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Person-to-Person Bacterial Transmission Can Influence Dry Eye Disease in Newly Married Couples: Six-Month Interim Results

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Person-to-Person Bacterial Transmission Can Influence Dry Eye Disease in Newly Married Couples: Six-Month Interim Results

DED Can Be Transmitted between Spouses

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Abstract: Background: The aim of this prospective comparative study was to determine whether alterations in ocular microbiota composition affect dry eye disease (DED) in newly married couples. **Methods**: This study comprised 392 spouses with confirmed dry eye disease (DED) who were either insomniac or hypersomniac, and 196 spouses as controls. We prospectively collected data on ocular surface parameters including tear osmolarity, Schirmer's test and Ocular Surface Disease Index (OSDI), sleep parameters and ocular microbiota composition on day 1 and after six months follow up. **Results**: The frequency of moderate-to-severe DED in spouses who had married with an insomniac person was significantly higher than healthy controls and hypersomoniacs ($P<0.001$). The DED phenotype was positively significantly correlated with species differences in each of the three groups (P value <0.001). At baseline, frequency of both mild and moderate-to-severe DED phenotype was significantly highest in insomniacs. Interestingly, in support of our hypothesis, the frequency of both mild and moderate-to-severe DED phenotype in healthy spouses was significantly increased after six months of marriage with an insomniac person (but not with a hypersomniac person), suggesting that there is a possibility that ocular microbiota alteration might have played a role in occurrence of DED in this group. **Conclusions**: We for the first time contribute to the existing literature by discovering an association between ocular gut microbiota and DED, possibly through person-to-person contact. In the framework of family medicine and personalized medicine, the practical and theoretical implications of this study cover vast areas of sleep therapy and microbiota-related ocular diseases.

Keywords: ocular microbiota; dry eye disease; insomnia; hypersomnia; person-to-person contact; bacterial transmission

Introduction

Sleep disorders are a prevalent and serious comorbidity in dry eye [1]. While insomnia severity is positively correlated with dry eye disease (DED) severity [2,3], results are not that straightforward for the hypersomnia situation. Only limited studies have investigated this association. Results of National Claim Data in South Korea demonstrated that sleep disorders were positively associated

with DES diagnosis. Positive trends were more common in younger patients, males, lower economic levels, and those with higher levels of comorbid in South Koreans [4]. Also, data from an Integrated Health Care Network in China showed that sleep disorder was associated with a three-fold increase in risk of DED [5]. However these two studies had not categorized their data by sleep disorder classifications [4,5]. Therefore, there is still uncertainty about the direction of this association, if there is any association at all.

These results are pertinent regarding the issue of whether there is a connection among (i) insomnia/hypersomnia, (ii) ocular conditions such as DED, and (iii) gut microbiota composition. Contemporary investigations infer the existence of a gut-eye microbiota axis [6,7]. There are even more interesting facts to observe. We recently reported that gut microbiota transmission change the sleep pattern in healthy spouses married to insomniac or hypersomniac spouses (ref). Furthermore, poor sleep has an important role in the development of DED and vice versa [8–10]. In addition to this, excessive yawning in many cases could be attributed to hypersomnia[11–13], while paradoxically, yawning frequently occurs in insomniac people mainly due to disturbed sleep [14]. On the other hand, yawning may facilitate lacrimation [15] and consequently may relieve eye dryness. Finally, from the perspective of microbiota studies, recent sophisticated studies using Metagenomics shows that there is substantial difference in terms of ocular microbiota between healthy eyes and those with DED [16].

Based on all these interconnected premises, we hypothesized that ocular microbiota alterations in newly married couples, possibly through person-to-person contact, may change the severity and/or occurrence of DED through ocular microbiota changes.

2. Materials and Methods

2.1. Participants

The study was approved by the Human Research Ethics Committee (IT. 24001670) and adhered to the tenets of the Declaration of Helsinki. Written and signed informed consents were obtained for all volunteer couples who were recruited from two private sleep clinics in Tehran, Iran. A total of 870 couples who had married during the past six months and were in a cohabiting relationship were invited to participate in this study together with their official spouses.

Exclusion criteria were as marriage below 18 years of age, antibiotic uses in the past month, ongoing, and active ocular infection, including conjunctivitis, and eyelid disorders affecting blinking. Couples were also required to not have any prior history with DED, a history of ophthalmic surgery, any systemic disease including autoimmune disease and diabetes, or use of systemic, photosensitizing, or ocular medications except unpreserved lubricants one month before and during the study. Those taking sleep medications or antidepressants, and contact lens use within three months of or during the study were excluded.

Overall, twelve couples were excluded because the women were taking medicines known to affect ocular microbiota composition. Thirteen couples were excluded due for personal reasons. The remaining 815 couples were all living with their spouses in a same house.

Assessment of Sleep Quality and DED

The participants voluntarily completed the validated Persian versions of the Pittsburgh Sleep Quality Inventory (PSQI) [17]. Insomnia was defined as coexistence of both daytime dysfunction and difficulty resuming sleep [18]. Hypersomnia was defined by a bed-rest total sleep time ≥19 hour during the 32-hour recording [19]. Participants were categorized as healthy spouses, insomniac spouses and hypersomniac spouses.

Ocular surface variables were assessed at Day 1 and Day 180. Participants were asked to fill out the validated Persian version of Ocular Surface Disease Index (OSDI) which is used to evaluate the DED severity, as recommended by the accepted guidelines [20]. According to the OSDI score, DED is classified as mild (13–22 points), moderate (23–32 points), or severe (33–100 points). We combined mild and moderate-to-severe DED. Schirmer's test without anesthesia was performed on both eyes

and the mean was calculated for all participants who had DED for use in the study. Finally, tear osmolarity was measured once from each eye using the TearLab Osmolarity System (TearLab Corporation, Inc, San Diego, CA) [21]. DED results are reported as the average of the two eyes of each participant ± SD.

Ocular Microbiora Sample Collection and Culture

The detailed procedure for sample collection and bacterial culture is describe elsewhere [16]. Briefly, culture of the lower conjunctival sac was collected by two qualified ophthalmologists using sterile nylon swabs (FloqSwabs, Copan, Italy), without any topical anesthetic. Tear samples were taken from both eyes. Then, a Floq swab was applied to both eyes and was used for culture analysis. Conjunctiva and tear samples were collected in a standard way by the same qualified ophthalmologist. Swabs were sealed in containers without any medium and placed on ice instantly after the collection and during transportation to the laboratory. Then, swabs from all participants were used to inoculate agar growth medium immediately upon arrival at the laboratory (about 4-6 h). For susceptibility testing of Staphylococcus sp. and Enterococcus sp. we applied a product protocol [22] using GPALL1F plates (Sensititre; Thermo Scientific).

*Data analyses of clinical samples and statistical analyses*

Statistical analysis was carried out using IBM SPSS version 24.0.0.2. The chi-square test was used to compare the frequency of DED among the groups. For normally distributed data, we used ANOVA to compare the means of the groups. For non-normally distributed data, we used the Kruskal–Wallis test and post hoc analyses were performed using the Tukey-Kramer protected least significant difference test.   Alpha and Beta diversity was evaluated using Shannon's diversity index and Bray-Curtis dissimilarity, respectively. Spearman's rank correlation coefficients were used to assess the correlation between ocular microbiota and DED. Age and sex differences were analyzed to discard any existing statistical influence. Statistical analyses of date related to ocular microbiota were done using microbiome R packages [23]. $P < 0.05$ represents statistical significance.

Results

This is an interim result and the detailed results will be published in full in a peer-reviewed journal. Men had an average age of 36.40 years, with a standard deviation (SD) of 7.20. 81.7% had attained a college degree. Women had an average age of 30.30 years (SD = 8.70), and 87.2% had attained a college degree. The couples had been married and living together for an average of 6.10 months (SD = 0.90).

The diagram of categorization and enrollment of participants is shown in **Figure 1.**

Figure 1 Diagram of categorization of participants according to sleep disturbance status and dry eye disuses
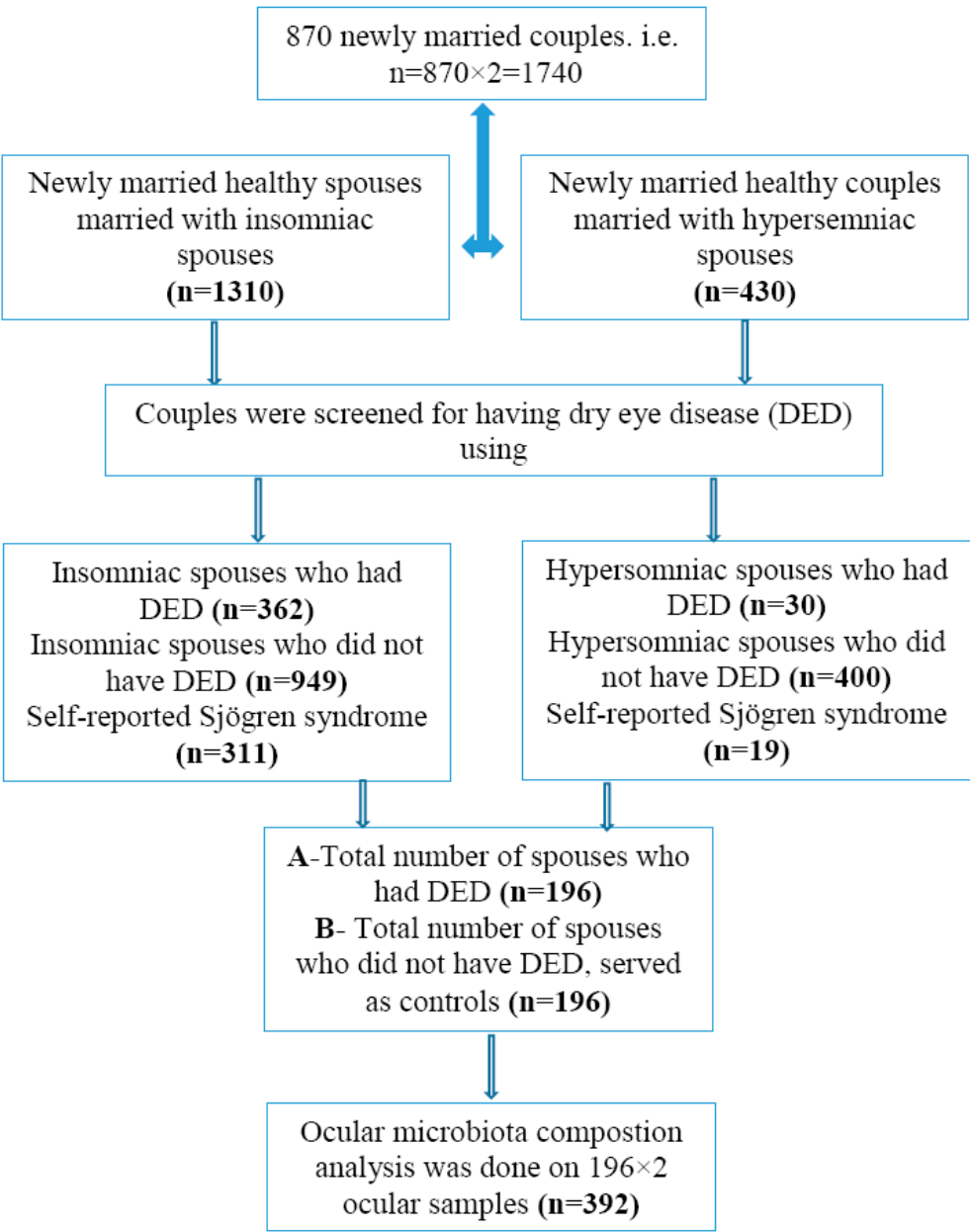
Overall, data drawn from 870 couples were analyzed for general characteristics (see Figure 1), however, as for ocular microbiota composition, we only analyzed the 392 participants who had definite DED.

Table 1 Baseline characteristics of the participants

Characteristic	Healthy controls (n=196)	Spouses married with an insomniac (n=196)	Spouses married with an hypersomnia (196)	P value
Sex (M: F)	1:1	1:1	1:1	-
Age				
Males	36.40 ± 7.20	36.40 ± 7.20	36.40 ± 7.20	NS
Females	30.30 ± 7	30.30 ± 8.70	30.30 ± 8.70	NS
OSDI*				
Moderate to severe	2.3%[a]	38.2%[b]	7.5%[c]	[a,b,c]0.001
Mild	15.3%[a]	44.8%[b]	4.5%[c]	[a,b,c] 0.003
Normal	82.4%[a]	17%[b]	88%[c]	[a,b& b,c]0.001, [a vs. c]NS
Schirmer's test (mm)	18.1 ± 3.0[a]	8.1 ± 4.3[b]	17.2 ± 3.1[c]	[a,b& b,c]0.001, [a vs. c]NS
Tear Osmolarity (mOsm/L)	304 ± 80	318 ± 20	299 ± 98	0.039
Total PSQI** score	3.5±3.[0a]	13.5±3.8[b]	5.6±2.8[c]	0.01
Self-reported Sjögren syndrome	0	27.84%	7.5%	0.0001
Sjögren syndrome –DED	0%	85.90%	63.33%	0.0001

* Ocular Surface Disease Index. According to the OSDI score, DED is classified as mild (13–22 points), moderate (23–32 points), or severe (33–100 points). We combined mild and moderate to severe DED. We classified DED as mild (13–22 points), moderate (23–32 points), or severe (33–100 points). We combined mild and moderate to severe DED and presented the data as percent. Variables related to DED are reported as the average of the two eyes of each participant.
** Pittsburgh Sleep Quality Inventory. Pittsburgh Sleep Quality Index: In scoring the PSQI, seven component scores are derived, each scored from zero (no difficulty) to three (severe difficulty). The component scores are summed to yield a global score (range 0-21). Higher scores demonstrate worse sleep quality.
Categorical data were compared with chi-square test. Quantitative data were compared with ANOVA (Tukey HSD).

Table 1 shows the baseline characteristics of the participants. As can be seen from this table, the frequency of severe DED in spouses who had married with an insomniac person was significantly higher than healthy controls at baseline. Self-reported Sjögren's Syndrome-related DED in spouses who had married to an insomniac and a hypersomniac person was 85.9% (311 out of 326) and 63.33 (19 out of 30) (63.33%), respectively.

As can be seen from **Table 2**, mean total PSQI score in a subsample of spouses who had married an insomniac person was significantly higher than that of the two other groups. In general, healthy controls, and spouses who had married a hypersomniac person were more similar to one another than were spouses who had married an insomniac person (**Table 2**).

Table 2 Sleep quality measures at baseline in three groups of participants

	Healthy spouses married with an insomniac person (n=49)		Healthy spouses married with a hypersomniac person (n=46)		Insomniacs (n=49)		Hypersomniacs (n=46)	
	Day 1	Day 90	Day 1	Day 90	Day 1	Day 90	Day 1	Day 90
Sleep quality	0.5±0.6	1.0±0.8	0.5±0.7	1.2±1.0	2.0±0.7	2.1±0.8	1.0±0.6	1.1±0.9
Sleep latency	0.8±0.9	1.4±0.9	0.9±1.0	1.2±1.1	2.3±0.9	2.2±1.1	1.0±0.9	0.9±1.1
Sleep duration	0.4±0.8	2.6±1.4	0.5±0.9	2.2±1.7	2.2±0.9	2.1±0.8	0.4±0.7	0.3±0.8
Sleep efficiency	0.3±0.6	2.2±1.7	0.3±0.8	1.1±1.2	1.8±1.1	1.8±1.1	0.5±0.8	0.5±0.9
Sleep disturbance	0.6±0.6	1.1±0.9	0.5±0.7	0.7±0.9	1.8±0.5	1.9±0.6	1.1±04	1.3±0.6
Sleep medication	0.03±0.2	2.4±1.7	0.01±0.3	0.2±1.1	1.6±1.4	1.7±1.3	0.2±0.7	0.2±0.9
Daytime dysfunction	0.7±0.9	3.1±1.7	0.7±1.0	1.1±0.9	1.7±0.9	1.8±1.1	1.1±0.7	1.2±0.9
Total PSQI** score	3.5±3.0	9.5±4.2	3.3±2.6	7.2±4.9	13.5±3.8	14.5±3.9	5.6±2.8	6.4±3.1

** Pittsburgh Sleep Quality Index: In scoring the PSQI, seven component scores are derived, each scored from zero (no difficulty) to three (severe difficulty). The component scores are summed to yield a global score (range 0-21). Higher scores demonstrate worse sleep quality.
Data were analyzed by the Kruskal–Wallis test. All Tukey-HSD post-hoc tests between groups were significant (P values < 0.001), with the exception of "Healthy spouses married with an insomniac person" vs. "Healthy spouses married with a hypersomniac (Day 1)".

Distribution of DED among Participants

Table 3 demonstrates the distribution of study participants according to dry eye symptoms and sleep status at baseline and six months follow up. At baseline, there were significant differences among the three groups in terms of ocular microbiota composition (see below) and DED frequency. However, six months after marriage, these significant differences were even more pronounced, i.e., individuals who were married with an insomniac spouse having DED phenotype (**see Figure 2**), were more significantly likely to develop DED after six months follow up. Interestingly, the case was reverse in spouses who had married with a hypersomniac person. In support of our initial hypothesis, these changes occurred in parallel to the changes in ocular microbiota composition (see below, P <0.0001). After six months, ocular microbiota composition in healthy spouses who did not have DED and had a normal sleep pattern were significantly changed and became similar to that of participant's

insomniac spouse. If the spouse was insomniac, then ocular microbiota composition became similar to his/her insomniac spouse (P < 0.001).

Table 3 Distribution of study participants according to dry eye symptom and sleep status at baseline and six months follow up.

	Healthy spouses married With an insomniac person	Healthy spouses married with a hypersomniac person
	*Baseline**	
Spouses with DED£	362 (295.13) [15.15]	30 (96.87) [46.16]
Spouses without DED	948 (1014.87) [4.41]	400 (333.13) [13.42]
	*Six months later***	
Spouses with DED	453 (356.86) [25.9]	21 (117.14) [78.9]
Spouses without DED	857 (953.14) [9.7]	409 (312.86) [29.54]

£ Dry eye disease. DED was evaluated using the Ocular Surface Disease Index and was further confirmed with Schirmer's test (score of less than 5 mm/5'). We combined mild and moderate to severe DED. We performed Schirmer's test for all participants. DED was confirmed in all the participants. A positive Schirmer's test (≤10 mm) was confirmed in all spouses with mild and moderate to severe DED. DED results are reported as the average of the two eyes of each participant.
*At baseline, there were significant differences among three groups in terms of ocular microbiota composition (data not shown) and DED (The chi-square statistic is 79.0338. The p-value is < 0.00001).
** Six months after the marriage, significant differences were even more pronounced, i.e., individuals who were married with an insomniac spouse who had an insomniac ocular composition phenotype were more significantly likely to develop DED, while the case was reverse in spouses who had married with a hypesomniac person (The chi-square statistic is 144.0408. The p-value is < 0.0001). These changes were parallel to the changes in ocular microbiota composition in spouses (P<0.0001).

Table 4 shows dry eye parameters in the three groups of participants at baseline and six months later. Notably, after six months follow-up, tear osmolality and OSDI scores in healthy spouses married with an insomniac spouse were significantly changed and becomes similar to that of participant's spouse (all P values <0.001), while the reverse was true in case of Schirmer's test (P <0.01). Interestingly, eye parameters were better for hypersomniac group and healthy spouses who had married with hypersomniacs. In these two groups, OSCDI score, Schirmer's and tear osmolality were close to normal values. Hypersominacs and healthy spouses who had married a hypersomniac had the best values both at baseline and after six months follow-up. Mean Schirmer's test values were significantly lower in spouses who had married an insomniac person. Mean tear osmolality in healthy controls and in spouses who had married a hypersomiac were similar. However mean tear osmolality values in spouses who had married an insomniac person were significantly higher than that of the two other groups (P<0.01).

Table 4 Dry eye parameters in three groups of participants at baseline and six month later

	HSMWI* (n=196)		HSMWH** (n=196)		Insomniacs (n=196)		Hypersomniacs (n=196)	
	D1	D180	D1	D180	D1	D180	D1	D180
OSDI score	12.4±13.0	28.5±20.5	11.4±11.0	11.9±12.2	32.5±22.6	33.4±23.7	9.4±11.1	10.4±9.2
Schirmer's Test (mm)	18.1 ± 3.0	10.2 ± 8.7	17.2 ± 3	15.2 ± 4	8.1 ± 4.3	8.8 ± 3.9	17.2 ± 3	16.9 ± 4
Tear Osmolarity (mOsm/L)	305 ± 70	310 ± 10	303 ± 90	290 ± 10	318 ± 20	319 ± 30	299 ± 95	300 ± 100

*HSMWI: Healthy spouses married with an insomniac, **HSMWH: Healthy spouses married with a hypersomniac.
At baseline, for all variables, there were no significant differences for HSMWI vs. HSMWH, HSMWI vs. Hypersomniacs, and HSMWH vs. Hypersomniacs. Variables related to DED are reported as the average of the two eyes of each participant.
Six months later, there was a significant differences for HSMWI vs. HSMWH, and HSMWI vs. Hypersomniacs (P<0.01). Within group changes for HSMWH, Insomniac and Hypersomniac groups were not significant after six months.
Data were analyzed by the Kruskal–Wallis test (Tukey-HSD post-hoc).

Alpha and Beta Diversity among Participants

There were significant differences in alpha diversity among the three groups of newly married couples who had married with an insomniac or hypersomniac spouse: the healthy control group with healthy sleep pattern, had the highest similarity (Chao1, Faith's phylogenetic diversity), while the mild DED group demonstrated the highest diversity (Shannon, Simpson), and the moderate-to-severe DED group had the lowest similarity for the above-mentioned indices.

Interestingly, reduction in beta diversity of the ocular microbial community was positively associated with DED severity, with the moderate-to-severe DED group exhibiting the lowest beta diversity. Linear discriminant analysis effect size showed marked dominant taxa that were significantly different among each group. In addition, random forest analysis showed that exacerbation of DED corresponds with the enrichment of certain pathogenic bacteria.

Identification of Species Differences and Marker Species

At baseline, the vast majority of the 392 analyzed ocular samples from healthy spouses and healthy hypersomniacs were highly enriched with Staphylococcus, whereas only in a few of them, other genera such as Corynebacterium, Bacillus and Pseudomonas predominated.

C. pseudogenitalium-tuberculostearicum and C. mastitidis were also abundant in these two groups.

On the other hand, in the group of insomniacs, Blautia wexlerae was found to be most associated with DED followed by different Corynebacterium species, namely C. accolens, C. bovis and C. kroppenstedtil. **Figure 2** shows the top 5 most abundant species in the three groups.

Figure 2 The top 5 most abundant species in the three groups.

Species by order of abundance	Healthy spouses	Hypersomniacs	Insomniacs
	Baseline		
	C.* pseudogenitalium-tuberculostearicum	C. pseudogenitalium-tuberculostearicum	Blautia wexlerae
	C. mastitidis	C. mastitidis	C. accolens
	Staphylococcus captis-caprae-epidemidis	Staphylococcus captis-caprae-epidemidis	Staphylococcus captis-caprae-epidemidis
	Cutibacterium acnes	Cutibacterium acnes	C. bovis
	Corynebacterium accolens-macginleyi	Corynebacterium accolens-macginleyi	C. kroppenstedtil
Baseline Phenotype	Healthy phenotype	Healthy phenotype	DED phenotype
	Six months follow up		
Species by order of abundance	Healthy spouses	Hypersomniacs	Insomniacs
	Blautia wexlerae	C. pseudogenitalium-tuberculostearicum	Blautia wexlerae
	C. mastitidis	C. mastitidis	C. accolens
	C. accolens	Staphylococcus captis-caprae-epidemidis	C. accolens
	C. bovis	Cutibacterium acnes	C. bovis
	Corynebacterium accolens-macginleyi	Corynebacterium accolens-macginleyi	C. kroppenstedtil
Six months Phenotype	A mixture of Healthy and DED phenotype	Mostly healthy phenotype	DED phenotype

*C.: Corynebacterium.

The frequency of both mild and moderate-to-severe DED phenotype in healthy spouses who were married with an insomniac person was significantly increased after six months follow up, suggesting that there is a possibility that ocular microbiota alteration might have played a role in occurrence of DED in this group (P<0.001). The changes were not significant for the hypersomniacs.

Correlation of DES with Ocular Microbiota

The DED phenotype was positively significantly correlated with species differences in each of the three groups (P value <0.001). At baseline, the frequency of both mild and moderate-to-severe DED phenotype was significantly highest in insomniacs. Interestingly, in support of our hypothesis, the frequency of both mild and moderate-to-severe DED phenotype in healthy spouses was significantly increased after six months of marriage with an insomniac person (but not with a hypersomniac person), suggesting that there is a possibility that ocular microbiota alteration might have played a role in the occurrence of   DED in this group.

Mediation Analysis

To better understand the role of ocular gut microbiota in DED and insomnia/hypersomnia status, we performed interaction effects assessment and mediation analyses in 588 participants (i.e., 196 spouses in each group).

We suspected interaction effects between these three variables with insomnia/hypersomnia status as the putative mediator of the link between ocular microbiota and DED/non-DED status. In fact, insomnia/hypersomnia is a significant predictor of DED/non-DED status (β = 0.42, P < 0.0001) which in turn is also a relevant predictor for insomnia/hypersomnia (β = 0.18, p < 0.0001). The mediation analysis explained 28% of the variability in the data (R^2 = 0.28).

Discussion

We found that the frequency of DED in spouses who married insomniac spouses with DED phenotype significantly increased over time, as compared to baseline values. In line with our initial hypothesis, the occurrence of DED in healthy spouses corresponded with the enrichment of certain pathogenic bacteria which was quite similar to the abundance pattern of ocular microbuta in their corresponding spouses. To the best of our knowledge, this is the first study to provide preliminary empirical evidence suggesting that ocular microbiota transmission between two people may play a role in the development of DED, particularly over such a short term, about 6 months follow-up.

Ocular microbiota composition in DED participants in our study was similar to the results of other reports [16,24–26], with highest resemblance to [16]. The reason may partly be that we applied the same methodology [16]. We noticed that as DED occurrence increases, microbial community diversity tends to decrease. This is in agreement with Zou et al [26]. DED frequency corresponds with alterations in microbial constituents, primarily characterized by diminished microbial diversity and a more uniform species composition.

We found that Blautia wexlerae was the most associated with DED followed by different Corynebacterium species, namely C. accolens, C. bovis and C. kroppenstedtil. Despite Staphylococcus captis-caprae-epidemidis [27], Corynebacterium bovis [28] and Corynebacterium kroppenstedtii [29] which are generally regarded as opportunistic pathogens, both C. accolens and Blautia wexlerae are generally considered as commensal bacteria [30,31]. Blautia wexlerae is an anaerobic and gram-positive bacteria that lives in the human intestine [31] and C. accolens is a lipophilic species which is commonly found in the oropharynx, nose, ears, and eyes [30]. These two species are not pathogenic per se. For instance, a recent comprehensive metagenomics study found that in Parkinson's disease (PD) patients, Blautia wexlerae was decreased by fivefold compared to neurologically healthy controls [32].

Self-reported Sjögren's Syndrome-related DED in spouses who had married to an insomniac and a hypersomniac person was 85.9%, which is relatively high compared to other studies [33] and might be partially related to severity of insomnia in participants of current study. Also, higher frequency of insomnia was found in insomniacs with symptomatic DED, and insomnia correlated significantly with DED symptoms, which is in agreement with other studies [34].

Sjögren's Syndrome (SS) is an autoimmune disorder that affects exocrine glands, typically the lacrimal and salivary glands, leading to dry mouth and dry eye [35]. On the other hand, SS may lead to decreased diversity of the ocular surface microbial community [36], which can be potentially

casually related to the pathophysiology of DED, particularly in patients with SS, as results of some Mendelian randomization studies suggest [37–39].

Regarding the possible role of gut microbiota in DED, our previous study [40] revealed that three months after the marriage, spouses with healthy sleep patterns were significantly more likely to resemble their insomniac or hypersomniac couples. Gut microbiota composition in participants with normal sleep patterns was significantly changed and becomes similar to that of the participant's spouse, i.e., if the spouse was insomniac or hypersomniac, then gut composition becomes similar to his/her insomniac or hypersomniac spouse, respectively. The results of mediation analysis confirmed the association between the changes in the sleep pattern and changes in the gut microbiota. This novel study provides indirect evidence suggesting that closeness between couples may potentially have a role in DED through changes in gut microbiota composition.

Goodman et al [41] in a case-control study, examined the composition of the gut microbiota in individuals with and without immune-mediated DED. They found clear differences in gut microbita composition in individuals with mostly early markers of SS compared with controls. There are other studies confirming that gut dysbiosis is prevailing in SS patients and is related to DED severity [42].

According to a review article by Moon et al [43], recent clinical studies have typically discovered an association between clinical manifestations of SS such as DED and gut dysbiosis, whereas environmental DED displays characteristics between normal and autoimmune. Furthermore, a decrease in both the genus Faecalibacterium and Firmicutes/Bacteroidetes ratio have most typically been seen in patients with SS. Interested readers may refer to a review article by Moon et al [43].

This paper is obviously not intended to provide a detailed discussion on mechanistic and metagenomic analysis. However, if it proves that the association between these species and increased frequency of DED in healthy spouses married to insomniac individuals is a cause-and-effect relationship, then, one possible mechanism is that following marriage and spouse-to-spouse contact, some species might competitively displace other native species, as **Figure 2** suggests. In support of this argument, in animal models of SS, it is shown that commensal bacteria protect lacrimal glands through immunoregulatory properties [44]. Indeed, one of the authors has elsewhere [45] discussed how closeness may facilitate bacterial transmission and consequently, change gut and ocular microbiota in people who are in close contact with one another such, as the couples in the present study. The animal models can provide platforms for further investigation of the underlying mechanism of DED and its association with gut and ocular microbiota.

The frequency of both mild and moderate-to-severe DED in hypersomniacs was similar to the healthy group. This might be related to higher yawning frequency in hypersomniacs, which in turn relates to higher ocular lacrimation [15,46]. However, we did not measure yawning in our study. We also did not take into account higher rates of yawning frequency in hypesomniac and their spouses, because these were all self-reported and we were not sure how reliable these self-reports were. Future studies may consider assessing yawning frequency quantitatively.

We could not find any study assessing ocular microbiota composition in insomniac and hypersomniac people. Therefore, our report contributes to the literature regarding the profile of ocular microbiota composition in sleep-related disorders. We suggest that within the framework of diagnostic, predictive, preventive, and personalized medicine, the practical and theoretical implications of this study may add to the understanding of broad areas of microbiota-host research.

Strengths

To our knowledge, this is the first study reporting parallel changes in ocular microbiota and DED frequency and severity. Also, we used a large sample size with minimum dropout rate. Finally, we propose a paradigm-changing hypothesis that DED can be partially a transmitted disease.

Limitations

Many other confounding factors, such as screen viewing, face mask use, occupation, personal hygiene, and contact lens use are involved in DED and sleep status. Future research could gather data on a larger sample size. We did not measure noninvasive Keratograph tear break-up time, tear meniscus height and many other relevant ocular parameters due to budget constraints. Another limitation of this study is that, due to the obligation to deliver the required treatment to the participants with DED, we could not observe long-term impact. We used a culture- based method. Future studies may use sequencing methods. Finally, our findings are cross sectional in nature and do not infer causality. Mechanistic studies in animal models are necessary.

Conclusions

In summary, most contemporary strategies are focused on increasing ocular surface

humidity and decreasing inflammation and osmolarity. Future medications and supplements may

improve ocular microbiota composition and tear osmolarity at the ocular surface. This novel strategy should be considered in future investigations and clinical trials. Furthermore, within the framework of diagnostic, predictive and preventive personalized medicine (and family medicine as well), the practical and theoretical implications of this study cover many areas of medicine, including host-microbiota research and sleep studies.

Future Directions

Recent studies suggest that gut microbiota profiles in myopes and nonmyopes may be different [47,48]. Other studies show an association with intraocular inflammation [49], corneal neovascularization [50], fungal micobiota [51] and other ocular surface pathophysiology and disorders [52]. This creates a unique opportunity for future microbiota and host research using animal models.

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