and summarized with respect to MDS findings.

Results

Forty-one patients were included in this study with 25 females (61.0%) and a mean age of 55.3 years (SD:±16). The anatomic distribution of uveitis included anterior uveitis in 13 patients (31.7%), anterior/intermediate uveitis in 7 patients (17.1%), posterior uveitis in 4 patients (9.76%), panuveitis in 15 patients (36.6%), and scleritis in 2 patients (4.9%). Intraocular fluid was collected from 25 aqueous humor samples (61.0%) and 16 vitreous samples (39.0%). MDS identified pathogens in 11 of 41 samples (26.8%). The MDS positivity results by the site of sampling were 20.0% (5/25) from the anterior chamber and 37.5% (6/16) from the vitreous cavity. Pathogens identified included bacterial species such as *Klebsiella pneumoniae*, *Serratia marcescens*, and *Streptococcus pneumoniae*; viral pathogens including Herpes Simplex Virus (HSV) and Varicella Zoster Virus (VZV); fungal organisms such as *Candida parapsilosis* and *Cladosporium* species; and parasites including *Toxocara canis*. Seven patients had an infectious etiology determined by MDS that was not detected by conventional diagnostics. Intraocular and systemic testing was concordant with MDS results in 4 patients.

Conclusion

Infectious organisms associated with inflammatory eye diseases presenting to a tertiary referral center are diverse and include fungi, bacteria, parasites, and viruses. The unbiased nature of MDS may be advantageous in these tertiary settings, assisting in diagnostics and guiding appropriate therapies.

Background

Inflammatory eye diseases are a leading cause of ocular morbidity and blindness globally, contributing up to 15% of blindness in developed countries and up to 25% in developing countries.^{1–3} However, the etiology of uveitis is unknown in several cases.⁴ Infectious etiologies are estimated to account for 15–20% in the United States⁵ and up to 50% in the developing world.⁶ Additionally, the distinction between infectious and inflammatory etiologies based on clinical features can be challenging, as inflammatory findings may be similar in both etiology categories. Currently, more than 50% of presumed intraocular infections (endophthalmitis) fail to have a pathogen identified using standard laboratory testing modalities.⁷ Challenges associated with traditional testing modalities for infectious uveitis include limited ocular fluid sample volume, a limited testing array with singleplex polymerase chain reaction (PCR), and poor sensitivity with culture, particularly for atypical organisms.⁸ The most readily available molecular diagnostic tests for ocular infections include a limited number of pathogen-directed PCRs, namely for cytomegalovirus (CMV), HSV, VZV, and *Toxoplasma gondii*.^{9,10}

Metagenomic deep sequencing (MDS) is a next-generation sequencing method that analyzes the full range of genomes within biological samples and enables comparison of sequenced libraries with pathogens whose reference sequences are available. This unbiased testing approach enables broad identification of known and unexpected pathogens and has previously shown concordant results with pathogen-directed PCRs, despite known challenges with sample volumes and handling conditions.^{11,12} The potential for MDS to refine the current diagnostic armamentarium and to improve characterization of the etiology of infectious inflammatory eye diseases prompted us to utilize this unbiased approach to assess presumptive infectious inflammatory eye diseases at a tertiary, university-based, referral center in the southeastern United States.

Methods

Study Design

We conducted a prospective study in accordance with the guidelines outlined by the Declaration of Helsinki as revised in 2013 and its later amendments, and this study was approved by the Institutional Review Board at Emory University (IRB00107236). Written informed consents were obtained from all participants. Inclusion criteria included those patients with intraocular inflammation and where a suspected infectious etiology was a consideration requiring sampling of the anterior chamber or vitreous cavity. Exclusion criteria included patients less than 18 years of age or unable to complete the informed consent.

Sample Collection and Processing

Patients underwent standard-of-care workup for uveitis or endophthalmitis as determined by the treating physician. Testing included serologic, blood, and/or radiologic testing, as deemed appropriate. Anterior chamber fluid or vitreous fluid from one eye was collected by a uveitis and/or retina-fellowship trained ophthalmologist for laboratory testing, and residual fluid was saved for MDS. Testing of intraocular pathogens included qualitative PCR for HSV-1/HSV-2, VZV, CMV and *Toxoplasma gondii*, and bacterial and fungal cultures. Intraocular fluid samples for MDS were stored at -80 degrees Celsius, and deidentified samples were sent to the Ralph and Sophia Heintz Laboratory at the Proctor Foundation at the University of California, San Francisco for deep sequencing analysis.

Samples underwent MDS using methods previously described.¹² Briefly, extracted RNA was prepared for sequencing libraries using the NEBNext Ultra II RNA Library Prep Kit per manufacturer's recommendations. Libraries were pooled and sequenced on the Novaseq X (Illumina) using 150-nucleotide paired-end sequencing. Human reads were removed from analysis, and the remaining non-host reads were subjected to taxonomic classification using the Centrifuge Taxonomic Classifier engine using an index created from the National Center for Biotechnology Information nucleotide non-redundant sequences.¹³

Data collected included demographic data, clinical characteristics including immune competency status, anatomical location of the uveitis according to the Standardization of Uveitis Nomenclature Working Group¹⁴, ophthalmic examination including baseline best corrected visual acuity (BCVA), clinical diagnosis, laboratory diagnostic testing results, and MDS results. Descriptive analyses were conducted to evaluate the frequency of pathogens and results from different sampling sites. Categorical variables were presented as percentages and/or frequencies, while continuous variables were depicted as means, ranges, or standard deviations.

Results

Baseline Characteristics

Forty-one patients were enrolled from January 2018 through March 2021. The cohort consisted of 61.0% females with a mean age of 55.3 years (SD $\pm$ 16.0) (Table 1). There were 8 patients who were immunocompromised, one of whom had an HIV infection. Of the other immunocompromised patients, 3 were being treated with chemotherapy for their associated cancer diagnoses, which included non-small cell lung cancer, multiple myeloma, low grade B-cell lymphoma. Two patients were being treated with systemic immunomodulatory therapy for underlying autoimmune conditions (rheumatoid arthritis, spondyloarthritis), and two patients were on systemic immunomodulatory therapy for chronic scleritis and uveitis. The majority of the patients had panuveitis (n = 15, 36.6%), which included acute retinal necrosis (n = 3, 7.3%) and endophthalmitis (n = 6, 14.6%), followed by anterior uveitis (n = 13, 31.7%), anterior-intermediate uveitis (n = 7, 17.1%), posterior uveitis (n = 4, 9.8%), and scleritis (n = 2, 4.9%). The predominant site of intraocular fluid sampling was the anterior chamber (n = 25, 61.0%) followed by the vitreous (n = 16, 39.0%) (Table 1).

Table 1
Baseline demographics and cohort characteristics

Patient-level baseline characteristics Total n (percent %)	
Patients	41
Age, mean +/- standard deviation (SD)	55.3 +/-16.0
Sex	
Female	25 (61.0%)
Male	16 (39.0%)
Race	
White or Caucasian	19 (46.3%)
Black or African American	15 (36.6%)
Hispanic	2 (4.9%)
Asian	1 (2.4%)
Other	4 (9.8%)
Immunocompromised	
Human immunodeficiency virus	1 (2.4%)
Other ¹	7 (17.1%)
Eye-level baseline characteristics Total n (percent %)	
Involved Eye	
Right eye	25 (61.0%)
Left eye	16 (39.0%)
Ocular fluid sample	
Aqueous humor aspirate	25 (61.0%)
Vitreous (Vitreotomy specimen)	14 (34.1%)
Vitreous aspirate (Vitreous tap)	2 (4.8%)
Anatomic Location of Uveitis	
Anterior	13 (31.7%)
Anterior/Intermediate	7 (17.1%)
¹ Immunocompromised from chemotherapy associated with malignancy and/or immunosuppressive agents for autoimmune or chronic uveitis	

Patient-level baseline characteristics Total n (percent %)	
Posterior	4 (9.8%)
Panuveitis	15 (36.6%)
<i>Panuveitis Syndromes</i>	
Acute retinal necrosis	3 (7.3%)
Endophthalmitis	6 (14.6%)
Scleritis	2 (4.9%)
Visual acuity in logMAR (mean +/- SD)	0.74 +/- 0.79
Anterior chamber cell grade	
0	11 (26.8%)
0.5+	3 (7.3%)
1 +	9 (22.0%)
2+	5 (12.2%)
3+	5 (12.2%)
4+	8 (19.5%)
Hypopyon	7 (17.1%)
Anterior chamber flare	
0	34 (82.9%)
1+	5 (12.2%)
2+	0
3+	1 (2.4%)
4+	0
Vitreous Haze	
0	15 (36.6%)
0.5+	4 (9.8%)
1+	6 (14.6%)
2+	5 (12.2%)
¹ Immunocompromised from chemotherapy associated with malignancy and/or immunosuppressive agents for autoimmune or chronic uveitis	

Patient-level baseline characteristics Total n (percent %)	
3+	8 (19.5%)
4+	3 (7.3%)
Other Characteristics	Total n (percent %)
Retinitis	16 (39.0%)
Cystoid macular edema	8 (19.5%)
Ocular hypertension	16 (39.0%)
¹ Immunocompromised from chemotherapy associated with malignancy and/or immunosuppressive agents for autoimmune or chronic uveitis	

Conventional Diagnostic Results

PCR testing was performed on most patients with 35 undergoing HSV and *Toxoplasma gondii* testing and 34 undergoing VZV and CMV testing. The HSV and *Toxoplasma gondii* PCR was negative in all patients. The CMV PCR was positive in one patient (2.9%), and the VZV PCR was positive in two patients (5.9%). Bacterial and fungal cultures were performed on 18 patients, including the six with suspected endophthalmitis. Bacterial cultures were negative in all patients. The fungal culture was positive in one patient (5.6%), which showed *Candida parapsilosis*. In the three patients with acute retinal necrosis, PCR testing was positive for VZV in two patients and negative for all organisms tested in the remaining patient.

Deep Sequencing Results

Twelve pathogens were identified by MDS from 11 patients (26.8%). Of the 25 aqueous humor samples, 5 (20%) were positive on MDS, while 6 (37.5%) of the 16 vitreous samples were positive (Table 2). Pathogen detection by sampling site is summarized in **Supplemental Table 1**. Deep sequencing identified diverse classes of microorganisms as the etiology of the intraocular inflammation (**Table 3**, Fig. 1). Bacteria were detected in two patients, with two bacterial organisms noted in one patient. Viruses were detected in four aqueous samples and two vitreous fluid samples. Fungi were detected in one aqueous and one vitreous sample.

Table 2
Deep sequencing result by ocular fluid sample

Procedural Sampling Site	MDS Positive (per eye)	MDS Negative (per eye)
Aqueous humor aspirate, n (%)	5 (20%)	20 (80%)
Vitreous humor aspirate, n (%)	6 (37.5%)	10 (62.5%)

Table 3: Comparison of deep sequencing positive results with orthogonal ocular and systemic testing

Classification	Deep Sequencing	Ocular Fluid Testing**	Systemic Testing***
Bacteria	Klebsiella pneumoniae*	Negative	
	Serratia marcescens*	Negative	
	Streptococcus pneumoniae	Negative	
Virus	Human retrovirus pathognomonic for multiple sclerosis	Negative	MRI brain and orbit unremarkable
	VZV (Patient 1)	Negative	
	VZV (Patient 2)	Positive VZV PCR	
	HSV-2	Negative	
	HTLV-1	Negative	HTLV-1 serum ELISA positive
	Rubella	Negative	
Fungi	Cladosporium	Negative	
	Candida parapsilosis	Fungal culture Candida parapsilosis	
Parasite	Toxocara canis	Negative	Toxocara antibody positive

Abbreviations: VZV – Varicella zoster virus; HSV-2 – Herpes simplex virus-2; HTLV-1 – human T-lymphotropic virus-1, polymerase chain reaction (PCR)

* Both positive from one sample

**Orthogonal testing: done based on clinical suspicion

***Systemic testing: when clinically relevant

Metagenomic sequencing compared with standard-of-care testing

Seven of the eleven patients (63.6%) had a pathogen identified by MDS that was negative by orthogonal testing (**Table 3**). Intraocular and systemic testing confirmed the etiology and was concordant with MDS results in four patients (36.4%). Two patients had concordant deep sequencing results with intraocular fluid testing: VZV by PCR of aqueous fluid and *Candida parapsilosis* from vitreous fungal culture. Two patients had MDS results concordant with systemic testing, including *Toxocara canis* antibody testing and human T-lymphotropic virus-1 by enzyme-linked immunosorbent assay.

Select Case Descriptions

A 59-year-old female with a history of hearing loss was referred for episodes of recurrent bilateral anterior uveitis for 10 years with a reported negative work-up. The patient's BCVA at her initial evaluation was 20/40 in the right eye (OD) and 20/20 in the left eye (OS) with intraocular pressures of 10 mmHg OD and 13 mmHg OS. Slit lamp examination showed inferior keratic precipitates and trace anterior chamber (AC) cell OD. Additionally, the patient had iris heterochromia (**Figure 2**). MDS testing showed rubella virus RNA, suggesting its contribution to the patient's disease manifestations.

An 18-year-old female from rural Florida was referred for panuveitis that was refractory to oral and topical corticosteroids and oral sulfamethoxazole/trimethoprim 800 mg/160 mg (Bactrim-DS) for five months. On initial evaluation, her BCVA was 20/20 OD and count fingers at 1 foot OS. Her anterior exam showed trace AC cell OD and 2+ AC cell with 1+ flare OS. Her dilated fundus examination of the left eye showed 4+ vitritis with a superonasal area of retinal whitening (**Figure 3**). Pars plana vitrectomy OS was performed with samples sent for PCR with VZV, HSV, toxoplasmosis, CMV, bacterial and fungal cultures, and MDS. PCR testing and culture were negative. MDS results were positive for *Toxocara*, and serology testing was subsequently performed, which was also positive for *Toxocara* antibody. The patient was referred to the Infectious Disease service and treated with albendazole, which led to resolution of the inflammation.

A 73-year-old female presented with signs of a chronic endophthalmitis OS four months after cataract surgery. She had previously received an intravitreal vancomycin injection with some improvement but had subsequent worsening of vision and symptoms. She also underwent a pars plana vitrectomy with the referring provider, with reported negative findings. At her initial evaluation, her vision was 20/50 OS with 4+ AC cell, 1+ flare, 0.5 mm hypopyon, and clumps of white material on the posterior chamber intraocular lens (PCIOL). Dilated fundus examination showed 2+ vitreous haze and vitreous opacities inferiorly. A repeat vitrectomy was performed with injections of vancomycin and ceftazidime. Fungal culture of the vitreous and MDS results were positive for *Candida parapsilosis*. Based on these results, PPV with IOL explant with voriconazole injection was performed with improvement in the patient's symptoms and resolution of inflammation.

Discussion

In this report, we observed that 11 patients (26.8%) showed positive findings by MDS. Pathogens were detected from both the anterior chamber and vitreous cavity. Of the 12 positive MDS pathogens detected, the majority were viral (50%, n = 6), followed by bacterial (25%), fungal (16.7%), and parasitic (8.3%). As demonstrated by the wide breadth of pathogens noted on deep sequencing, MDS has an advantage in detecting a range of organisms that otherwise may not be detected by singleplex PCR and/or cultures. Additionally, in patients with organisms that may be difficult to culture including fungi or fastidious organisms, MDS provides an opportunity to detect pathogens through the highly sensitive detection technique that does not rely on culture and its associated limitations.

In this study, seven patients who underwent MDS had pathogens detected that were not otherwise detected by orthogonal testing. Pathogens detected by MDS that were missed by orthogonal testing include bacteria, viruses, parasites, and fungal organisms. In cases where MDS was concordant with orthogonal testing (i.e., VZV uveitis), the findings were supportive of the diagnosis and provided additional confirmation of the presumed infectious etiology. Viruses that have been increasingly recognized as causes of infectious uveitis include rubella virus and HTLV-1.¹⁵ Detection of rubella by reverse transcriptase (RT)-PCR has been reported to be positive in only a minority of cases, due to the chronic, low-grade infection typically seen in patients.¹⁶ The assay, however, is not typically assessable to most tertiary centers in the United States. Organisms like HTLV-1 have previously been difficult to diagnose, as they can masquerade as other etiologies and often are a diagnosis of exclusion. Similarly, ocular toxocariasis is often mistaken for idiopathic uveitis, as patients may have low parasite levels, which may go undetected on testing.¹⁷ A thorough history and high clinical suspicion are needed to aid in diagnosis. Accurate diagnosis of the infectious etiology of intraocular inflammatory diseases is critical to management.

A recent multi-center, randomized controlled trial, the Ocular Pathogen and Transcriptome Investigation Using Comprehensive Sequencing (OPTICS)¹⁸ study, was conducted to assess the impact of MDS on the clinical outcomes of patients with suspected infectious inflammatory eye diseases. Patients were randomized for their physicians to receive or not receive MDS results following completion of testing. The trial results showed modest clinical improvements in a subset of patients who received the MDS results compared to patients in whom physicians did not have access to MDS results. Notably, the majority of patients in OPTICS did not require MDS for appropriate disease management, although the MDS results increased confidence by the treating physician of the leading diagnosis. Larger studies will be needed to fully elucidate the impact of MDS on clinical outcomes in patients with suspected intraocular infection.

This study was limited by its small sample size and absence of a control group. Moreover, selection bias may have influenced enrollment, as the decision of which patients could benefit from MDS analyses may have varied by physicians who referred patients for assessment. In addition, while MDS is unbiased, potential pitfalls include the risk of detecting pathogens that may not be associated with the active disease process.¹² Clinical correlation remains critical, regardless of access to testing.

In summary, our study demonstrates the potential for MDS to detect organisms that may not be captured on currently available systemic and intraocular fluid testing. Besides the identification of known pathogens that are difficult to detect with conventional testing, novel pathogens may also be observed. Further research is ongoing to understand the potential for MDS as a diagnostic or adjunctive tool for pathogen detection in cases of a suspected intraocular infection.

Declarations

Acknowledgements

A. Funding/Support: This work is supported by National Eye Institute/ National Institutes of Health K23 EY030158 (Shantha), NIH P30EY06360 (Core grant to the Emory Eye Center), and NIH EY029594 (Yeh). This research was supported, in part, by the UCSF Vision Core shared resource of the NIH/NEI P30 EY002162, and by an unrestricted grant from Research to Prevent Blindness, New York, NY.

Funding

Support: This work is supported by National Eye Institute/ National Institutes of Health K23 EY030158 (Shantha), NIH P30EY06360 (Core grant to the Emory Eye Center), and NIH EY029594 (Yeh). This research was supported, in part, by the UCSF Vision Core shared resource of the NIH/NEI P30 EY002162, and by an unrestricted grant from Research to Prevent Blindness, New York, NY.

Author Contribution

JT: writing - original draft, writing - review and editing KS: data collection, prepared figures, writing - review and editing CC: writing - review and editing, data analysis LZ: writing - review and editing, data analysis YL: writing - review and editing, data analysis TA: writing - review and editing, data analysis DY: writing - review and editing, data analysis AH: writing - review and editing, data analysis SA: writing - review and editing SY: writing - original draft, writing - review and editing, data analysis, preparing figures and tables JGS: writing - original draft, writing - review and editing, data analysis, preparing figures and tables

Data Availability

The datasets generated and analysed during the current study are not publicly available to protect patient privacy but are available from the corresponding author on reasonable request

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Figures

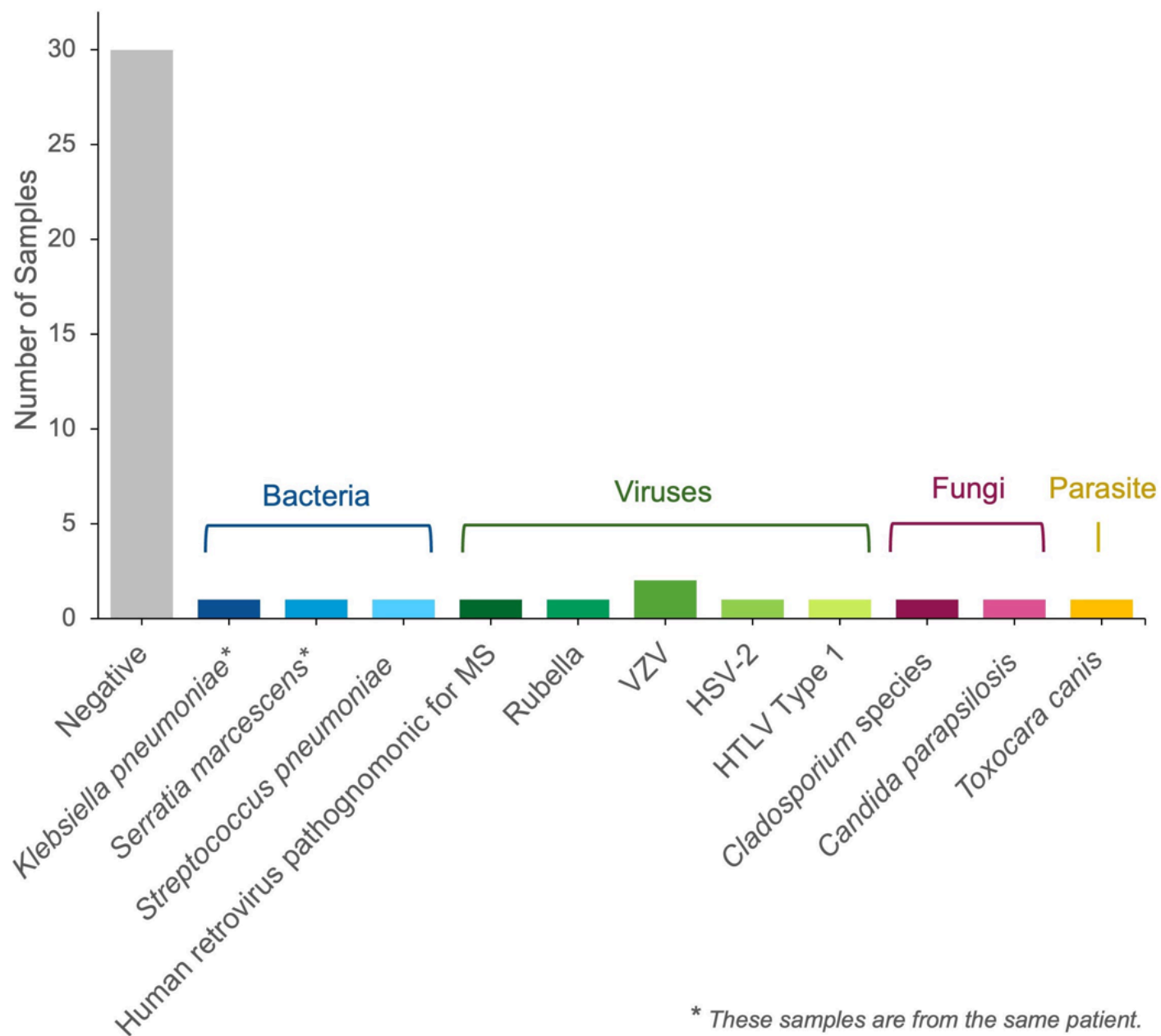


Figure 1

Categories of pathogens detected by metagenomic sequencing in the aqueous and vitreous samples of 41 patients.

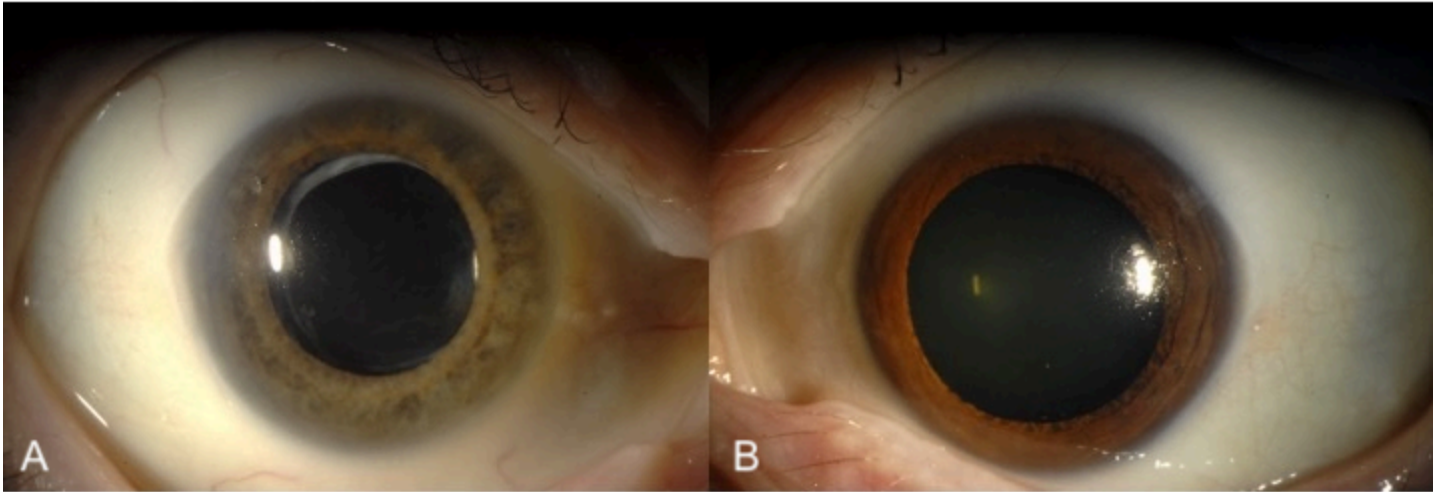


Figure 2

Anterior segment photos demonstrating iris heterochromia (A right eye, B left eye) in a patient with rubella-associated uveitis that was determined by metagenomic sequencing.

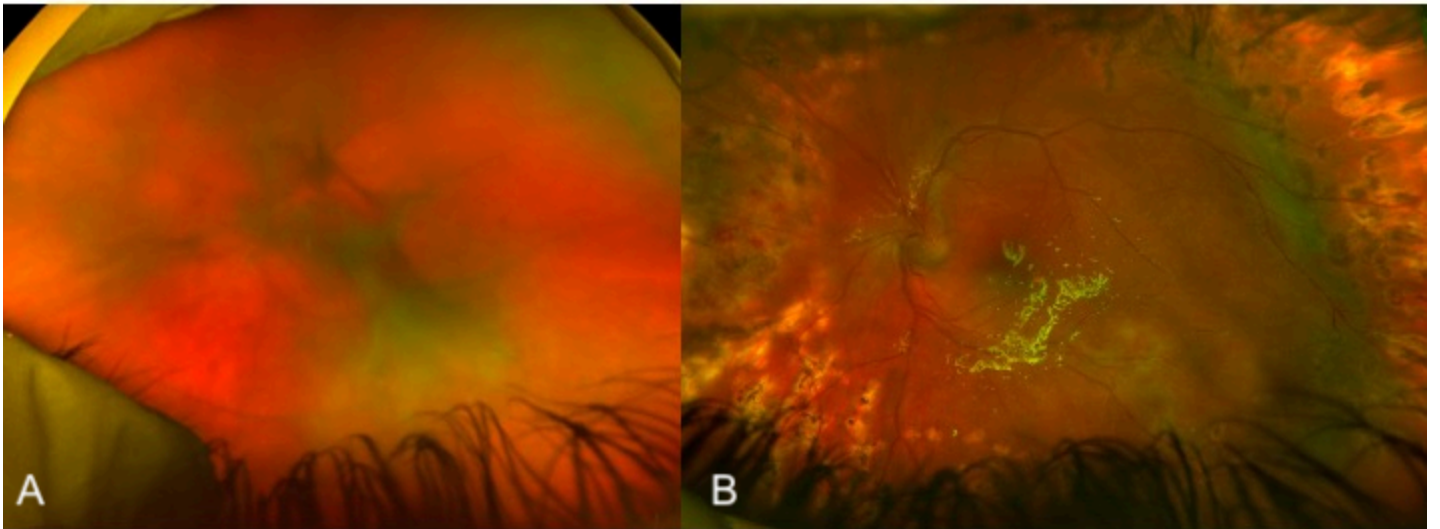


Figure 3

Fundus photos of the left eye of a patient diagnosed with *Toxocara canis* by deep sequencing. Figure A is a color fundus photo of the left eye showing dense vitreous haze. Figure B shows the resolution of inflammation and vitreous haze after para-planar vitrectomy and systemic therapy.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementalTable1.docx](#)