Pain Sensitivity Associated With the Length of the Maximum Interblink Period

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PURPOSE. Pain sensitivity has been identified as a factor that affects how individuals answer dry eye questionnaires, but it is unknown how it affects ocular discomfort. This study used the time that individuals could refrain from blinking as an indicator of ocular discomfort and set out to determine whether it was related to pain sensitivity, while adjusting for ocular surface conditions.

METHODS. Subjects first completed the Pain Sensitivity Questionnaire to quantify pain sensitivity levels. Exposed interpupillary area, tear meniscus height, tear-film lipid layer thickness, ocular surface cooling, and noninvasive tear breakup were assessed. Subjects were then asked to refrain from blinking until the initial onset of discomfort, which was termed “the maximum interblink period” (MIBP), while ocular surface cooling rate was simultaneously measured. Subjects were seen for four visits over a course of 2 days.

RESULTS. Forty-two subjects (36 females, 6 males) completed the study, with a mean (SD) age of 23.2 (3.8) years. A longer MIBP was associated with decreased pain sensitivity ($P = 0.04$), lower ocular surface cooling rate ($P < 0.001$), and Asian ethnicity ($P = 0.005$). Based on the results from the mixed-effect model, it is estimated that individuals would be able to refrain from blinking for an additional 4 seconds if they had the lowest (0.6) compared to the highest (6.1) pain sensitivity in the study cohort.

CONCLUSIONS. The Pain Sensitivity Questionnaire was associated with the MIBP length even after adjusting for ocular surface conditions, which suggests that pain sensitivity plays a role in influencing how ocular discomfort is perceived.

Keywords: pain sensitivity, blink refrainment, ocular surface cooling, blink, dry eye
ocular discomfort, with length of blink refrainment as a measure of ocular discomfort. Therefore, the purpose of this study was to determine if pain sensitivity was associated with how long subjects could refrain from blinking. It was hypothesized that a subject with lower pain sensitivity would be less perceptive to ocular discomfort and could refrain from blinking for a longer period of time than a subject with higher pain sensitivity. It would also be important to factor in ocular surface parameters, as they have been shown to influence the length of blink refrainment, and to consider dry eye questionnaires, as they reflect the ocular discomfort that subjects encounter in their daily lives.10,11,16

### Materials and Methods

#### Study Population

Subjects were recruited from the University of California, Berkeley, and the surrounding community. Subjects taking systemic or ocular medication, or having a history of systemic or ocular disease or surgery, were excluded from the study. Subjects were also excluded if they had undergone refractive surgery, had a history of ocular allergies, or had an eyelid or conjunctival abnormality.

Subjects aged 18 to 39 years were eligible for the study and consisted of individuals who were of Asian or non-Asian descent. These two groups were selected on the basis of studies that have found interethnic differences in pain sensitivity.17 and dry eye prevalence rate.18,19 Individuals were considered to be Asians if they were of Chinese, Taiwanese, Japanese, or Korean descent, or a mixture of these ethnicities. Individuals were considered to be non-Asians if they were of any other ethnicity (e.g., European white, Latin American, Indian, African descent). Informed consent, with a complete description of the goals, risks, benefits, and procedures of the study was obtained from all participants. This study observed the tenets of the Declaration of Helsinki and was approved by the University of California, Berkeley Committee for Protection of Human Subjects.

#### Instrumentation and Measurements

Subject pain sensitivity was measured by using the PSQ, which has been validated in normal and chronic pain populations.20,21 and in ocular surface research.2 Subject symptoms associated with dry eye and contact lens discomfort were assessed by using the OSDI, Contact Lens Dry Eye Questionnaire-8 (CLDEQ-8),22 and the University of California, Berkeley Clinical Research Center Dry Eye Flow Chart (DEFC).23 These questionnaires were included as it was thought that subject symptomology might influence MIBP length. Anterior ocular surface health was evaluated with slit lamp biomicroscopy (SL120; Carl Zeiss Meditec, Inc., Jena, Germany) under white light. Noninvasive tear breakup time (NIBUT) was measured by using the Medmont e300 corneal topographer (Medmont International Pty Ltd., Victoria, Australia). Tear meniscus height was measured by using the Oculus Keratograph 5m (Oculus, Inc., Arlington, WA, USA). Average tear-film lipid layer thickness was assessed by using the LipiView interferometry system (TearScience, Inc., Morrisville, NC, USA).

Ocular surface temperature was measured by using a combination digital thermometer and hygrometer (General Tools & Instruments, Secaucus, NJ, USA). Room temperature and humidity were typically at 25°C and 48%, respectively.

#### Study Protocol

Seasoned contact lens wearers were administered a set of baseline questionnaires, which was composed of the PSQ, OSDI, CLDEQ-8, and the DEFC. Subjects were seen for four visits over the course of 2 days (two visits per day, consisting of a morning and an afternoon visit), providing four measurements per subject. All visits had the same set of procedures except that the afternoon visits included an assessment of corneal staining type, depth, and extent with sodium fluorescein instillation using the Cornea and Contact Lens Research Unit (CCLRU) grading scale. Subjects were asked to discontinue contact lens wear for at least 24 hours before their visits. All measurements were done on the right eye only and on an ocular surface without contact lenses. Digital photos were taken of the subject’s eye in primary gaze position, using a smartphone (iPhone 6; Apple, Cupertino, CA, USA) while the subject held a United States 1-cent coin (diameter = 1.91 cm) in the same plane as the eye to help calibrate the magnification. Digital images were then processed with Adobe Photoshop CS5.5 (Adobe Systems, San Jose, CA, USA) to determine the exposed interpalpebral area (conjunctiva and cornea) in square centimeter (cm²) by pixel counting.25

Anterior ocular health was assessed with slit lamp biomicroscopy under white light to ensure there was no evidence of active or preexisting ocular pathology (e.g., corneal scars, infiltrates, excessive corneal epithelial irritation). Subjects were then taken to a different examination room and acclimated to the ambient environment for a minimum of 10 minutes before testing started. Tear-film lipid layer thickness was measured first and then tear meniscus height was measured, with the investigator (WL) marking the height of the tear meniscus directly below the 6-o’clock position of the cornea. NIBUT was measured three times, with a 30-second break between each measurement and an endpoint consisting of the first visible disruption noted on the placido mires or upon a blink. Ocular surface temperature and MIBP measurements were conducted at the same time, where subjects were placed in a slit lamp head- and chinrest assembly, which minimized head movement during recordings; no slit lamp lighting was introduced during this measurement. Instructions to subjects during ocular surface temperature and MIBP measurements were scripted, so during each visit they were asked to “please close your eyes for 2 minutes and after opening your eyes, refrain from blinking until you experience the initial onset of ocular discomfort.” The tone of voice for giving directions was standardized to a neutral affect in an attempt to minimize potential influence on the MIBP (e.g., subjects may try to hold their eyes for longer if the investigator
uses a more forceful voice). The ocular surface temperature recording was later reviewed to determine the MIBP length, using time stamps on the recording.

Statistical Methods

The PSQ provides three numerical values: the overall pain sensitivity score (PSQ-Total), and scores for sensitivity to situations with minor (PSQ-min) and moderate (PSQ-mod) pain. Our previous study determined that the PSQ-min score most accurately reflects the influence of pain sensitivity on dry eye questionnaires. Therefore, the analysis will only focus on the PSQ-min score and will be referred as the “PSQ score.”

Data were analyzed with R statistical package (version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria).

A thorough exploratory and descriptive preliminary analysis was conducted by assessing bivariate plots and univariate models to examine for possible significant associations between explanatory and outcome variables, which guided how multivariate modeling was used. The statistical models factored the MIBP length as the outcome variable, with sex, ethnicity, exposed interpalpebral area, tear meniscus height, NITBUT, ocular surface cooling rate, average tear-film lipid layer thickness, and PSQ score as potential explanatory variables. Linear mixed-effects modeling was used to account for potential within-subject correlations related to repeated measurements. Upon examining residual plots, the MIBP length was natural-log transformed to better approximate normality to meet key assumptions for statistical modeling. The normality to meet key assumptions for statistical modeling. The results were reported after back-transformation. In all tests, results with $P \leq 0.05$ and $P \leq 0.10$ were considered statistically significant and borderline significant, respectively.

Univariate linear mixed-effects modeling identified explanatory variables that were associated with the outcome variable. Significant or borderline significant explanatory variables identified in univariate modeling were then examined by using multivariate linear mixed-effects modeling. A stepwise regression procedure with consideration of F-test $P$ values and examination of residual and other diagnostic plots was used to determine accurate multivariate regression models.

RESULTS

Subject Characteristics

Forty-two subjects completed the study (36 females, 6 males), with a mean (SD) age of 23.2 (3.8) years and a range of 18 to 34 years. Table 1 shows the clinical measurements from the study cohort. There were 22 subjects of Asian descent and 20 subjects of non-Asian descent. Table 2 shows the measurements between Asians and non-Asians, with no interethnic difference noted in PSQ score ($P = 0.79$), OSDI score ($P = 0.96$), DEFC score ($P = 0.87$), CLDEQ-8 score ($P = 0.52$), ocular surface cooling rate ($P = 0.42$), NITBUT ($P = 0.78$), average tear-film lipid layer thickness ($P = 0.25$), tear meniscus height ($P = 0.12$), and years of contact lens wear ($P = 0.15$). Asians were found to have a longer MIBP (12.4 s vs. 9.2 s; $P = 0.02$), less exposed interpalpebral area ($2.7 \text{ cm}^2$ vs. $2.9 \text{ cm}^2; P = 0.04$), slightly older (24.5 years versus 21.6 years; $P = 0.02$), and had greater aggregate corneal staining type (0.8 vs. 0.1; $P = 0.01$), extent (0.5 vs. 0.1; $P = 0.04$), and depth (0.5 vs. 0.1; $P = 0.03$) as compared to non-Asians. Subjects were all contact lens wearers.

Univariate and Multivariate Modeling

In univariate modeling (Table 3), a longer MIBP was associated with a reduced ocular surface cooling rate ($P < 0.001$; Fig. 1), a lower PSQ score ($P = 0.04$; Fig. 2), Asian ethnicity ($P = 0.02$; Fig. 3), and male sex ($P = 0.01$); it was borderline associated with a longer NITBUT ($P = 0.06$) and greater aggregate corneal staining depth ($P = 0.08$). No association was noted with age ($P = 0.35$), years of contact lens wear ($P = 0.34$), aggregate corneal staining type ($P = 0.15$), aggregate corneal staining extent ($P = 0.19$), tear meniscus height ($P = 0.64$), average tear-film lipid layer thickness ($P = 0.61$), exposed interpalpebral area ($P = 0.67$), OSDI score ($P = 0.26$), DEFC score ($P = 0.72$), and CLDEQ-8 score ($P = 0.60$). Interaction terms were considered but none were found to be significant.

In multivariate modeling (Table 4), a longer MIBP was associated with a reduced ocular surface cooling rate ($P < 0.001$), a lower PSQ score ($P = 0.04$), and Asian ethnicity ($P = 0.003$). No association was noted with aggregate corneal staining depth ($P = 0.72$), NITBUT ($P = 0.21$), and sex ($P = 0.22$). Based on the model, there would be an estimated 8.7-second increase in the MIBP length when comparing the lowest (0.002°C/s) to the highest (0.27°C/s) ocular surface cooling rate. In addition, there would be an estimated 3.6-second increase in the MIBP length when comparing the lowest PSQ score (0.6) to the highest PSQ score (6.1). Supplementary Video S1 shows an example of how MIBP was conducted by assessing bivariate plots and univariate models to examine for possible significant associations between explanatory and outcome variables, which guided how multivariate modeling was used. The statistical models factored the MIBP length as the outcome variable, with sex, ethnicity, exposed interpalpebral area, tear meniscus height, NITBUT, ocular surface cooling rate, average tear-film lipid layer thickness, and PSQ score as potential explanatory variables. Linear mixed-effects modeling was used to account for potential within-subject correlations related to repeated measurements. Upon examining residual plots, the MIBP length was natural-log transformed to better approximate normality to meet key assumptions for statistical modeling. The results were reported after back-transformation. In all tests, results with $P \leq 0.05$ and $P \leq 0.10$ were considered statistically significant and borderline significant, respectively.

Univariate linear mixed-effects modeling identified explanatory variables that were associated with the outcome variable. Significant or borderline significant explanatory variables identified in univariate modeling were then examined by using multivariate linear mixed-effects modeling. A stepwise regression procedure with consideration of F-test $P$ values and examination of residual and other diagnostic plots was used to determine accurate multivariate regression models.

DISCUSSION

Research on ocular discomfort related to dry eye has primarily focused on understanding how conditions on the ocular surface (e.g., ocular surface cooling, tear hyperosmolarity) influence the level of discomfort experienced. Advances in technology will improve the accuracy of measuring ocular surface conditions, but it would be unsurprising if a weak-to-moderate correlation between measurements and dry eye symptoms is still observed. There are similar parallels to when clinical tests measuring skin conductance and cytokine levels were hailed as potential gold standards for measuring...
pain because they objectively assess physiological markers for pain. Tellingly, these tests were never readily adopted by clinics and have been relegated to niche use.31,32

Nevertheless, although the development of new diagnostic technology is important, the results from the study argue that it may also be important to understand how intrinsic factors such as pain sensitivity, ethnicity, and sex influence the relationship between signs and symptoms of dry eye. When assessing the multivariate model, ocular surface cooling rate had the greatest estimated effect size on the MIBP length, which is not surprising as ocular surface cooling represents a noxious stimulus on the ocular surface.33 Of interest, although PSQ is unrelated to ocular surface conditions and is considered an intrinsic factor of a subject’s characteristic, it had a relatively large effect size on MIBP length, as the maximum effect size for the PSQ on MIBP length of 3.6 s represents nearly a third of the

| TABLE 2. | Mean, Standard Deviation, and Range for Clinical Measurements of Asian and Non-Asian Subjects, and P Values From Comparing the Two Groups |
|---------------------------------|---------------------------------|---------------------------------|------------------|
| | Asian (n = 22) | Non-Asian (n = 20) | P Value |
| Age | Mean (SD): 24.5 (4.5) y | Mean (SD): 21.6 (2.6) y | 0.02 |
| | Range: 19–34 y | Range: 18–27 y | |
| Years of contact lens wear | Mean (SD): 8.8 (4.9) | Mean (SD): 7.1 (2.2) | 0.15 |
| | Range: 1–20 | Range: 4–11 | |
| Corneal staining type (aggregate) | Mean (SD): 0.8 (1.7) | Mean (SD): 0.1 (0.3) | 0.01 |
| | Range: 0.0–7.5 | Range: 0.0–1.0 | |
| Corneal staining extent (aggregate) | Mean (SD): 0.5 (0.9) | Mean (SD): 0.1 (0.4) | 0.04 |
| | Range: 0.0–3.5 | Range: 0.0–2.0 | |
| Corneal staining depth (aggregate) | Mean (SD): 0.5 (0.9) | Mean (SD): 0.1 (0.3) | 0.03 |
| | Range: 0.0–3.5 | Range: 0.0–1.0 | |
| PSQ score | Mean (SD): 2.9 (1.3) | Mean (SD): 2.8 (1.4) | 0.79 |
| | Range: 0.7–5.6 | Range: 0.6–6.1 | |
| OSDI score | Mean (SD): 9.7 (9.3) | Mean (SD): 9.6 (8.2) | 0.96 |
| | Range: 0.0–31.3 | Range: 0–31.3 | |
| DEFC score | Mean (SD): 2.8 (1.4) | Mean (SD): 2.7 (1.5) | 0.87 |
| | Range: 1.0–5.0 | Range: 1.0–5.0 | |
| CLDEQ8 score | Mean (SD): 10.1 (6.1) | Mean (SD): 11.5 (7.1) | 0.52 |
| | Range: 1.0–22.0 | Range: 1.0–25.0 | |
| Ocular surface cooling rate | Mean (SD): 0.08 (0.06)°C/s | Mean (SD): 0.09 (0.06)°C/s | 0.42 |
| | Range: 0.006°C/s–0.27°C/s | Range: 0.002°C/s–0.26°C/s | |
| NITBUT | Mean (SD): 10.6 (9.9) s | Mean (SD): 11.1 (9.0) s | 0.78 |
| | Range: 2.9–87.0 s | Range: 4.0–48.6 s | |
| Average tear-film lipid layer thickness | Mean (SD): 55 (15) nm | Mean (SD): 59 (18) nm | 0.25 |
| | Range: 34–100 nm | Range: 32–100 nm | |
| Tear meniscus height | Mean (SD): 0.24 (0.07) mm | Mean (SD): 0.27 (0.09) mm | 0.12 |
| | Range: 0.12–0.44 mm | Range: 0.09–0.52 mm | |
| MIBP length | Mean (SD): 12.4 (9.0) s | Mean (SD): 9.2 (8.2) s | 0.02 |
| | Range: 3.3–57.1 s | Range: 2.3–57.9 s | |
| Exposed interpalpebral area | Mean (SD): 2.7 (0.3) cm² | Mean (SD): 2.9 (0.4) cm² | 0.04 |
| | Range: 2.0–3.5 cm² | Range: 2.1–3.6 cm² | |

| TABLE 3. | Separate Univariate Models Showing the Association Between Natural-Log-Transformed MIBP Length and Explanatory Variables |
|---------------------------------|---------------------------------|---------------------------------|------------------|
| | Intercept | Coefficient | P Value |
| Ocular surface cooling rate | 2.68 | –4.84 (per 1°C/s) | <0.001 |
| PSQ score | 2.59 | –0.10 (per unit on the PSQ score) | <0.05 |
| Ethnicity: Asian | 2.12 | 0.34 | 0.02 |
| Sex: Female | 2.72 | –0.50 | 0.01 |
| NITBUT | 2.09 | 0.01 (per s of NITBUT) | 0.06 |
| Age | | | 0.35 |
| Years of contact lens wear | | | 0.34 |
| Corneal staining type (aggregate) | | | 0.15 |
| Corneal staining extent (aggregate) | | | 0.19 |
| Corneal staining depth (aggregate) | 2.25 | 0.12 | 0.08 |
| Tear meniscus height | | | 0.64 |
| Average tear-film lipid layer thickness | | | 0.61 |
| Exposed interpalpebral area | | | 0.67 |
| OSDI score | | | 0.26 |
| DEFC score | | | 0.72 |
| CLDEQ8 score | | | 0.60 |

The arbitrary reference groups for ethnicity and sex were Asian and female, respectively.
mean MIBP length of 10.8 s. This agrees with studies that have found similar trends in other areas of the body and supports our previous work in which the PSQ has been found to influence OSDI by up to 9 points and up to 31 points on a 100-point visual analog scale for comfort and dryness, respectively. It should be noted that Kaido et al. have found that greater corneal sensitivity to pain is associated with increased dry eye symptomology. Although greater corneal sensitivity to pain is a byproduct of dry eye, it is possible that baseline corneal sensitivity to pain (unaffected by contact lens wear or dry eye) is associated with an individual’s pain sensitivity, and further study is warranted on this topic.

In addition to pain sensitivity, ethnicity was another intrinsic factor associated with MIBP length, with Asians having a longer MIBP than non-Asians. Although ocular surface parameters and symptoms play a mediating role in when blinks occur, there was only a minor interethnic difference in corneal staining, which has been previously noted, and no differences in dry eye symptoms. Therefore, the interethnic difference in MIBP length appears to be primarily mediated by ethnicity as an intrinsic attribute.

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First, it may be related to distinct differences in eyelid anatomy between the two groups, with Asians having a smaller vertical palpebral aperture, more oblique palpebral fissure, and
greater herniation of orbital fat in the eyelids.\textsuperscript{37} This causes a smaller surface area of the eye to be exposed to tear-film evaporation, which assuming a similar tear film, would allow Asians more time to refrain from blinking before they experience ocular discomfort.\textsuperscript{38} In this study, Asian subjects were found to have a smaller exposed interpalpebral area than non-Asian subjects; however, the difference in the exposed area was small (\(<10\%\)). More important, the exposed interpalpebral area was not found to be associated with the MIBP length in both univariate and multivariate models, possibly owing to a small range of interpalpebral area obtained in this cohort. Further studies are warranted to improve our understanding about the relationship among exposed interpalpebral area, MIBP, ocular surface cooling, and comfort.

Second, the previous reports of greater tear-film instability\textsuperscript{39,40} and corneal staining in Asians compared to non-Asians\textsuperscript{23} may suggest that Asians are continually exposed to a higher frequency and greater magnitude of noxious stimuli on the ocular surface.\textsuperscript{25,41,42} It is possible that Asians might adapt to a greater proclivity for noxious stimuli by decreasing corneal nociceptor sensitivity as a compensatory mechanism.\textsuperscript{43} Evidence to support this reasoning is seen in the study of Tran et al.,\textsuperscript{23} where dryness symptoms are associated with the severity of corneal staining in non-Asians, but not in Asians. However, owing to the relatively small sample size and makeup of the study cohort, it is impossible to make any conclusive statements on the role of ethnicity on dry eye symptoms and further research is needed. It should be noted that the subjects were all contact lens wearers; studies suggest that contact lens wear may influence corneal sensation.\textsuperscript{55} In addition, studies suggest that contact lens wearers perceive ocular discomfort and dryness differently than non–contact lens wearers, so the results found in this study may not hold true in non–contact lens wearers and further investigation is warranted for different subgroups.\textsuperscript{56,57}

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In addition to pain sensitivity and ethnicity, there was evidence in the univariate model to suggest that sex may be another intrinsic factor that influenced the MIBP length. It is difficult to draw any conclusions regarding sex owing to the small number of males in the study cohort. However, there is significant research that has demonstrated that males and females process pain differently.\textsuperscript{20,45–47} This may offer an insight on why females have a significantly higher dry eye prevalence rate, are more apt to be affected by dry eye in their everyday lives with a greater likelihood of seeking dry eye treatment than males, even though studies have not necessarily found a pronounced ocular surface difference.\textsuperscript{25,48–50} The role of hormones likely plays a key reason in the disparity in dry eye prevalence rates, but the difference in pain perception between males and females should be considered as a possible mediating factor.\textsuperscript{51,52}

The results from this study provide evidence to suggest that pain sensitivity and other intrinsic factors play a role in how ocular discomfort is perceived. A limitation of the study was that the cohort was composed of young and healthy subjects, so it is difficult to know if the same association is seen in older individuals. Nevertheless, one benefit of having a younger cohort is that it is unlikely that any subjects had corneal allodynia or hyperalgesia from nociceptor sensitization, owing to the extended period of time it takes to develop.\textsuperscript{53,54} It should also be noted that the subjects were all contact lens wearers; studies suggest that contact lens wear may influence corneal sensation.\textsuperscript{55} In addition, studies suggest that contact lens wearers perceive ocular discomfort and dryness differently than non–contact lens wearers, so the results found in this study may not hold true in non–contact lens wearers and further investigation is warranted for different subgroups.\textsuperscript{56,57}

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\begin{table}
\centering
\caption{Multivariate Model Showing the Association Between the Natural-Log-Transformed MIBP Length and Significant Explanatory Variables, With Coefficients From the Model Also Listed}  
\begin{tabular}{lccc}
\hline
Outcome & Intercept & Ocular Surface Cooling Rate & PSQ Score & Ethnicity: Asian \\
\hline
\text{ln(MIBP length)} & 2.74 & $-4.63$ ($P < 0.001$) & $-0.077$ ($P = 0.04$) & 0.31 ($P = 0.003$) \\
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\end{tabular}
\end{table}

\begin{figure}
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\includegraphics[width=\textwidth]{figure3}
\caption{Box plot comparing the lnMIBP length between Asians and non-Asians ($P = 0.02$).}
\end{figure}
the exact role that pain sensitivity has on the relationship between signs and symptoms of dry eye. If pain sensitivity is found to play an important role, then it may require a paradigm shift in how dry eye is diagnosed and treated.

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