Potential Role of Ocular Microbiome, Host Genotype, Tear Cytokines, and Environmental Factors in Corneal Infiltrative Events in Contact Lens Wearers

Cecilia Chao,[1,2] Lakshmi Akileswaran,[3] Jessica N. Cooke Bailey,[4] Mark Willcox,[2] Russell Van Gelder,[3] Carol Lakkis,[5] Fiona Stapleton,[2] and Kathryn Richdale[6]

[1]Tufts Medical Center, Center for Translational Ocular Immunology, Boston, Massachusetts, United States
[2]University of New South Wales School of Optometry and Vision Science, Kensington, New South Wales, Australia
[3]Department of Ophthalmology, University of Washington Medical School, Seattle, Washington, United States
[4]Department of Population and Quantitative Health Sciences, Case Western Reserve University, Institute for Computational Biology, Cleveland, Ohio, United States
[5]Johnson & Johnson Vision Care, Inc., Jacksonville, Florida, United States
[6]University of Houston College of Optometry, Houston, Texas, United States

Correspondence: Kathryn Richdale, University of Houston College of Optometry, 4901 Calhoun Road, Houston, TX 77024, USA; richdale@uh.edu.

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Purpose. The purpose of this study was to explore differences in genotype, ocular surface microbiome, tear inflammatory markers, and environmental and behavioral exposures in soft contact lens (SCL) wearers with and without a history of corneal infiltrative events (CIEs).

Methods. Nine SCL wearers with a recent CIE and nine age-, sex-, and SCL material- and modality-matched controls were enrolled. The Contact Lens Risk Survey, slit-lamp examination data, basal tears, conjunctival microbial cultures, and peripheral blood samples were collected. Tear inflammatory mediator concentrations, genomic DNA from swabs, and whole exome sequencing of blood samples were quantified.

Results. There were no marked differences in SCL wear behaviors or exposures between case and control subjects. Predominant organisms detected among case and control subjects were *Staphylococcus*, *Propionibacterium*, *Streptococcus*, and *Corynebacterium*. Marginally higher levels of *Neisseria* were found in three of nine cases but zero of nine control samples ($P = 0.056$). A potentially deleterious missense single nucleotide polymorphism (SNP) variant in IL-6 Signal Transducer (*IL6ST*) was found in seven of eight cases and zero of nine controls (rs2228046; $P = 0.03$). The concentration of tear IL-6 was significantly higher in cases (4.5 [range, 2.1 to 6.2] pg/mL) versus controls (3.5 [range, 2.5 to 6.6] Pg/mL; = 0.02).

Conclusions. Tear IL-6 concentration was higher, and SNP variants were detected in subjects with a history of CIEs compared with healthy controls. The synthesis, signaling, and ocular surface cytokine concentration of IL-6 may be related to susceptibility to CIE. A larger study population is required to further explore relationships between genetic variations, the ocular surface microbiome, inflammatory mediators, and environmental exposures.

Keywords: tear cytokines, ocular microbiome, whole exome sequencing, soft contact lenses, corneal infiltrative events

Corneal infiltrative events (CIEs) occur in 7% to 44% of soft contact lens wearers per year and can be associated with significant morbidity and economic cost.[1,2] Nearly 41 million adults in the United States wear contact lenses, and the annual rate of contact lens complications translates into approximately 1 million doctor visits at a cost of more than $175 million dollars per year.[3,4] Contact lens wear can lead to CIEs due to mechanical, chemical, or hypoxic stress or via introduction of bacteria and associated toxins.[5–8]

Pioneering work by Keijser et al. and Carnt et al. demonstrated that genetic variations in single nucleotide polymorphism (SNP) variants of *IL-1β*, *IL-6*, *IL-10*, and *IL-12* were associated with susceptibility to and severity of CIEs in soft contact lens wearers.[9–11] Carriers of a SNP in the intron region of the *TLR5* gene have been associated with a higher risk of developing keratitis, and an SNP in the exome of *TLR4* has been associated with a lower risk of keratitis (Willcox M, et al. *IOVS* 2015;56:ARVO E-Abstract 4812). The human leukocyte antigen *(HLA)-DQ* genotype has been shown to influence staphylococcal colonization of the gut.[12] To our knowledge, these are the only genetic variations that have been studied in patients with a history of CIEs.

Tear inflammatory mediators are proteins expressed by cells of the ocular surface during inflammation and are critical to understanding the eye's inflammatory/immune response to contact lens wear.[13,14] Both routine contact lens wear and contact lens–related complications can regulate the levels of inflammatory mediators in tears.[13,15–19]

It is thought that the ocular surface may not elicit a full immune response against certain core microbes.[20,21] However, routine soft contact lens wear may increase the number and diversity of bacteria on the ocular surface.[22,23] Contact lens

wearers with active CIEs harbor higher levels of bacteria on their ocular surface compared to healthy contact lens wearers.[7,23] The presence of colonized bacteria on contact lenses increases the risk of CIE by three to eight times.[23] It is known that many microbes cannot be grown in the laboratory, and therefore, genetic-based deep sequencing techniques (i.e., 16/18s rRNA) have recently been used to identify the presence of bacteria, fungi, or viruses on the ocular surface.[24–26] Biome representational in silico karyotyping (BRiSK) detects a large number of DNA tags, including those of human, microbial, fungal, viral, and parasitic origin. This improves the accuracy of mapping of amplifications and deletions compared with 16s metagenomic techniques.[27,28]

This pilot study aimed to (1) demonstrate feasibility of study methodology and (2) explore differences in key genetic exome sequences, the ocular surface microbiome, and tear inflammatory proteins between contact lens wearers with and without a history of CIEs.

Methods

A single-visit cohort study was conducted at the State University of New York (SUNY), College of Optometry. Subjects were recruited from the SUNY College of Optometry and the surrounding New York City metropolitan area. The study followed the tenets of the Declaration of Helsinki. The Institutional Review Board at the SUNY College of Optometry approved the study before data collection began. Written informed consent was obtained from all participants prior to participation in the study.

Nine soft contact lens wearers with a medical record documented history of a CIE (contact lens peripheral ulcer [CLPU], contact lens–induced acute red eye [CLARE], infiltrative keratitis [IK], or microbial keratitis [MK]) were enrolled 3 to 15 months after treatment. As a control group, nine healthy established full-time soft contact lens wearers ($\geq$1 year of full-time wear, >5 days/wk) were also enrolled, and matched by sex, contact lens material (silicone hydrogel or hydrogel), and modality (daily or reusable wear). All subjects were between 18 and 40 years of age. Exclusion criteria for all subjects included active eye infection or inflammatory disease (e.g., allergy, blepharitis, meibomian gland disease), a prior history of refractive/eye surgery, ocular trauma or systemic disease likely to affect the ocular surface (e.g., thyroid disease, diabetes, autoimmune disease), family history of systemic autoimmune or inflammatory disease, oral or ocular treatment with anti-inflammatory or antibiotic within 1 month prior to the study visit, and pregnancy or breastfeeding during the study period.

Measurements were carried out in the order of least to most invasive as described below. The Contact Lens Risk Survey (CLRS)[29,30] was used to collect data on demographics, environmental exposures, and behaviors that may affect the ocular surface microbiome (i.e., water exposure and use of multipurpose solutions).[4] A slit-lamp examination was conducted, and limbal and conjunctival redness and palpebral conjunctival roughness (papillae/follicles) were graded using the Cornea and Contact Lens Research Unit (CCLRU) scale (0 to 4 each eye); a total score from both eyes was recorded for analysis.[31] Approximately 15 µL basal tears were collected from both eyes and prepared as previously described for batch analysis (see Tear Analysis section).[32,33]

Anesthetic (0.5% proparacaine; AKORN, Lake Forest, IL, USA) was instilled prior to culturing of the ocular surface as previously described.[26] Briefly, two DNA free swabs (SK-2S-T swabs; Baca Scientific, Boca Raton, FL, USA) were used to collect samples from the upper and lower bulbar and palpebral conjunctiva of each eye. Negative controls were also prepared after every fourth subject by applying one drop of anesthetic to a new swab. All swabs were stored at –80°C until analysis (see Microbiome Analysis section). A second slit-lamp examination was conducted to assess corneal (0 to 5 per eye) and conjunctival staining (0 to 5 per region at temporal and nasal per eye) using sodium fluorescein, Lissamine green, and the modified Oxford grading scale.[34] Approximately 10 mL peripheral blood was collected in EDTA tubes and stored at –80°C for batch analysis (see Whole Exome Sequencing section).

Tear Analysis

Tear inflammatory mediator concentrations (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12(p70), IL-13, and TNFα) were determined using V-plex Multiplex assays (Meso Scale Discovery Japan, Tokyo, Japan) in 1:10 dilution. The concentration of secretory IgA (sIgA) was analyzed using standard ELISA (human secretory IgA ELISA; Alpco, Salem, NH, USA). Tear sIgA concentration has been used to discriminate basal tears from reflex tears.[35] Therefore, sIgA concentration was compared between groups to ensure there was no difference in the type of tears collected, which may confound interpretation of the inflammatory mediators between controls and cases.

Microbiome Analysis

Genomic DNA collected from swabs were extracted using the DNeasy Blood and Tissue Kit (Qiagen, Inc., Venlo, the Netherlands), quantified using the NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and analyzed using BRiSK and 16SrDNA metagenomic amplification and sequencing.[27] Quantitative PCR (qPCR) for actin and 16SrDNA was performed to determine total bacterial load on individual swabs as previously described by comparing with a database in the GenBank data for known microbial species using the basic local alignment software tool.[36] Phi29 amplification was performed for BRiSK analysis on genomic DNA and obtained microbial DNA.[27] The 33-bp DNA sequence tags obtained from BRISK were compared with a database for known microbial species.[27]

Raw sequence data from 16SrDNA were processed with MOTHUR (https://www.mothur.org, provided by the University of Michigan, Ann Arbor, MI, USA).[37] Taxonomic assignment and RDP II Classifier were used for the filtered sequences that were classified with the TUIT algorithm from the GenBank database. BRiSK analysis was performed using custom built scripts and databases. The reconstruction of ocular surface microbiomes allows the analyses of diversity, relative abundance per subject, and between-group comparisons to assess CIE-related differences. Visual comparison of the groups was performed with MEGAN4 (www-ab.informatik.uni-tuebingen.de/software/megan) (Shalabi NM, et al. *IOVS* 2014;55:ARVO E-Abstract 6288).

Whole Exome Sequencing Analysis

Blood samples were whole exome sequenced on the Illumina HiSeq 3000 (Illumina, San Diego, CA, USA) at the University of Miami John P. Hussman Institute for Human Genomics. Base calls were determined by the Illumina CASAVA pipeline. After filtering for base quality and adapter sequences, the sequencing reads were aligned to the human reference genome (hg19) using bowtie2 and formatted for input into the Genome Analysis Toolkit (GATK) from the Broad Institute (Cambridge, MA, USA; https://software.broadinstitute.org/gatk/). The pipeline is based on the Broad Institutes' Best Practices Guideline including local realignment, removal of PCR duplicates, and

Table 1. Primary Demographic Information and Contact Lens Exposures for the Study Population

Question	Response	Case ($n = 9$)	Control ($n = 9$)
Ethnicity/race	African American	5	0
	Hispanic	2	0
	Asian	1	3
	Caucasian	1	5
	Other	0	1
Days/week of contact lens wear	<1 day/wk	0	0
	1–3 days/wk	2	0
	4–6 days/wk	4	5
	Everyday	3	4
Contact lens replacement schedule	Daily	6	6
	Biweekly	1	1
	Monthly	2	2
Wear contact lens >18 h/day	Always/fairly often	1	2
	Sometimes	4	0
	Infrequently/never	4	7
Wash hand before handling lens	Always/fairly often	9	9
	Sometimes	0	0
	Infrequently/never	0	0
Sleep in contact lenses	Always/fairly often	1	0
	Sometimes	1	4
	Infrequently/never	7	5
Sleep in contact lenses more often after alcohol consumption	Yes	5	1
	No	4	8
Shower in contact lenses	Always/fairly often	3	0
	Sometimes	3	4
	Infrequently/never	3	5
Rinse/store contact lenses in tap water	Always/fairly often	0	0
	Sometimes	0	0
	Infrequently/never	9	9

base quality recalibration. Single nucleotide variants and small insertion-deletion variants (indels) were called by GATK's HaplotypeCaller, and variants for each sample were consolidated with GenotypeGVCFs. The combined variant call format file (VCF) was then annotated with ANNOVAR (http://annovar.openbioinformatics.org/en/latest/, available in the public domain) and filtered for quality with VCFtools (http://vcftools.sourceforge.net/, available in the public domain). Variants were annotated with their frequency in the European population using the National Heart, Lung, and Blood Institute (NHLBI), Exome Sequencing Project's Exome Variant Server (ESP), Exome Aggregation Consortium (ExAC), and 1000 Genomes databases using ANNOVAR. Variants were annotated for region and exonic function by reference to refSeq and annotated for predicted impact by reference to PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/, available in the public domain) (benign, possibly damaging, probably damaging) and SIFT (http://sift.jcvi.org/, provided by the J. Craig Venter Institute, Rockville, MD, USA) (tolerated or damaging) using ANNOVAR. Variants with genotype quality (GQ) <30, depth (DP) <8, or Phred-scaled likelihood of reference genotypes (PL) <99 were excluded. Variants in *IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12(p70), IL-13, TNF-α, TLR-4,* and *HLA-DQ* and related genes were prioritized for relevance based on previous studies. Additional hard filtering was applied as an alternative to VQSR using values suggested by GATK best practices.[38] Filters included the following: quality by depth (QD) <2.0, Fisher strand (FS) >60.0, root mean square of the mapping quality (MQ) <40.0, Mapping quality rank sum test (MQRankSum) <−12.5, read position rank sum test (ReadPosRankSum) <−8.0, strand odds ratio (SOR) >3.0. Filters were applied manually in R version 3.4.2.[39]

Statistical Analysis

Descriptive data of the CLRS were generated to describe the demographic, environmental, and behavioral risk factors of CIEs of the study sample.[30] A Mann-Whitney *U*-test was carried out to examine differences in tear concentration of inflammatory mediators and clinical indicators between case and control subjects. Relative abundance of pathogens detected in BRiSK were calculated, and significant differences between case and control subjects were determined after estimating the false discovery rate using a Monte Carlo analysis permutation test with 100,000 permutations.[40] The Mann-Whitney *U* test with false discovery rate correction (Benjamini-Hochberg) was carried out to confirm the differences in the microbial compositions and diversity between two groups. For the whole exome sequencing, variants were analyzed with Fisher's exact test (P_{FET}) according to recessive and dominant models. Significance was determined as $P < 0.05$.

Results

Sample Population and Clinical Results

Nine case and nine control subjects completed the study (four male in each group). Blood samples with adequate volume were only able to be acquired from eight of the nine case subjects. The case subjects all had a history of at least one serious or significant CIE (three subjects with CLPU and six subjects with CLARE with infiltrates). There were no reported cases of microbial keratitis. Each group was comprised of six daily disposable (three silicone hydrogel and three hydrogel) and three reusable silicone hydrogel contact lens wearers. All reusable contact lens wearers reported using multipurpose solutions. The mean age of case and control subjects were 25.9 ± 5.0 and 25.4 ± 2.3 years, respectively ($P = 0.79$). The mean age at which subjects began SCL wear was at 15.4 ± 5.5 years in the case group and 14.4 ± 2.5 years in the control group ($P = 0.75$). Table 1 shows other subject demographic information and some of the primary contact lens–related risk factors obtained from the CLRS. There were five (56%) African-American subjects in the case group compared with zero in the control group. Case subjects also reported sleeping in contact lenses more often after alcohol consumption than control subjects (Table 1). There were no other differences seen in any contact lens related risk factors between groups, and further statistical testing was not done (not all data shown). All subjects had normal ocular adnexa via slit-lamp examination, and bulbar and limbal redness and corneal and conjunctival staining were not significantly different between case and control groups (Table 2). Corneal scars were observed in seven of nine cases but zero controls.

Tear Cytokine Results

IFN-γ, IL-2, IL-4, IL-10, IL-12(p70), IL-13, and TNF-α were not detected in tear samples. There was no difference in IL-1β and IL-8 between groups, but there was a significantly higher concentration of IL-6 in the case group (Table 2). There were no significant differences in tear sIgA between groups (Table 2).

Ocular Microbiome Results

Actin and 16S bacterial ribosomal DNA were examined in all the conjunctival samples and negative controls using PCR (Fig.

Table 2. Summary of Group Mean and SD or Median and IQR, as Appropriate, for the Slit-Lamp Findings and Tear Proteins

Outcome Measure	Case ($n = 9$)	Control ($n = 9$)	P
Slit-lamp findings			
Overall bulbar redness (0–8)	5.0 ± 0.9	5.2 ± 1.1	0.73
Overall limbal redness (0–8)	4.8 ± 2.1	5.1 ± 1.1	0.73
Overall palpebral roughness (0–8)	2.0 ± 1.7	2.4 ± 1.4	0.55
Overall corneal staining (0–10)	1.6 ± 1.3	1.2 ± 0.8	0.61
Overall conjunctival staining (0–20)	2.3 ± 3.0	1.8 ± 3.2	0.61
Total corneal scars	**1.8 ± 1.6**	**0 ± 0**	**0.004**
Tear analysis			
IL-1β (pg/mL)	1.7 (0.1–3.3)	0.0 (0.0–1.3)	0.13
IL-6 (pg/mL)	**4.5 (2.1–6.2)**	**3.5 (2.5–6.6)**	**0.02**
IL-8 (pg/mL)	563 (337–672)	347 (220–647)	0.14
Secretory IgA (mg/mL)	4.5 (2.7–8.7)	4.8 (2.8–7.6)	0.34

Bold entries represent $P < 0.05$.

1). All conjunctival samples showed strong actin results (Fig. 1A); some contamination was seen in the negative control samples but at much lower levels than the subject samples. Bacteria were recovered from all samples and very slight bands often seen from reagent contamination were observed in the negative controls (Fig. 1B).

Table 3 shows the qPCR results in actin and 16S copies/ng, as well as the ratio of bacteria to human cells for each subject. There was no significant difference in the bacteria/human cell ratio between groups (Table 3; case average: 0.0052 ± 0.0056, control average: 0.0043 ± 0.0047; $P = 0.74$). Even though individual variance was observed in bacterial loads, the proportions of human DNA findings were consistent between subjects.

All samples yielded good sequences from BRiSK, and 263,550 tags were recovered, of which 99.1% mapped to human (mammalian) sequences. The distribution of the human sequences per chromosome had an r^2 of 0.988 from expected, indicating high correlation. Tables 4 and 5 show the BRiSK and 16S results. Limited contamination was observed in the negative controls (see Supplementary Tables S1 and S2 for absolute levels of 16S bacterial rDNA results and the proportions of bacteria loads in all subjects). No significant differences were found in absolute levels or proportions of bacterial loads in the six major genera (Table 5) except for the proportion of *Neisseria*. The *Neisseria* genera was marginally higher in the case versus control group ($P = 0.056$); however, 16S bacterial rDNA results tend to be unreliable in extremely paucibacterial samples, therefore, the differences in *Neisseria* proportion between groups would need to be confirmed by directed qPCR.

Whole Exome Sequencing Results

A total of 2,097,409 variants were available. After filtering on mapping quality (MQ) >30.0 and a maximum missing value of 0.5, 794,187 variants remained, and 767,401 had available annotation data. Variants were restricted to those with exonic function within the prespecified regions of interest (*IL-1β*, *IL-2*, *IL-4*, *IL-6*, *IL-8*, *IL-10*, *IL-12*, *IL-13*, *HLA-DQ*, *TLR-4*, *TNF-α*, and related genes) including a total of 229 exonic variants, 111 of which satisfied all filters (Table 6).

Two variants reached a nominal significance threshold ($P_{FET} < 0.05$; Table 7). rs2228046 ($P_{FET} = 0.0294$) is a missense single nucleotide variation (SNV) in *IL6ST* transcript variant 1 mRNA, located on chromosome 5q11.2 (position 55954899) that results in an Ile→Thr substitution at amino acid position 454 of exon 11. Three cases had one copy of the alternate allele, and one case had two copies of the alternate allele. Zero control subjects had copies of the alternate allele. rs2230926 ($P_{FET} = 0.0294$) is a missense SNV in *Homo sapiens TNFα-induced protein 3* (*TNFAIP3*), transcript variant 1, mRNA, located on chromosome 6q23.3 (position 137874929) that results in an Phe→Cys substitution at amino acid position 127 of exon 3. Three cases had one copy of the alternate allele, and one case had two copies of the alternate allele. Zero control subjects had copies of the alternate allele. Allele frequencies across varying populations included in the ExAC browser (http://exac.broadinstitute.org/; the Broad Institute), as well as

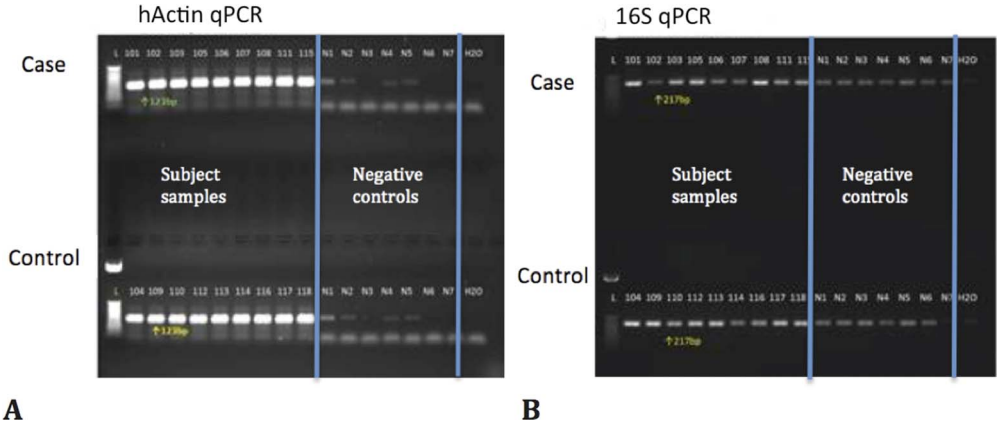

Figure 1. PCR results for actin (**A**) and 16S bacterial ribosomal DNA (**B**).

Table 3. Quantitative PCR Actin and 16S Bacterial rDNA Copies/ng and the Ratio of Bacteria to Human Cells in All Subjects

Group	Sample ID	Actin Copies/ng	16S Copies/ng	Bacteria/Human Cells
Case	M101	198,749	6930	0.0174
	M102	230,807	309	0.0007
	M103	220,252	1404	0.0032
	M105	216,967	2084	0.0048
	M106	218,848	528	0.0012
	M107	198,962	364	0.0009
	M108	201,787	5073	0.0126
	M111	209,877	1011	0.0024
	M115	211,731	1218	0.0029
Control	M104	193,517	5540	0.0143
	M109	202,150	4121	0.0102
	M110	235,218	1131	0.0024
	M112	259,981	1382	0.0027
	M113	254,063	1464	0.0029
	M114	250,305	399	0.0008
	M116	264,943	418	0.0008
	M117	228,152	958	0.0021
	M118	242,926	1175	0.0024

predictions from various additional prediction sources for these variants, as annotated by ANNOVAR, are in Tables 7 and 8.

Correlations Between Exome Sequencing, Tear Cytokines, and Microbiome Results

Subjects with a present (1/1 or 0/1) or absent (0/0) allele variation in *IL-6* were plotted against the statistically significant tear cytokine and ocular microbiome data (Fig. 2). Given the pilot nature of the study, statistical tests were not performed; however, the direction of the tear cytokine concentration is in line with the hypothesis that a deleterious SNP in *IL-6* receptor would lead to subsequent overproduction of cytokines at the ocular surface. There was no clear relationship between the genetic variation and *Neisseria* levels.

Discussion

The first aim of this pilot study was to demonstrate the ability to collect high-quality ocular and blood samples from subjects and send the samples for processing at remote sites. Nine subjects with a history of CIE and nine matched controls meeting all of the prespecified entry criteria were successfully enrolled. All enrolled subjects completed all study procedures; however, an adequate blood sample volume could not be obtained from one subject. The tears, ocular surface cultures, and blood samples obtained were of high quality and able to be processed by multiplex assay, BRISK/16srDNA, and whole exome sequencing.

The second aim of the study was to explore differences in key genetic exome sequences, the ocular surface microbiome, and tear inflammatory proteins between contact lens wearers with and without a history of CIEs. The sample population enrolled was matched by age, sex, and contact lens material and modality. Slit-lamp examination revealed that subjects had no active clinical signs of inflammation. The reported contact lens wear and care behaviors and environmental exposures associated with CIEs have been reported previously,[41–43] and this pilot study was not powered to analyze these factors.

No significant difference in ocular surface microbiome was shown between groups in this sample of 18 soft contact lens wearers. A previous study using traditional culturing techniques has suggested an increased risk of harboring Gram-negative bacteria with contact lens wear.[44] There have been only a few reports of the ocular surface microbiome by deep sequencing, which is known to show more diverse bacterial findings than traditional culturing methods.[25,27,44] The ocular surface of contact lens wearer in this study harbored primarily *Staphylococcus*, *Neisseria*, *Propionibacterium*, *Streptococcus*, and *Corynebacterium* spp. This is in agreement with previous studies conducted in our laboratories that quantified the ocular surface microbiome of non–contact lens wearers using similar techniques.[24,26] Shin et al. reported higher levels of *Methylobacterium*, *Lactobacillus*, *Acinetobacter*, and *Pseudomonas* spp. and lower levels of *Haemophilus*, *Streptococcus*, *Staphylococcus*, and *Corynebacterium* spp. among 9 soft contact lens wearers compared with 11 non–contact lens wearers.[45] Zhang et al.[46] reported no statistically significant differences in

Table 4. BRiSK Reads for Human, Bacteria, and Virus for Control Subjects and Case

	Case								
Sample ID	**M101**	**M102**	**M103**	**M105**	**M106**	**M107**	**M108**	**M111**	**M115**
Total reads	338,926	248,757	431,901	1,115,223	852,360	133,324	205,035	847,783	1,129,111
Human reads	335,723	246,181	427,805	104,256	844,803	132,099	203,066	839,560	1,118,404
% human/total	99.1	99.0	99.1	99.0	99.1	99.1	99.0	99.0	99.1
Bacteria mapped	1	0	0	4	0	0	5	1	14
Virus/TTV	0	0	0	4	0	0	49	0	0
r^2	0.978	0.988	0.978	0.997	0.996	0.989	0.993	0.995	0.99
	Control								
Sample ID	**M104**	**M109**	**M110**	**M112**	**M113**	**M114**	**M116**	**M117**	**M118**
Total reads	574,407	726,742	381,811	575,690	845,304	1,367,913	263,550	187,552	363,867
Human reads	569,363	719,747	377,890	570,672	837,287	1,355,540	261,180	185,650	360,618
% human/total	99.1	99.0	99.0	99.1	99.1	99.1	99.1	99.0	99.1
Bacteria mapped	9	22	5	4	4	0	0	0	3
Virus/TTV	0	0	8	0	2	0	0	0	0
r^2	0.986	0.985	0.983	0.981	0.989	0.991	0.987	0.982	0.994

Table 5. Group Mean and SD in Absolute Levels and Proportions of Bacterial Loads Using 16S Bacterial rDNA Analysis

	Absolute Levels			Proportions of Bacterial Loads		
Bacterial Species	**Case**	**Control**	***P***	**Case**	**Control**	***P***
Staphylococcus spp.	38,931 ± 52,388	82,216 ± 79,693	0.19	0.23497 ± 0.22201	0.40227 ± 0.28142	0.18
Neisseria spp.	87,135 ± 189,830	280 ± 395	0.19	***0.23351 ± 0.33721***	***0.00170 ± 0.00142***	***0.056***
Propionibacterium acnes	14,602 ± 9,174	56,174 ± 71,100	0.10	0.24167 ± 0.17903	0.31228 ± 0.21360	0.46
Streptococcus spp.	7,680 ± 10,381	33,724 ± 95,872	0.43	0.10850 ± 0.17387	0.05960 ± 0.15506	0.54
Corynebacterium spp.	15,057 ± 16,186	25,077 ± 29,165	0.38	0.15748 ± 0.14067	0.15537 ± 0.13466	0.97
Pseudomonas spp.	1,213 ± 943	3,776 ± 6,073	0.23	0.02388 ± 0.02340	0.06879 ± 0.10041	0.21

Bold italic entries represent $P < 0.10$.

the ocular surface microbiome of 12 soft contact lens wearers compared with 12 non–contact lens wearers; however, they suggested levels of *Delftia* spp. may be decreased and *Elizabethkingia* spp. increased in contact lens wearers.

The presence of resident viruses on the ocular surface is even less well understood. Two case subjects and one healthy control harbored the torque teno virus (TTV). TTV was previously reported on the ocular surface of healthy adults in our study of healthy non–contact lens wearers.[26] Given TTVs association with seasonal hyperacute panuveitis, culture-negative endophthalmitis and ability to induce an adaptive immune response, further research is needed to explore potential clinical implications of resident TTV on the ocular surface.

Differences in the ocular surface microbiome have been reported by region, sex, and age.[24–26] There is some evidence of a minimal core microbiome.[20,24] The potential effects of daily disposable or reusable contact lens wear, and use of hydrogen peroxide or multipurpose solution are yet to be fully explored; however, a study using similar techniques suggests that the ocular surface microbiome of contact lens wearers with or without a history of CIE may not be substantially different from each other or from non–lens wearers.[46]

Table 6. Examined Variants by Gene Region Before and After Hard Filtering

Gene of Interest	Gene (or Related)	All Regions ($N = 229$)	After Hard Filtering ($N = 111$)
HLA-DQ	*HLA-DQA1*	42	5
	HLA-DQA2	8	5
	HLA-DQB1	69	0
	HLA-DQB2	11	4
INF-γ	*IFNGR1*	3	3
	IFNGR2	2	2
IL-1β	*IL1B*	1	1
IL-2	*IL2*	1	1
	IL2RA	5	5
	IL2RB	3	3
IL-4	*IL4*	1	1
	IL4I1	2	2
	IL4R	14	14
IL-6	*IL6*	3	2
	IL6R	5	5
	IL6ST	6	5
IL-8	*CXCL8*	1	1
IL-10	*IL10RA*	9	9
	IL10RB	1	1
IL-12	*IL12B*	1	1
	IL12RB1	9	9
	IL12RB2	8	8
IL-13	*IL13*	1	1
TLR-4	*TLR4*	4	4
TNF-α	*TNFAIP2*	5	5
	TNFAIP3	2	2
	TNFAIP6	6	6
	TNFAIP8	3	3
	TNFAIP8L2	1	1
	TNFAIP8L3	2	2

The tear cytokine analysis showed higher levels of IL-6 in case subjects versus controls. Previous work has shown no significant day to day variation in IL-6 after discontinuing contact lens wear in healthy subjects, nor changes in IL-6 with one continuous day and night of hydrogel or silicone hydrogel contact lens wear among established contact lens wearers.[32,47–49] Increases in IL-6 have been associated with the use of certain multipurpose cleaning solutions,[50] and reuseable wearers have been shown to have higher levels of IL-6 than daily disposable soft contact lens wearers.[51] Poyraz et al.[52] examined changes in tear cytokines when neophytes were first fitted with either hydrogel or silicone hydrogel contact lenses and found early increases in IL-6. Taken together, these findings could indicate an initial upregulation of the ocular inflammatory status occurs on initiation of lens wear, which may become chronic in some wearers, especially those using multipurpose cleaners and reuseable lenses. A previous study also explored individual inflammatory cytokine responses of established wearers and showed a few subjects with much larger than average changes in IL-6 with overnight contact lens wear.[49] An exaggerated or long-term inflammatory response to contact lens wear may be an early signal that some patients are predisposed to ocular inflammatory complications and are not good candidates for extended or continuous wear lenses.

Whole exome sequencing analysis revealed a significant variation in SNPs in *IL-6* signal transducer for cases versus controls. We found that rs2228046 is predicted to be deleterious by the SIFT prediction tool and to be possibly damaging by the PolyPhen-2 prediction tool. The overall allele frequency in the ExAC is 0.2903 for this variant. rs2230926 is predicted to be tolerated by the SIFT prediction tool and to be benign by the PolyPhen-2 prediction tool. The overall allele frequency in the ExAC is 0.0613 for this variant. Carnt et al.[10] found that variations in rs1800795 and rs1800797 in the *IL-6* promoter region were associated with greater risk of and more severe microbial keratitis in a cohort of more than 2500 contact lens wearers. Importantly, these genetic variations were not present in cases with sterile (versus microbial) keratitis.[10] In this study, all four cases with *IL-6* variations in SNPs were African Americans. Coe et al.[53] found that African Americans had higher serum *IL-6* and lower soluble *IL-6* receptor concentrations than Japanese Americans and whites. They suggested that higher body mass index (BMI) was a significant confounder related to higher levels of IL-6 in African Americans[53] and that further research was needed in this area. No previous study has reported variations in *IL-6* by race or ethnicity, and there is no reported difference in risk of CIE by

TABLE 7. Single Nucleotide Variants in Genes of Interest With Suggestive $P_{FET} < 0.05$

Gene.refGene	avsnp147	UID	ExonicFunc.refGene	AAChange.refGene	CytoBand	Chromosome	Position	REF	ALT
IL6ST	rs2228046	ID_24_rs2228046	Nonsynonymous_SNV	IL6ST:NM_002184:exon11:c.T1361C:p.I454T	5q11.2	5	55954899	A	[G]
TNFAIP3	rs2230926	ID_169_rs2230926	Nonsynonymous_SNV	TNFAIP3:NM_001270507:exon3:c.T380G:p.F127C, TNFAIP3:NM_001270508:exon3:c.T380G:p.F127C, TNFAIP3:NM_006290:exon3:c.T380G:p.F127C	6q23.3	6	1.38E+08	T	[G]

CtyoBand, location of the chromosome.

TABLE 8. Single Nucleotide Variants in Genes of Interest With Suggestive $P_{FET} < 0.05$

	Case			Control												
Gene.refGene	0/0	0/1	1/1	0/0	0/1	1/1	P_{FET} Value (dom)	ExAC_ALL	ExAC_AFR	ExAC_AMR	ExAC_EAS	ExAC_FIN	ExAC_NFE	ExAC_OTH	ExAC_SAS	Func.refGene
IL6ST	4	3	1	9	0	0	0.0294	0.0265	0.2903	0.0122	0	0	0.0005	0.0154	0.0002	exonic
TNFAIP3	4	3	1	9	0	0	0.0294	0.0613	0.3652	0.0339	0.0403	0.0157	0.0339	0.0441	0.0299	exonic

0/0, zero copies of the alternate (G) allele; 0/1, one copy of the alternate allele; 1/1, two copies of the alternate allele; P_{FET} value, *P* value of Fisher exact test; E_xAC, exome aggregation consortium.

race/ethnicity. Obesity has been identified as a risk factor for inflammatory disease. A larger, more diverse study sample with balanced racial groups and information on BMI is needed to explore the relationship between race/ethnicity/BMI and genetic variations in *IL-6*.

IL-6 is a pleotropic mediator with both pro- and anti-inflammatory properties.[54,55] It has been shown to be directly involved in both the defense and homeostatic maintenance of the ocular surface.[14] IL-6 helps regulate the initial acute phase of the inflammatory response by recruiting polymorphonuclear cells (PMNs) and also the later clearing of inflammation via lowering the production of IL-1 and TNF.[56] Thakur et al.[57] used a mouse model to demonstrate that concentrations of IL-6 varied significantly by type of *Pseudomonas aeruginosa* infecting the cornea. Cole et al.[58] showed more severe *Pseudomonas* keratitis in the absence of IL-6. Hume et al.[59] found that administration of exogenous IL-6 during infection with *Staphylococcus aureus* in mice decreased the numbers of *Staphylococci* and improved outcomes. Although not found in this pilot study, mutations in IL-6 could affect the diversity and density of bacterial loads on the ocular surface. CLPU is commonly associated with *S. aureus* colonization of lenses,[6,23,60] whereas CLARE is commonly associated with Gram-negative, including *P. aeruginosa* bacterial colonization of contact lenses.[23,61,62] However, as only three CLPU (presumed Gram positive) and six CLARE (presumed Gram negative) subjects were included in this study, subanalysis by bacterial type could not be conducted. Larger studies are needed to examine the potential effect of *IL-6* SNPs on specific corneal infiltrative events and microbial colonization.

SNP variants in *IL-6* have been associated with chronic inflammatory diseases including arthritis, diabetes, and cardiovascular disease.[63–65] *IL-6* and *IL-6* receptors bind together with the signal-transducing subunit (also known as *gp130*).[56] Impaired *IL-6* trans-signaling (*gp130*) can lead to impairment of bacterial response including impaired influx of monocytes, excessive retention of neutrophils, and greater tissue damage.[54,56] Thus, the SNP variants in *IL-6ST* found in case subjects in this study may support an increased inflammatory response and increased risk for the development of CIEs or other microbial infections. Longitudinal cohort studies would be needed to identify the direct causal relationship between possession of *IL-6* SNPs, concentration of IL-6 in tears, and the risk of ocular surface infection or inflammation.

There are limitations to interpretation of the study results due to the small sample size. All 18 subjects were recruited from a single urban site and included a mix of contact lens wearers (i.e., material, modality, solution use) and races/ethnicities. In this pilot study, four of the nine cases were African American, whereas none of the controls were African American. Future studies with racially balanced samples are required to further explore potential associations between SNP variants and the ocular surface cytokine and bacterial levels. Variations in genes may lead to production of more or less proteins at the ocular surface, thus affecting the ocular inflammatory state and response. It is likely that changes in ocular surface proteins may also affect the ocular surface microbiome, especially because certain cytokines are known to have antimicrobial functions.

In summary, in this small pilot study, we demonstrated feasibility for measuring multiple internal and external risk factors for CIEs using a multisite collection and analysis protocol. Contact lens wear can serve as a useful "trigger" to elicit ocular surface inflammatory responses, as contact lenses are the most common cause of CIEs in the United States.[4] Further research in large, diverse populations, enrolled from multiple geographic sites, is necessary to understand the complex relationship between variations in genetic profiles,

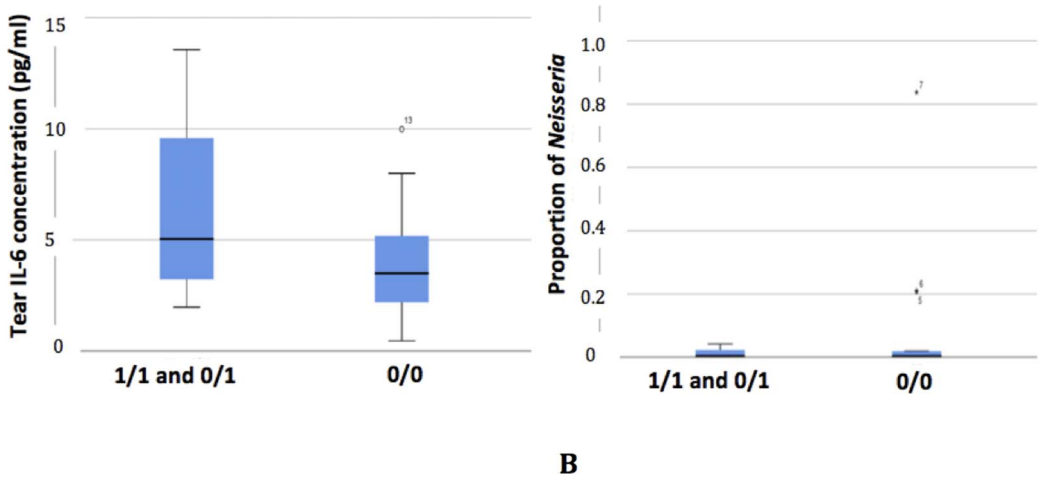

FIGURE 2. The concentration of IL-6 in tears (**A**) and proportion (**B**) of *Neisseria* spp. isolated from ocular swabs in people homologous or heterologous for the IL-6ST variant. 1/1, those homologous for mutation; 1/0, those heterologous for the mutation; 0/0, subjects who possess the normal gene; *box*, interquartile range and median; *whiskers*, 1.5 times interquartile range. Outliers are indicated.

ocular surface protein expression, and the ocular surface microbiome and how such changes may predispose patients to ocular surface infection and inflammation.

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