Ocular surface quantitative microbiota of mild and moderate meibomian gland dysfunction before and after intense pulsed light therapy: A case report

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Abstract

Background Intense pulsed light (IPL) has been a promising treatment option for patients with meibomian gland dysfunction (MGD). While the ocular surface microbiome is associated with various eye conditions including MGD, evidence of the effect of IPL on the ocular surface microbiome has been lacking.

Methods Patients with mild and moderate MGD underwent IPL treatment and standard eyelid hygiene every two weeks for a total of four sessions. Eyelid scraping and eyelash samples before and after IPL therapy were evaluated by microbiome analysis based on 16S ribonucleic acid gene sequencing as well as dry eye speed score, lid margin abnormalities, corneal and conjunctival fluorescein staining, meibum grading score, and meibography.

Results Before IPL, the relative abundance of *Propionibacterium acnes* (12.79%) in mild MGD was the highest, followed by *Faecalibacterium prausnitzii* (6.35%) and family *Lachnospiraceae* (4.74%). In moderate MGD, the relative abundance of unclassified *Bifidobacterium* (8.46%) was the highest, followed by unclassified *Rhodoplane* (6.65%) and A. Ellin 6513 (4.95%). After IPL treatment, the relative abundances of *Bifidobacterium adolescentis* and *Mitsuokella multacida* in mild MGD were significantly reduced from 0.88 % to 0.0044% and from 0.2 to 0.0065%, respectively. The relative abundance of family *Bradyrhizobiaceae*, order *Rhizobiale*, order *Solibacterales,* and unclassified *Bacillus* in moderate MGD were significantly reduced from 1.6% to 0.196%, from 4.99% to 0.93%, 2.37% to 0.44%, and 1.18% to 0.28%, respectively, whereas the relative abundance of unclassified *Bifidobacterium* has increased five times (8.46% to 45%). In mild MGD, the abundance of phylum *Proteobacterium* after IPL was the most positively correlated with clinical parameters (correlation coefficient 0.25 to 0.36) and the abundance of order *RB41* after IPL was significantly negatively correlated with clinical parameters (correlation coefficient -0.7 to -0.84). In moderate MGD, the abundance of several taxa after IPL was significantly positively correlated with clinical parameters (correlation coefficient 0.20 to 0.60), whereas *Bifidobacterium*, family *Bifidobacteriaceae*, class *Actinobacteria,* were significantly negatively correlated with clinical parameters (correlation coefficient -0.50 to -0.70).

Conclusion The microbiota of patients with mild and moderate MGD changed significantly from before to after IPL treatment. IPL therapy likely increases the relative abundance of bacteria as well as alters bacterial metabolites. The findings facilitated an understanding of the role of IPL that affected bacterial microbiota in MGD.

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Keywords: *Dry eye; intense pulsed light therapy; meibomian gland dysfunction; microbiome.*

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Introduction

Meibomian gland dysfunction (MGD) is defined as a chronic, diffuse functional abnormality of the meibomian glands, with varying estimated prevalence from 3.5% to 19.9% among Caucasians, and as high as 69.3% among Asians.[1] Terminal duct obstruction, qualitative and quantitative changes in meibum could result in an alteration of the tear film, eye irritation, inflammation, and ocular surface diseases.

Several behavioral, hormonal, metabolic, environmental, as well as microbial factors could influence the physical and functional status of meibomian glands. Despite bacterial diversity of healthy human eyes,[2] samples collected from MGD patients had a more complex bacterial profile, predominantly with *Staphylococcus epidermidis* and *Propionibacterium acnes*, than healthy individuals.[3]

The composition of bacteria becomes more complex as the severity of MGD increases.[4] Also, MGD patients can have various degrees of bacterial microbiota imbalance in the conjunctival sac, in which *Staphylococcus*, *Corynebacterium*, and *Sphingomonas* may play roles in the pathophysiology of MGD.[5]

Conventional treatments for MGD including eye drops, artificial tears, nonsteroidal anti-inflammatory drugs, corticosteroids, antibiotics, and physical therapy with hot compression following eyelid hygiene treatment, have achieved only temporary improvement for 30-90 days. Recently, intense pulsed light (IPL) has become an option for MGD treatment as it improves dry eye symptoms by selective photothermolysis and reduction of bacteria and parasitic growth.[6] To date, there is no information on the ocular surface quantitative microbiota (culture-independent diversity and quantity of bacteria) in MGD patients, before and after IPL. We aimed to comprehensively assess the quantitative microbiota before and after IPL by using metagenomics coupled with 16S rRNA gene qPCR and next-generation sequencing.

Materials and Methods

Two patients with mild and moderate MGD, diagnosed based on clinical signs and symptoms, such as tear break-up time, slit-lamp microscopy, meibography, and meiboscale, participated in this study. Both patients were clinically assessed by the same experienced ophthalmologist (NW) using standard equipment. They provided written consent and underwent IPL, standard eyelid hygiene, and self-eye care with tea tree oil (Cleanradex®) every two weeks for a total of four sessions.[7] The IPL treatment (Lumenis® M22, Lumenis Ltd., Yokneam, Israel) utilized intensity from 10 J/cm^2 to 12 J/cm^2 (depending on local severity) and a 590-nm filter with 6-mm SapphireCool cylindrical light for the upper and lower eyelids, provided that other parameters were set based on patient's skin type as determined by NW.

Sterile cotton swab was used to wipe the upper and lower conjunctival sac and eyelid margin of each eye. Samples were extracted for metagenomes using DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany), and the metagenome quality and quantity were determined by agarose gel electrophoresis and nanodrop spectrophotometry. Then, multiple displacement amplification (MDA) using REPLI-g Mini Kit (Qiagen, Hilden, Germany) followed by V3-V5 16S rRNA gene sequencing using universal prokaryotic primers 342F and 895R, and MiSeq600 platform (Illumina, California, USA) was performed according to standard protocols. An unused swab (a negative control to the microbiota experiment) showed no bacterial DNA amplification. A copy number of bacteria were quantified via 16S rRNA gene qPCR using Rotor-Gene Q PCR system (Qiagen, Hilden, Germany). The 16S rRNA gene sequences (NCBI SRA accession no. PRJNA642342) were analyzed according to Mothur's standard operating procedures, which included processing for quality reads, operational taxonomic unit (OTU) classification, good's coverage index (percent coverage of sequences to estimate true diversity), alpha diversity (Shannon index), and beta diversity indices. Potential

Table 1 Clinical characteristics, high quality sequencing and total bacteria number results of mild and moderate MGD, before and after IPL treatment

	Before IPL	After IPL 2	After IPL 3	After IPL 4
Mild MGD	**D1b**	**D1a2**	**D1a3**	**D1a4**
Demographic data				
MGD grading (0-4+)	2+	2+	2+	2+
Mucocutaneous junction (%)	40	20	10	10
Corneal & conjunctival fluorescein score (%)	20	20	10	5
Conjunctival reaction (0-4+)[a]	1+	1+	1+	1+
Debris grading (0-4+)	1+	1+	1+	1+
Demodex grading (0-4+)	1+	0.5+	0	0
High quality reads	62,564	64,869	107,717	68,287
Good coverage (%)	99.93	99.90	99.95	99.92
Alpha diversity (Shannon index)	4.22	3.54	3.68	3.95
Total bacteria (copies/eye)	6.17×10^6	1.93×10^7	1.03×10^7	1.59×10^7
Moderate MGD	**D2b**	**D2a2**	**D2a3**	**D2a4**
Demographic data				
MGD grading (0-4+)	3+	2+	2+	2+
Mucocutaneous junction (%)	40	30	20	10
Corneal & conjunctival fluorescein score (%)	10	5	10	5
Conjunctival reaction (0-4+)[a]	1.5+	1.5+	1+	1+
Debris grading (0-4+)	2+	2+	1+	0
Demodex grading (0-4+)	2+	1+	0.5+	0
High quality reads	45,457	63,283	40,000	-
Good coverage (%)	99.84	99.89	99.84	-
Alpha diversity (Shannon index)	4.19	3.31	2.62	-
Total bacteria (copies/eye)	1.63×10^7	1.82×10^7	6.83×10^6	-

MGD, meibomian gland dysfunction; IPL, intense pulsed light;
[a] 0 = none, 1+ = minimal, 2+ = mild, 3+ = moderate, 4+ = severe

metabolic functions of bacteria and statistical analysis between quantitative microbiota profiles were performed using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) and Statistical Analysis of Metagenomic Profiles (STAMP), respectively.

Results

Clinical characteristics, high-quality sequencing, and total bacteria number results before and after IPL treatment were shown in Table 1. The sequencing numbers were sufficient and yielded over 99.84-99.95% Good's coverage. The alpha diversity was found relatively lower after the IPL compared with before the IPL for mild and moderate MGD patients (Table 1). These lower microbiome diversity of Shannon indices correlated with the fewer number of OTUs in the patients after IPL (Figure 1). Figure 1 summarizes the relative abundance of the dominant microbiota and quantitative profile in mild and moderate MGD patients. While the percent relative abundance showed the composition and the lower alpha diversity OTUs after the IPL treatments, the quantitative profile suggested that the copy number of bacteria might not be involved in the IPL treatment.

Before IPL treatment

At the phylum level, 4 phyla were detected in both patients. The predominant phyla in mild MGD were *Actinobacteria* followed by *Firmicutes*, *Proteobacteria*, and *Acidobacteria* whereas the predominant phyla in moderate MGD were *Proteobacteria* followed by *Actinobacteria*, *Acidobacteria*, and *Firmicutes*.

At the species level, the relative abundance of *Propionibacterium acnes* (12.79%) in mild MGD before IPL was the highest, followed by *Faecalibacterium prausnitzii* (6.35%) and Family *Lachnospiraceae* (4.74%). In moderate MGD, the relative abundance of unclassified *Bifidobacterium* (8.46%) was the most prevalent, followed by unclassified *Rhodoplane* (6.65%) and A. Ellin 6513 (4.95%).

After IPL treatment

The relative abundance of *Bifidobacterium adolescentis* and *Mitsuokella multacida* in mild MGD were significantly reduced from 0.88% to 0.0044% and from 0.2 to 0.0065%, respectively. The relative abundance of family *Bradyrhizobiaceae*, order *Rhizobiale*, order *Solibacterales* and unclassified *Bacillus* in moderate MGD were significantly reduced from 1.6% to 0.196%, 4.99% to 0.93%, 2.37% to 0.44% and 1.18% to 0.28%, respectively. On the other hand, the relative abundance of unclassified *Bifidobacterium* has increased 5 times (8.46% to 45%). Noted the D4a4 sample was insufficient for metagenomic sequencing, and thus the microbiome data was not available.

Microbiota and clinical Parameters

We combined the relative abundance of microbiota in mild and moderate MGD after IPL and associated with changes in various clinical parameters (Figure 2A). In mild MGD, the relative abundance of phylum *Proteobacterium* after IPL was the most positively correlated with improved clinical parameters (correlation coefficient 0.25 to 0.36), whereas the relative abundance of order *RB41* after IPL was significantly negatively correlated with clinical parameters (correlation coefficient -0.70 to -0.84). In moderate MGD, the relative abundance of most taxa after IPL (such as class *Alphaproteobacteria*, order *RB41*, *Coprococcus*, or *Propionibacterium*) were significantly positively correlated with clinical parameters (correlation coefficient 0.20 to 0.60), while those significantly negatively correlated with clinical parameters were *Bifidobacterium*, family *Bifidobacteriaceae*, class *Actinobacteria* (correlation coefficient -0.50 to -0.70). The bacterial metabolite potential in mild MGD tends to decrease after IPL treatment whereas the bacterial metabolite potential in moderate MGD tends to increase after IPL treatment (Figure 2B).

Discussion

IPL has been increasingly recognized as an effective alternative treatment for MGD, but its mechanisms of action are not fully elucidated.[6] The decrease of the bacterial and parasitic load over eyelids and eyelashes has been considered the principal ones, but more recently the alteration of microbiome or bacterial dysbiosis have also been proposed[6] and supported by the findings from our study. The diversity of microbiome after IPL treatment could be determined in mild and moderate MGD, with some taxa being higher and some being lower than before treatment. Another important observation was the association between the diversity of microbiota in mild and moderate MGD after IPL and the changes in various clinical parameters.

The decreased bacterial metabolite potential in mild MGD after IPL treatment suggested compositional change of microbiota as a potential mechanism of action of IPL on MGD. However, this mechanism might not be as strong in moderate MGD as the dysbacteriosis might be tightly linked with the disease process.

Although the findings revealed significant changes in the microbiota profiles in response to the IPL therapy, further studies with larger samples are needed to explore whether there are common ocular surface microbiota changes by IPL therapy.

Conclusion

Findings from our study suggested that the microbiota of patients with mild and moderate MGD changed significantly from before to after IPL treatment. IPL therapy likely increases the relative abundance of bacteria as well as alters bacterial metabolites. The findings facilitated an understanding of the role of IPL that affect bacterial microbiota in MGD.

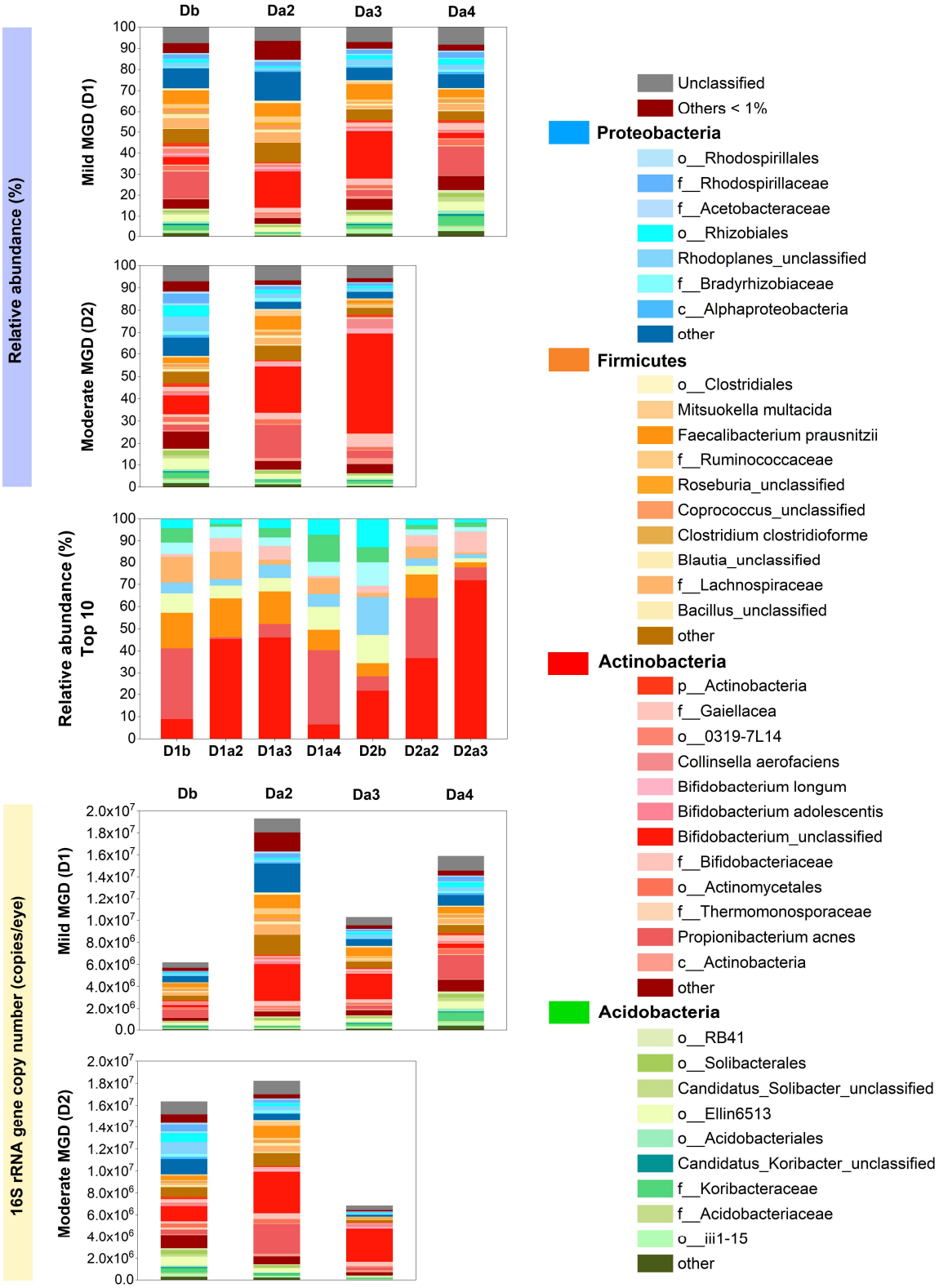

Figure 1 Ocular surface microbiota of mild and moderate MGD, displayed as percent relative abundance and bacterial copy number. Ocular surface microbiota of mild and moderate MGD, displayed as percent relative abundance and bacterial copy number. Top panel shows bacterial relative abundance; middle panel shows top 10 bacterial relative abundance; and lower panel shows bacterial quantitative profile. D1, mild MGD; D2, moderate MGD; Db, before IPL; Da2, 2 weeks after IPL; Da3, 4 weeks after IPL; Da4, 6 weeks after IPL.

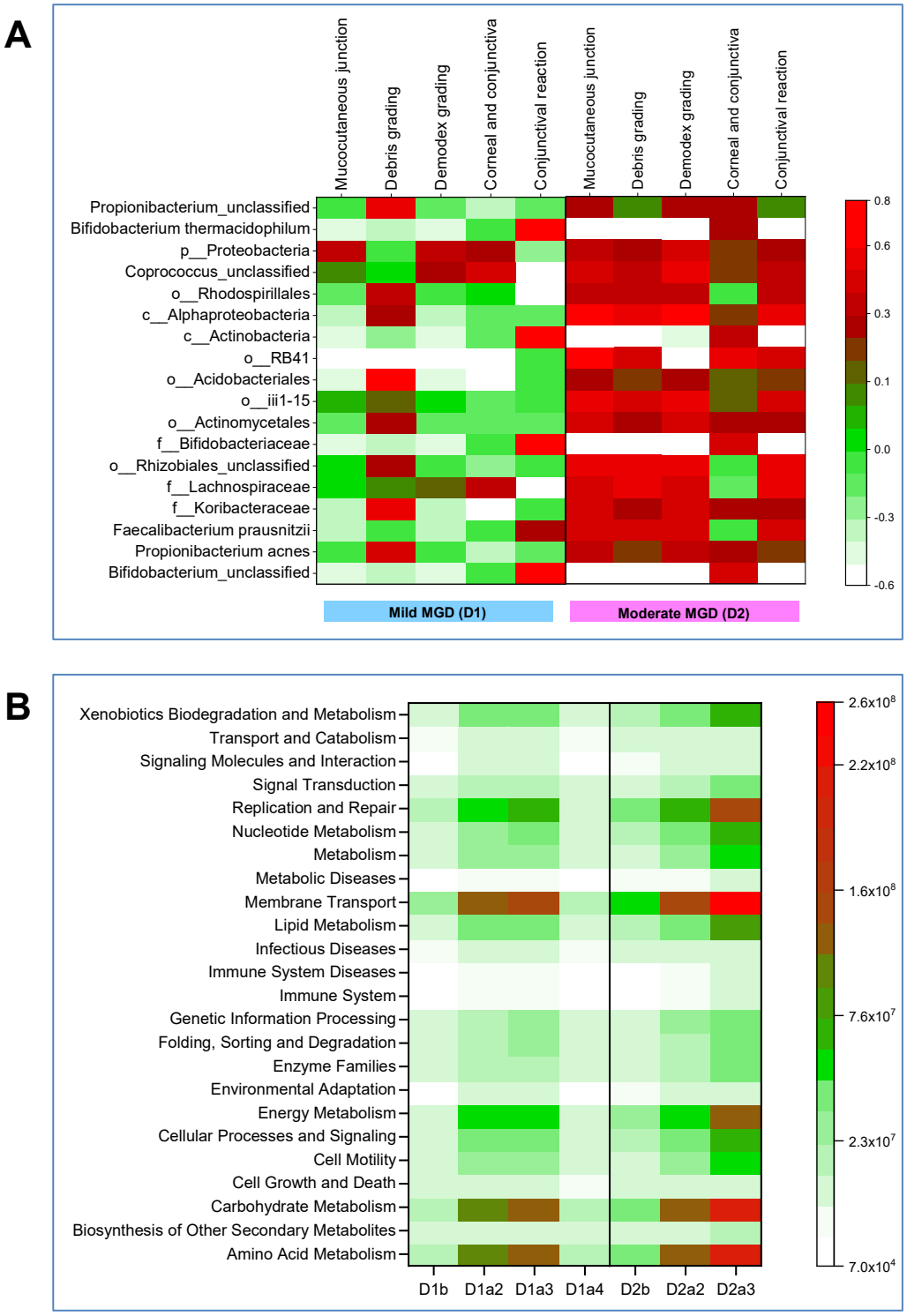

Figure 2 Correlations between (***A***) mild and moderate MGD after IPL, and in associated with changes in clinical eye exam characteristics, and (***B***) bacterial metabolic potentials. D1, mild MGD; D2, moderate MGD; Db, before IPL; Da2, 2 weeks after IPL; Da3, 4 weeks after IPL; Da4, 6 weeks after IPL.

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Conflict of Interest

None to declare.

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