Metagenomic sequencing provides evidence of the nasal microbiota's influence on idiopathic orbital myositis: a case control study

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Research Article

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Abstract

Background: Nasal microbiota is a key factor in the development of chronic rhinosinusitis (CRS). Its influence on orbital autoimmune diseases remains unknown. This study aims to investigate the influence of CRS-related nasal dysbiosis on extraocular muscle (EOM) microbiota in patients with or without idiopathic orbital myositis (IOM).

Methods: Sixteen subjects were recruited, including 4 cases in each study group [IOM(+) CRS(+) group, IOM(−) CRS(+) group, IOM(+) CRS(−) group, IOM(−) CRS(−) group]. The EOM tissues and corresponding incision tissues were sampled and processed with metagenomic next-generation sequencing (mNGS). The nasal swaps from middle meatus were collected and analyzed with 16s rRNA sequencing. The sequencing data were calculated with Shannon index for microbial diversity, principal component analysis for microbial abundance and linear discriminant analysis effect size for dominant species.

Results: A total of 42 species of bacteria, 3 species of fungi and no virus were detected. Both microbial diversity and microbial abundance differed between the EOM and incision samples in IOM(−) CRS(+) and IOM(−) CRS(−) subjects, with *Staphylococcus* predominant in the EOM samples and *Micrococcus luteus* predominant in the incision samples. The microbiota also differed between EOM and swap samples in the IOM(−)CRS(+) subjects, and the most distinguishable species in the swab samples was *Cutibacterium acnes*. Further analysis of the swab samples confirmed nasal dysbiosis in the CRS(+) subjects with *Staphylococcus haemolyticus* representating as the dominant species. The microbiota of EOM samples differed between the IOM(−)CRS(+) and IOM(−)CRS(−) subjects, resulting into preponderance of *Cutibacterium acnes* in the IOM(−)CRS(+) subjects. Small discrepancies of EOM microbiota were detected between the IOM(+)CRS(+) and IOM(+)CRS(−) subjects, but data analysis showed no statistical significance. The above findings demonstrate an alteration of EOM microbiota in CRS patients, providing evidence that nasal dysbiosis may play a role in regulating microbial communities in EOM.

Conclusions: This study provided a reference spectrum of EOM microbiota in IOM patients and offered insights into the nasal microbiota's influence on orbital autoimmune conditions.

(Trial registration: *Chinese Clinical Trial Registry, ChiCTR2100047731*. Registered 24 June 2021 - *Retrospectively registered, https://www.chictr.org.cn/showproj.aspx?proj=127896*)

Background

Orbital myositis, the second most common cause of extraocular muscle (EOM) diseases, is defined as nonspecific inflammation or pathogenic infection of one or more EOMs [1]. Infectious orbital myositis is typically attributed to direct spreading of pathogens from adjacent structures such as the paranasal sinuses. In patients with acute rhinosinusitis, orbital involvment accounts for approximately 95% of cases with complications [2]. Systemic antibiotic therapy, along with surgical removable of pathologic tissues, are considered as effective treatments [3]. Apart from infection, the cases with no known causative

pathogens are referred to as idiopathic orbital myositis (IOM), and the autoimmune system plays a key role during IOM pathogenesis. Even though both infectious and noninfectious cases are characterized with pre-septal inflammation and acute onset of painful diplopia, the IOM cases are distinguishable by a lack of microbial evidence, limited inflammatory edema surrounding the affected EOMs and preponderance of chronic inflammatory cells within muscle fibers [4]. IOM patients generally respond to high dose corticosteroids, immunosuppressive drugs and orbital radiation therapy [1]. However, the recurrent rate was reported as high as 8.0-41.0% in different medical centers [5, 6]. To achieve better treatment outcome requires more targeted therapy and deeper understanding of the disease mechanism.

More and more efforts have been devoted to unveil the immune triggers responsible for the development of idiopathic myositis. Recent findings provided insights into a potential interaction between the immune system and the exposure to microorganism antigens [7]. Notably, some particular IOM cases coexist with chronic rhinosinusitis (CRS), which is a common condition with high prevalence of 5.5-10.0% in general population of Asian countries [8]. Based on clinical experience, several case reports demonstrated that CRS treatment exerted positive effects on the coexisting IOM [9–11]. The role of nasal microbiota on CRS has been deeply explored, and a growing evidence supports the concept that dysfunction of the microbiota has a significant impact on the occurrence of CRS [12]. In general, pathogens of all kinds, including viruses, bacteria and parasites, not only cause direct damage to organs and tissues resulting in infectious diseases, but also interfere with the innate as well as the adaptive immune system leading to autoimmune diseases or even oncogenesis [13, 14]. The ocular surface microbiome is considered as a significant participant in regulating the pathophysiology of ocular surface diseases, such as Stevens-Johnson Syndrome and mucosa-associated lymphoid tissue lymphoma [15, 16]. The microbiome within aqueous humor and vitreous fluid is also closely related to intraocular diseases, including uveitis, retinitis, and vitreal lymphoma [17–19]. It is reasonable to speculate that a causal relationship exists between the nasal microbiota and the pathogenesis of IOM.

Before the development of metagenomic next-generation sequencing (mNGS), the progress of microbiome study was impeded by the culture-dependent techniques which bias towards easy culturable species [20]. The mNGS technology enables analysis of the entire microbiome as well as the human host genome in clinical samples, contributing to precision diagnosis of infectious diseases as well as detection of all potential pathogens in noninfectious conditions. For instance, mNGS analyses of neural tissues revealed a variety of fungal and bacterial species in samples obtained from Alzheimer's disease, providing research basis for further exploration of the causative pathogens during disease evolution [21]. Since little is known about the taxonomic composition in IOM samples, mNGS technique may serve as an effective and efficient tool to assist demonstrating the microbiota in EOM tissues.

Our study hypothesized that the dysbiosis of nasal microbiota may play a role in IOM development. To test the hypothesis, we prospectively recruited 4 groups of patients, including the IOM(+) CRS(+) subjects, the IOM(−) CRS(+) subjects, the IOM(+) CRS(−) subjects and the IOM(−) CRS(−) subjects. Both the EOM samples and the nasal swap samples were collected and processed for microbiota analysis. The

microbial diversity and microbial abundance were compared among the 4 study groups so as to evaluate the potential influence of nasal microbiota the pathogenesis of IOM.

Methods

Subject Recruitment

We prospectively recruited 16 age- and gender-matched subjects into 4 study groups [IOM(+) CRS(+) group, IOM(−) CRS(+) group, IOM(+) CRS(−) group, IOM(−) CRS(−) group] from January 2021 to May 2021 at Fudan Eye & ENT Hospital. The inclusion criteria of IOM(+) groups were acute onset of unilateral IOM within 1 month presenting with the typical symptoms such as conjunctival edema, eyelid erythema, orbital pain evoked by ocular motility, paretic or restrictive diplopia, and EOM enlargement on computed tomography (CT) scan. The inclusion criteria of IOM(−) groups were patients with concomitant strabismus who underwent muscle resection surgery. The inclusion criteria of CRS(+) groups were inflammation of the nose and paranasal sinuses for more than 3 months in accordance with the European Position Paper on Rhinosinusitis and Nasal Polyps guidelines (https://www.rhinologyjournal.com/Search.php; accessed October 4, 2021). The inclusion criteria of IOM(−) groups were CT and endoscopy-confirmed absence of inflammation within nasal cavity and paranasal sinuses. The exclusion criteria were ocular surface diseases such as conjunctivitis and keratitis, eyelid conditions such as Meibomian gland dysfunction and blepharitis, and specific causes of myositis including thyroid-associated ophthalmopathy, IgG4-related ophthalmic disease, other systemic autoimmune conditions such as sarcoidosis, myositis-specific autoantibodies, confirmed infections, drug reactions and paraneoplastic syndrome. The medical records of each subject were collected, including demographics, chief complaints, clinical symptoms and signs, medical treatments and 3-month follow-up results. The study was retrospectively registered on Chinese Clinical Trial Registry website with the registration number ChiCTR2100047731 on 24 June 2021 (https://www.chictr.org.cn/showproj.aspx?proj=127896; accessed October 4, 2021). The study protocol was in accordance with the Declaration of Helsinki, and informed consents were obtained from all patients.

Sample Collection

The sequencing samples were collected in Class 1000 surgical operating room by an experienced surgeon. After topical anesthesia, both the conjunctival sacs and the skin of upper face were disinfected with iodophor. For IOM(+) subjects, surgical incisions of the affected eyes were made along limbal conjunctiva, and the surrounding conjunctival tissues were collected as the Incision samples. The most severely affected EOMs were sampled at muscle belly, and the EOM samples were kept no more than 10 mm^3 to minimize iatrogenic injury. For IOM(−) subjects, both the incision tissues and the resected muscle tissues were collected during strabismus surgery. A small part of each EOM sample was processed with hematoxylin-eosin (H&E) stain, and the remaining part was subjected to mNGS analysis. The ocular motility function was examined immediately after surgery to ensure no presence of new onset diplopia. For both CRS(+) and CRS(−) subjects, nasal swab samples were immediately collected prior to the

removal of nasal secretions and the application of topical mucosal vasoconstrictors. Sterile swabs were endoscopically guided to the middle meatus region to avoid contamination from the anterior nasal cavity, rotated for 5 full turns and kept in place for at least one minute until fully saturated. Any swabs that were contaminated through contact with a nontarget region were discarded. All collected samples were placed in sterile Eppendorf tubes without enzyme on ice temporarily, transported to the laboratory within 2 h and stored at − 80°C in liquid nitrogen until DNA extraction was performed.

DNA Extraction and mNGS

DNA was extracted using the TIANamp Micro DNA Kit (DP316, TIANGEN BIOTECH ,Beijing, China) according to the manufacturer's instructions. HeLa cell lines with or without *Acinetobacter baumannii* were subjected to the same procedure as positive and negative controls. The DNA samples, along with the negative and positive controls, were processed with mNGS according to the MGISeq2000 sequencing protocol. Briefly, samples were purified using AmPure beads (Qiagen, Germany) and quantitated by Qubit Fluorometer 3.0 (Life Technologies, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies Inc. USA). A total of 100 ng DNA was fragmented into 100-150 bps by Bioruptor® Pico (Diagenode, Belgium) and was constructed into the library following the standard protocol provided by the manufacture using PMseq DNA/RNA Library Preparation Kit (BGI-Wuhan, China). DNA libraries were sequenced to a depth of at leats 20 million reads per sample on the MGISeq-2000 platform.

16S rRNA sequencing

Microbial DNA was extracted from the swab samples using the DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocols. Amplification of the V3-V4 hypervariable region of the bacterial 16S rRNA gene was performed using the extracted DNA template with the following primers: 338F (5'- ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The purified products were sequenced (2 × 250) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols. The raw reads were deposited into the National Center for Biotechnology Information Sequence Read Archive database.

Data Analysis

After quality control by FastQC, the sequence reads were preprocessed with removal of human reads by Scalable Nucleotide Alignment Program to obtain clean nonhuman sequences. The nonhuman sequences were aligned to the National Center for Biotechnology Information nucleotide reference database (updated 2021.1) and grouped into distinct taxonomic units according to their species level classification. The relative abundance of each species was calculated by the ratio of the total mapped reads of each species, normalized by their genome size and the total mapped microbial reads within each sample. Microbial diversity (Shannon index) was calculated according to the method described in the Mothur program after using a subsampling cutoff of 500 bacterial sequences per sample [22]. Microbial abundance was calculated with principal component analysis (PCA) using Ade4 package in R statistical software (v3.1.1). The differences in microbiome characterizing the groups were evaluated by linear discriminant analysis (LDA) effect size [23].

Statistics

Data analysis was carried out with Statistical Product and Service Solutions. Continuous variables were described as mean and range and compared with the two-tailed *t*-test. Categorical variables were described as frequencies (number (%)) and compared with the two-tailed chi-square test or Fisher's exact test as appropriate. The Shannon index was analyzed by Mann-Whitney U test, and the PCA results were compared with Wilcoxson Rank-Sum test. A value of $P < 0.05$ was considered statistically significant.

Results

Clinical Characteristics

Sixteen cases were prospectively recruited in the study (Table 1). The clinical symptoms of IOM(+) subjects included painful diplopia (87.5%, 7/8), congestion and edema of ocular surface (75.0%, 6/8), eyelid swelling and erythema (62.5%, 5/8), proptosis (37.5%, 3/8), and ptosis (25.0%, 2/8). The oculomotor disorders initially started with paretic dysfunction within the first week, moved into a combined paretic and restrictive phase during 2-3 weeks, and finally ended as restrictive strabismus after 1 month (Fig. 1a). Based on CT scan, the most frequently affected EOMs were medial rectus (75.0%, 6/8) and inferior rectus (62.5%, 5/8) (Fig. 1b), and the superior and inferior oblique muscles were not affected in our case series. Compared with the IOM(−) subjects, the pathologic findings of the IOM(+) subjects typically presented as focal infiltration of inflammatory cells, perimysial edema within muscle fibers and mild accumulation of collagen strands (Fig. 1c). Oral corticosteroids were prescribed for all IOM(+) cases. The IOM(+)CRS(+) subjects were concurrently treated with intranasal corticosteroids to manage rhinosinusitis. Two patients (25.0%) in the IOM(+)CRS(−) group underwent orbital radiotherapy to correct muscle dysfunction. All patients responded to treatments, and 3 patients (37.5%) achieved full recovery after 3 months of follow-up.

Table 1
Clinical characteristics of the recruited subjects

	IOM(+) CRS(+) (n=4)	IOM(−) CRS(+) (n=4)	IOM(+) CRS(−) (n=4)	IOM(−) CRS(−) (n=4)
Male	3	3	3	3
Age (yr)	47.8 ± 11.6	48.0 ± 12.7	46.5 ± 15.0	47.8 ± 4.9
General medical history				
Previous antibiotics	0	1	0	0
Smoking	2	1	0	1
Allergic rhinitis	3	2	1	0
Asthma	0	0	0	0
Blood eosinophil(%)	2.6 ± 1.3	4.5 ± 0.4	1.8 ± 1.5	0.9 ± 0.1
Sampled extraocular muscle				
Medial rectus	2	3	3	2
Inferior rectus	1	0	1	0
Superior rectus	1	0	0	0
Lateral rectus	0	1	0	2
CRS-related medical history				
Nasal polys	1	2	–	–
Lund-Mackay CT score	11.3 ± 3.6	13.8 ± 0.9	–	–
Treatment				
Corticosteroids	4	4	–	–
Functional endoscopic sinus surgery	1	2	–	–
Follow-up at 3 months				
Full recovery	1	2	–	–
Partial recovery	3	2	–	–
IOM-related medical history				
Affected orbit				
Left	3	–	2	–

	IOM(+) CRS(+) (n=4)	IOM(−) CRS(+) (n=4)	IOM(+) CRS(−) (n=4)	IOM(−) CRS(−) (n=4)
Right	1	–	2	–
Affected extraocular muscle				
Medial rectus	2	–	4	–
Inferior rectus	2	–	3	–
Superior rectus	1	–	1	–
Lateral rectus	0	–	0	–
Muscle dysfunction				
Paretic	1	–	2	–
Mixed paretic-restrictive	1	–	1	–
Restrictive	2	–	1	–
Treatment				
Corticosteroids	4	–	4	–
Radiotherapy	0	–	2	–
Follow-up at 3 months				
Full recovery	2	–	1	–
Partial recovery	2	–	3	–

IOM idiopathic orbital myositis, *CRS* chronic rhinosinusitis, *CT* computer tomography

Microbiome Results

The distribution of microbial species in the EOM samples, the surgical incision samples and the nasal swab samples were processed with mNGS or 16s rRNA sequencing in each subject (Fig. 2). Taken together, 42 species (18 genera) of bacteria and 3 species (2 genera) of fungi were detected, and no virus was present in any sample. Regarding to the IOM(+) subjects, the top three microorganisms were *Cutibacterium acnes*, *Malassezia restricta* and *Acinetobacter johnsonii* in the EOM samples, and *Cutibacterium acnes*, *Brevundimonas* and *Sphingomonas echinoides* in the Incision samples. Regarding to the IOM(−) subjects, the top three microorganisms were *Cutibacterium acnes*, *Staphylococcus* and *Acinetobacter johnsonii* in the EOM samples, and *Ralstonia*, *Cutibacterium acnes* and *Micrococcus luteus* in the Incision samples. In terms of the Swab samples, the predominant species were *Cutibacterium*

acnes and *Staphylococcus haemolyticus* in the CRS(+) subjects, and *Cutibacterium acnes* and *Staphylococcus hominis* in the CRS(−) subjects.

Taxonomic Changes

The microbial diversity in each group was measured with Shannon index (Fig. 3a). Both the Incision samples and the Swab samples exhibited higher microbial diversity than the EOM samples. In terms of the Incision samples, no significant difference was detected among the four study groups, suggesting that the microbiome within conjunctival tissues was probably not affected by IOM or CRS conditions. The microbial diversity of the EOM samples was statistically higher in the IOM(+)CRS(+) subjects compared with the IOM(−) subjects, but no statistical difference was detected between the IOM(+)CRS(−) subjects and the IOM(−) subjects, indicating that microbiotal dysbiosis may be responsible for myositis development in the IOM(+)CRS(+) group but not in the IOM(+)CRS(−) group.

The taxonomic changes were further analyzed with PCA and LDA effect size. PCA analysis revealed that microbial abundance significantly differed between the EOM and Incision samples in the IOM(−) subjects (Fig. 3b, Wilcoxson Rank-Sum test, $P = 0.011$) but not in the IOM(+) subjects. LDA effect size screened out the most distinguishable species (LDA score > 3) as *Staphylococcus* in the EOM samples and *Micrococcus luteus* in the Incision samples (Fig. 3b). Comparison of the EOM and Swab samples detected statistical differences of microbial abundance in the IOM(−)CRS(+) subjects (Fig. 3c, Wilcoxson Rank-Sum test, $P = 0.003$) but not in the IOM(+)CRS(+) or CRS(−) subjects, and the most distinguishable species (LDA score > 3) were *Cutibacterium acnes*, *Malassezia restricta* and *Staphylococcus haemolyticus* in the Swab samples (Fig. 3c). Further analysis of the Swab samples confirmed distinctive microbiome between the CRS(+) and CRS(−) subjects (Fig. 3d, Wilcoxson Rank-Sum test, $P = 0.006$), with *Staphylococcus haemolyticus* predominant in the CRS(+) subjects and *Staphylococcus hominis* predominant in the CRS(−) subjects (Fig. 3d). Notably, the microbiota in EOM samples was statistically different between the IOM(−)CRS(+) and IOM(−)CRS(−) subjects (Fig. 3d, Wilcoxson Rank-Sum test, $P = 0.018$), and LDA effect size revealed preponderance of *Cutibacterium acnes* in the IOM(−)CRS(+) subjects. We also noticed discrepancies of EOM microbiota between the IOM(+)CRS(+) and IOM(+)CRS(−) subjects, but data analysis showed no statistical significance (Fig. 3d). The above findings collectively demonstrated an alteration of microbiota in both nasal cavity and EOMs of the CRS subjects, providing evidence that the microbiotal dysbiosis may contribute to IOM pathogenesis in patients with concurrent IOM and CRS.

Discussion

Microbiome, which refers to the entirety of organisms that colonize individual sites in the human body, has been considered as an essential participant in the the surveillance and monitoring of immune system. Microbiota encompasses a relatively narrower range of microorganisms than microbiome, and the prokaryotes and eukaryotes in microbiota are active within the range of microbial structures, metabolites and movable genetic elements [12]. Many studies have confirmed that dysfunctional

microbiota inhabiting the airway is a key factor in the pathology of upper respiratory tract diseases such as CRS [24, 25]. Consistent with previous studies, our data also demonstrated microbiotal dysbiosis in the nasal cavities of CRS subjects. Furthermore, we discovered an alteration of microbiota in EOM tissues obtained from IOM(−)CRS(+) subjects compared with the IOM(−)CRS(−) subjects. Our results indicate that the nasal microbiota may interfere with the balance of microbial communities in EOM tissues and the imbalanced microbiota within muscle fibers in turn contributes to the development of IOM. This new finding provides insights into the role of nasal microbiome in the development of orbital autoimmune diseases. Further study is necessary to investigate the interaction between microbiotal dysbiosis in nasal cavity and autoimmune reaction within orbit.

It is disappointing that no significant differences were detected between the EOM samples obtained from IOM(+)CRS(+) subjects and IOM(+)CRS(−) subjects. This result is seemingly paradoxical to the hypothesis that nasal microbiota influences the pathogenesis of IOM. It is reasonable to presume that the negative results are attributed to small sample size and the drawbacks of mNGS technique. The major challenge of mNGS is immature validation of sequencing process and lack of experience in bioinformatic analysis [26]. Many efforts have been made to develop analytical tools to aid in all aspects of data analysis. We believe that the rapid progress of bioinformatics may remarkably improve the performance of mNGS in future studies. Up to date, mNGS has been successfully applied in dozens of sample types, such as cerebrospinal fluid, respiratory secretions, feces, urine, blood and other human tissues [27, 28]. The advantages of high-throughput, culture-independence and unbiased performance have enabled mNGS as a routine diagnostic test in some medical centers [29]. The mNGS technology not only opens another window for the diagnosis of infectious diseases but also offers a precious opportunity to identify the whole genome of pathogens within a certain sample. Many researchers have utilized mNGS to screen out the potential causative pathogens in previously-considered idiopathic inflammatory conditions. For instance, in inflammatory bowel disease, mNGS analysis identified a common skin resident fungus *Malassezia restricta* as a specific pathogen, and mouse model experiments confirmed its pathogenic roles as eliciting innate inflammatory responses and exacerbating colitis [30]. The mNGS technique has also been applied to investigate periocular and intraocular conditions [31]. However, few studies focus on intraorbital diseases. To this point, our study provides a reference spectrum for further experiments to explore the microbiome within orbital tissues.

Another drawback of our study is unbalanced confounding factors. Many studies have proved that the ocular surface microbiome differs by age, sex, geographic area, ocular surface conditions and antibiotic drugs [22, 32–34]. Since the conjunctival incision may affect the results of EOM tissues, our study recruited age- and gender-matched subjects to minimize the confounding effects of ocular surface microbiome. Other potential confounding factors include the sampled EOM, the histologic endotype of CRS, the affected sinuses, previous usage of antibiotics and so forth. Owing to a small sample size, these factors were not balanced in our study. We anticipate large sample study with case-control design to balance more confounding effects and clarify the diverse microbiota during different clinical phases of IOM.

Conclusions

Our study recruited a cohort of age- and gender-matched subjects with IOM and/or CRS, performed mNGS analyses of the EOM tissues and 16s rRNA sequencing of the nasal swap samples, and explored the influence of CRS on EOM microbiota in subjects with/without IOM. Our data demonstrated distinctive microbiota in EOM tissues between subjects with CRS and without CRS, providing evidence that nasal dysbiosis may play a role in the development of IOM.

Abbreviations

CRS
Chronic rhinosinusitis
CT
Computed tomography
EOM
Extraocular muscle
H&E
Hematoxylin-eosin
IOM
Idiopathic orbital myositis
LDA
Linear discriminant analysis
mNGS
Metagenomic next-generation sequencing
PCA
Principal component analysis

Declarations

Ethics approval and consent to participate: The study protocol was approved by the Ethics Committee of Fudan EENT Hospital. Written informed consents were obtained from all patients.

Consent for publication: All authors have approved the manuscript for publication. The patients depicted in the figures have given written consent for their personal or clinical details along with any identifying images to be published in this study.

Availability of data and materials: The original dataset is available upon request to the corresponding authors.

Competing interests: None of the authors have any conflicts of interest to report.

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Authors' contributions: XL and RM were involved in analysis and interpretation of data and drafting the manuscript. LG and ZP made contribution to acquisition of data and drafting. JQ and RZ contributed to conception and design, analysis and interpretation of data, drafting and revising the manuscript. All authors read and approved the final manuscript.

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References

1. McNab AA. Orbital Myositis: A Comprehensive Review and Reclassification. Ophthalmic Plast Reconstr Surg. 2020;36(2):109–17.
2. Snidvongs K, Chitsuthipakorn W, Akarapas C, Aeumjaturapat S, Chusakul S, Kanjanaumporn J, Seresirikachorn K. Risk factors of orbital complications in outpatients presenting with severe rhinosinusitis: A case-control study. Clin Otolaryngol. 2021;46(3):587–93.
3. Trivić A, Cevik M, Folić M, Krejovic-Trivić S, Rubino S, Micić J, Stevanović G, Milovanović J, Jotić A, Barać A. Management of Orbital Complications of Acute Rhinosinusitis in Pediatric Patients: A 15-Year Single-Center Experience. Pediatr Infect Dis J. 2019;38(10):994–8.
4. Ben Artsi E, Mckelvie PA, McNab AA. Histopathologic Findings in Idiopathic Orbital Myositis. Ophthalmology. 2021;128(4):609–16.
5. Yan J, Wu P. Idiopathic orbital myositis. J Craniofac Surg. 2014;25(3):884–7.
6. Avni-Zauberman N, Tripathy D, Rosen N, Ben Simon GJ. Relapsing migratory idiopathic orbital inflammation: six new cases and review of the literature. Br J Ophthalmol. 2012;96(2):276–80.
7. Miller FW, Lamb JA, Schmidt J, Nagaraju K. Risk factors and disease mechanisms in myositis. Nat Rev Rheumatol. 2018;14(5):255–68.
8. Gan W, Zhang H, Yang F, Liu S, Liu F, Meng J. The influence of nasal bacterial microbiome diversity on the pathogenesis and prognosis of chronic rhinosinusitis patients with polyps. Eur Arch Otorhinolaryngol. 2021;278(4):1075–88.
9. Rana K, Juniat V, Selva D, Psaltis AJ. Diplopia in a patient with chronic rhinosinusitis: think about the orbit. BMJ Case Rep. 2021;14(1):e240127.
10. Ikeda M, Iwahashi C, Hirai T, Hattori K, Mitarai K. A Case of Eosinophilic Chronic Rhinosinusitis Associated with Choroidal Folds and Ocular Motility Disorder. Case Rep Ophthalmol. 2019;10(1):89–94.

11. Qazi ZU, Latif S, Awan SM. Orbital Involvement In Sinonasal Diseases. J Ayub Med Coll Abbottabad. 2016;28(4):687–93.
12. Tai J, Han MS, Kwak J, Kim TH. Association Between Microbiota and Nasal Mucosal Diseases in terms of Immunity. Int J Mol Sci. 2021;22(9):4744.
13. Christen U. Pathogen infection and autoimmune disease. Clin Exp Immunol. 2019;195(1):10–4.
14. Zitvogel L, Ayyoub M, Routy B, Kroemer G. Microbiome and Anticancer Immunosurveillance. Cell. 2016;165(2):276–87.
15. Kittipibul T, Puangsricharern V. The Ocular Microbiome in Stevens-Johnson Syndrome. Front Med (Lausanne). 2021;8:645053.
16. Asao K, Hashida N, Ando S, Motooka D, Kurakami H, Nakamura S, Yamashita D, Maruyama K, Kawasaki S, Yamada T, Iida T, Nishida K. Conjunctival dysbiosis in mucosa-associated lymphoid tissue lymphoma. Sci Rep. 2019;9(1):8424.
17. Doan T, Wilson MR, Crawford ED, Chow ED, Khan LM, Knopp KA, O'Donovan BD, Xia D, Hacker JK, Stewart JM, Gonzales JA, Acharya NR, DeRisi JL. Illuminating uveitis: metagenomic deep sequencing identifies common and rare pathogens. Genome Med. 2016;8(1):90.
18. Arunasri K, Mahesh M, Sai Prashanthi G, Jayasudha R, Kalyana Chakravarthy S, Tyagi M, Pappuru RR, Shivaji S. Comparison of the Vitreous Fluid Bacterial Microbiomes between Individuals with Post Fever Retinitis and Healthy Controls. Microorganisms. 2020;8(5):751.
19. Gonzales J, Doan T, Shantha JG, Bloomer M, Wilson MR, DeRisi JL, Acharya N. Metagenomic deep sequencing of aqueous fluid detects intraocular lymphomas. Br J Ophthalmol. 2018;102(1):6–8.
20. Chiu CY, Miller SA. Clinical metagenomics. Nat Rev Genet. 2019;20(6):341–55.
21. Alonso R, Pisa D, Fernández-Fernández AM, Carrasco L. Infection of Fungi and Bacteria in Brain Tissue From Elderly Persons and Patients With Alzheimer's Disease. Front Aging Neurosci. 2018;10:159.
22. Deng Y, Wen X, Hu X, Zou Y, Zhao C, Chen X, Miao L, Li X, Deng X, Bible PW, Ke H, Situ J, Guo S, Liang J, Chen T, Zou B, Liu Y, Chen W, Wu K, Zhang M, Jin ZB, Liang L, Wei L. Geographic Difference Shaped Human Ocular Surface Metagenome of Young Han Chinese From Beijing, Wenzhou, and Guangzhou Cities. Invest Ophthalmol Vis Sci. 2020;61(2):47.
23. Wen X, Miao L, Deng Y, Bible PW, Hu X, Zou Y, Liu Y, Guo S, Liang J, Chen T, Peng GH, Chen W, Liang L, Wei L. The Influence of Age and Sex on Ocular Surface Microbiota in Healthy Adults. Invest Ophthalmol Vis Sci. 2017;58(14):6030–7.
24. Grayson JW, Hopkins C, Mori E, Senior B, Harvey RJ. Contemporary Classification of Chronic Rhinosinusitis Beyond Polyps vs No Polyps: A Review. JAMA Otolaryngol Head Neck Surg. 2020;146(9):831–8.
25. De Boeck I, Spacova I, Vanderveken OM, Lebeer S. Lactic acid bacteria as probiotics for the nose? Microb Biotechnol. 2021;14(3):859–69.

26. Schlaberg R, Chiu CY, Miller S, Procop GW, Weinstock G, Professional Practice Committee and Committee on Laboratory Practices of the American Society for Microbiology. Microbiology Resource Committee of the College of American Pathologists. Validation of Metagenomic Next-Generation Sequencing Tests for Universal Pathogen Detection. Arch Pathol Lab Med. 2017;141(6):776–86.
27. Miller S, Naccache SN, Samayoa E, Messacar K, Arevalo S, Federman S, Stryke D, Pham E, Fung B, Bolosky WJ, Ingebrigtsen D, Lorizio W, Paff SM, Leake JA, Pesano R, DeBiasi R, Dominguez S, Chiu CY. Laboratory validation of a clinical metagenomic sequencing assay for pathogen detection in cerebrospinal fluid. Genome Res. 2019;29(5):831–42.
28. Charalampous T, Kay GL, Richardson H, Aydin A, Baldan R, Jeanes C, Rae D, Grundy S, Turner DJ, Wain J, Leggett RM, Livermore DM, O'Grady J. Nanopore metagenomics enables rapid clinical diagnosis of bacterial lower respiratory infection. Nat Biotechnol. 2019;37(7):783–92.
29. Ung L, Bispo PJM, Doan T, Van Gelder RN, Gilmore MS, Lietman T, Margolis TP, Zegans ME, Lee CS, Chodosh J. Clinical metagenomics for infectious corneal ulcers: Rags to riches? Ocul Surf. 2020;18(1):1–12.
30. Limon JJ, Tang J, Li D, Wolf AJ, Michelsen KS, Funari V, Gargus M, Nguyen C, Sharma P, Maymi VI, Iliev ID, Skalski JH, Brown J, Landers C, Borneman J, Braun J, Targan SR, McGovern DPB, Underhill DM. Malassezia Is Associated with Crohn's Disease and Exacerbates Colitis in Mouse Models. Cell Host Microbe. 2019;25(3):377–88.
31. Delbeke H, Younas S, Casteels I, Joossens M. Current knowledge on the human eye microbiome: a systematic review of available amplicon and metagenomic sequencing data. Acta Ophthalmol. 2021;99(1):16–25.
32. Fan C, Yang B, Huang Y. Efficacy of 0.5% Levofloxacin and 5.0% Povidone-Iodine Eyedrops in Reducing Conjunctival Bacterial Flora: Metagenomic Analysis. J Ophthalmol. 2020;2020:1780498.
33. Kang Y, Zhang H, Hu M, Ma Y, Chen P, Zhao Z, Li J, Ye Y, Zheng M, Lou Y. Alterations in the Ocular Surface Microbiome in Traumatic Corneal Ulcer Patients. Invest Ophthalmol Vis Sci. 2020;61(6):35.
34. Leonardi A, Modugno RL, Cavarzeran F, Rosani U. Metagenomic analysis of the conjunctival bacterial and fungal microbiome in vernal keratoconjunctivitis. Allergy. 2021;76(10):3215–7.

Figures

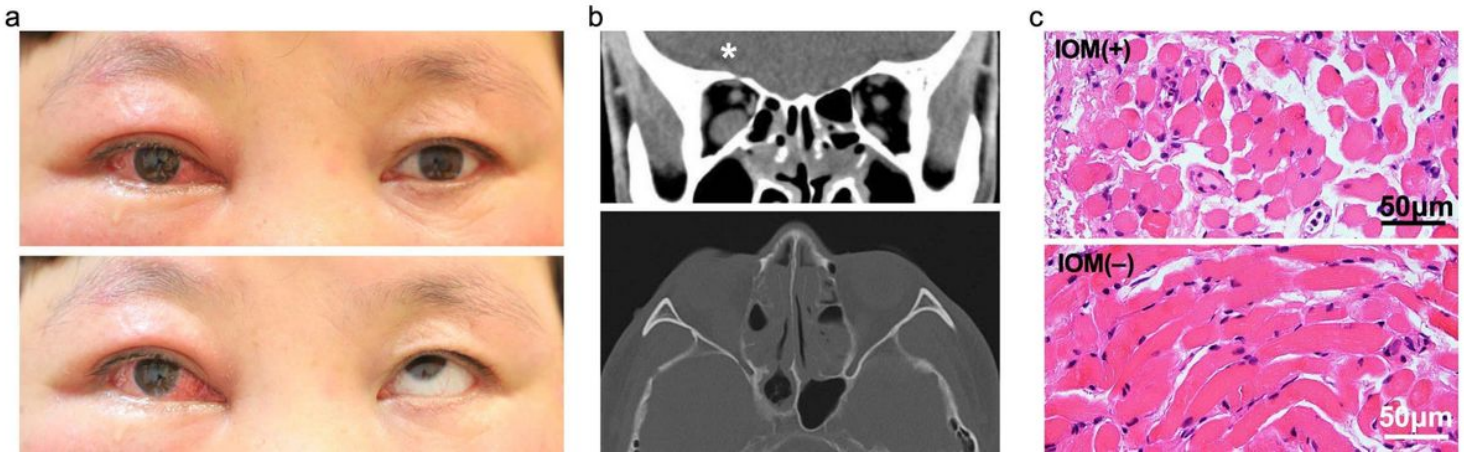

Figure 1

A typical IOM(+)CRS(+) subject. **(a)** A 51-year-old female complaint of double vision and motility evoked orbital pain in the right eye for 1 month. Physical examination showed periorbital inflammation and restrictive motility on upgaze. **(b)** CT scan demonstrated irregular enlargement of the inferior rectus in the right eye along with bilateral ethmoidal sinusitis. **(c)** The right inferior rectus was sampled for pathologic analysis, presenting with focal inflammation, perimysial edema and mild fibrosis (upper; stain, hematoxylin-eosin; original magnification, x400). Muscle tissues from IOM(–) subjects were harvested during concomitant strabismus surgery as healthy controls (lower; stain, hematoxylin-eosin; original magnification, x400). IOM = idiopathic orbital myositis, CRS = chronic rhinosinusitis, CT = computed tomography

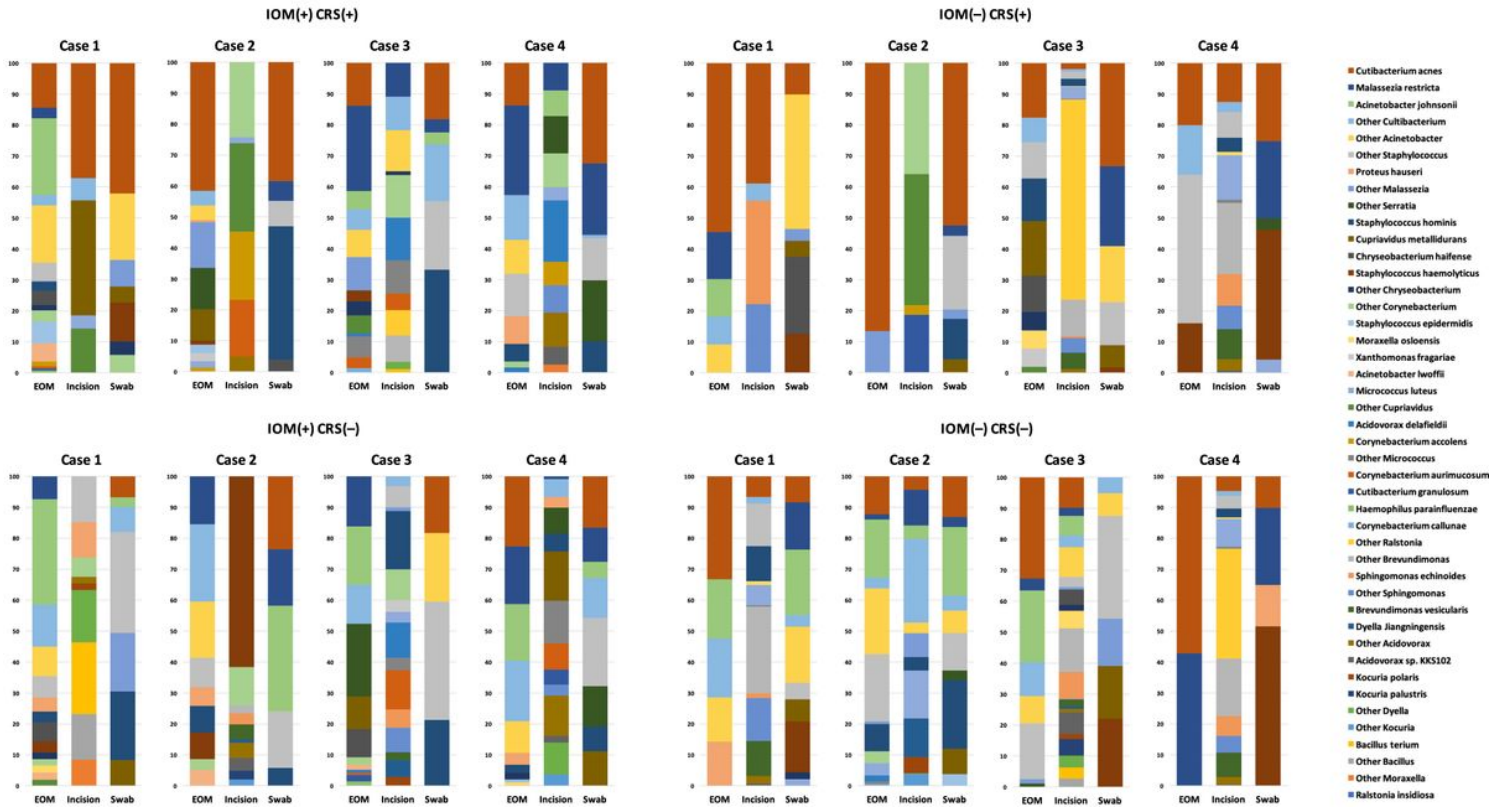

Figure 2

Distribution of microorganisms in each subject. The proportions of identified species in the EOM samples, the conjunctival incision samples and the nasal swap samples are listed as stacked columns. IOM = idiopathic orbital myositis, CRS = chronic rhinosinusitis, EOM = extraocular muscle

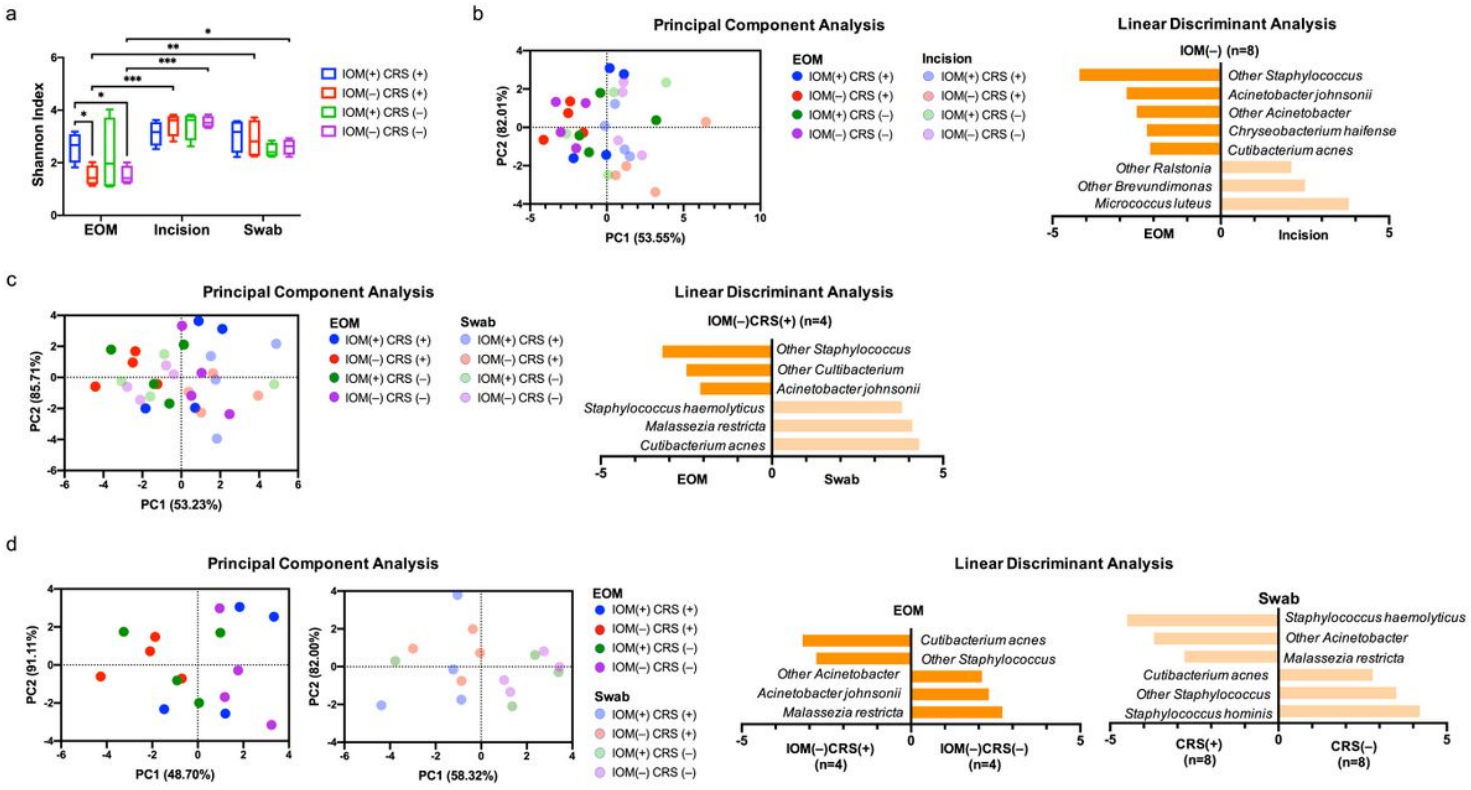

Figure 3

Analysis of microbial diversity and abundance. **(a)** The microbial diversity was calculated by Shannon index. * as $P < 0.05$, ** as $P < 0.01$, *** as $P < 0.001$. **(b-d)** The microbial abundance was evaluated by principal component analysis [b: IOM(−), EOM vs Incision, $P = 0.011$; c: IOM(−)CRS(+), EOM vs Swab, $P = 0.003$; d: Swab, CRS(+) vs CRS(−), $P = 0.006$; D: EOM, IOM(−)CRS(+) vs IOM(−)CRS(−), $P = 0.018$]. The most distinguishable species were identified by linear discriminant analysis (LDA) effect size program (LDA score > 3). IOM = idiopathic orbital myositis, CRS = chronic rhinosinusitis, EOM = extraocular muscle