The Ocular Surface 31 (2024) 31–42

Contents lists available at ScienceDirect

The Ocular Surface

journal homepage: www.elsevier.com/locate/jtos

Gene expression changes in conjunctival cells associated with contact lens wear and discomfort

Andrés Ángel Calderón-García [a,b], Laura Valencia-Nieto [a,b], Cristina Valencia-Sandonis [a,b], Alberto López-de la Rosa [a,b], Marta Blanco-Vazquez [a], Itziar Fernández [a,c,d], Carmen García-Vázquez [a], Cristina Arroyo-del Arroyo [a,b], María J. González-García [a,b,c,*], Amalia Enríquez-de-Salamanca [a,c]

[a] Institute of Applied Ophthalmobiology (IOBA), Universidad de Valladolid, Valladolid, Spain
[b] Department of Theoretical Physics, Atomic and Optics, Universidad de Valladolid, Valladolid, Spain
[c] Networking Research Center on Bioengineering Biomaterials and Nanomedicine (CIBER-BBN), Valladolid, Spain
[d] Department of Statistics and Operations Research, Universidad de Valladolid, Valladolid, Spain

ARTICLE INFO

Keywords:
Conjunctival cells
Contact lens
Discomfort
Gene expression
Inflammation
Pain

ABSTRACT

Purpose: This study aimed to analyze the differences in the expression of pain-related genes in conjunctival epithelial cells among symptomatic contact lens (CL) wearers (SCLWs), asymptomatic CL wearers (ACLWs), and non-CL wearers (non-CLWs).

Methods: For this study, 60 participants (20 non-CLWs, 40 CLWs) were enrolled. The CLW group comprised 20 ACLWs and 20 SCLWs according to the Contact Lens Dry Eye Questionnaire short form©. Conjunctival cells were collected using impression cytology, and RNA was isolated and used to determine the expression levels of 85 human genes involved in neuropathic and inflammatory pain. The effects of CL wear and discomfort were evaluated using mixed-effects ANOVA with partially nested fixed-effects model. Gene set enrichment analysis was performed to assign biological meaning to sets of differentially expressed genes.

Results: Six genes (*CD200*, *EDN1*, *GRIN1*, *PTGS1*, *P2RX7,* and *TNF*) were significantly upregulated in CLWs compared to non-CLWs. Eleven genes (*ADORA1*, *BDKRB1*, *CACNA1B*, *DBH*, *GRIN1*, *GRM1*, *HTR1A*, *PDYN*, *PTGS1*, *P2RX3,* and *TNF*) were downregulated in SCLWs compared to ACLWs. These genes were mainly related to pain, synaptic transmission and signaling, ion transport, calcium transport and concentration, and cell-cell signaling.

Conclusions: CL wear modified the expression of pain- and inflammation-related genes in conjunctival epithelial cells. These changes may be in part, along with other mechanisms, responsible for CL discomfort in SCLWs.

1. Introduction

Contact lenses (CL) can effectively correct visual alterations, but many users cease their use, CL discomfort (CLD) being the main reason [1]. CLD is defined as "a condition characterized by episodic or persistent adverse ocular sensations related to lens wear, either with or without visual disturbance, resulting from reduced compatibility between the contact lens and the ocular environment, which can lead to decreased wearing time and discontinuation of contact lens wear" [2]. However, clear evidence of a correlation between clinical signs and CLD symptoms is lacking [3,4]. Additionally, the cause of CLD is not clear, causing a significant impact on CL wearers and clinicians.

Several studies have reported changes in cytokine, growth factor, and inflammatory mediator levels in tear fluid of CL wearers [5–10] and with CLD [11–13]. These changes may suggest the presence of an underlying inflammation on the ocular surface during CL wear and that this may be a potential cause of CLD. However, contradictory results have been reported. On the one hand, some studies analyzing a considerable number of inflammatory molecules in tears found no differences between asymptomatic and symptomatic CL wearers [10,14]. On the other hand, an upregulation of nerve growth factor (NGF), transforming growth factor-β, leukotriene B4 and interleukin (IL)-17 A has been detected in tears of CL wearers with CLD [11–13]. Moreover, conjunctival cells have altered inflammatory gene expression related to ocular surface diseases, e.g., graft-versus-host disease [15], Sjögren's

* Corresponding author. Institute of Applied Ophthalmobiology (IOBA), Universidad de Valladolid, Valladolid, Spain.
E-mail address: mjgonzalez@ioba.med.uva.es (M.J. González-García).

https://doi.org/10.1016/j.jtos.2023.12.004
Received 1 September 2023; Received in revised form 27 October 2023; Accepted 9 December 2023
Available online 20 December 2023

Abbreviations

ACLW	asymptomatic contact lens wearer
CCLRU	Cornea and Contact Lens Research Unit
CIC	conjunctival impression cytology
CL	contact lens
CLD	contact lens discomfort
CLDEQ	Contact Lens Dry Eye Questionnaire
CLW	contact lens wearer
DED	dry eye disease
DEG	differentially expressed gene
GO	Gene Ontology
GO:BP	GO biological process
GO:CC	GO cellular component
GO:MF	GO molecular function
HP	Human Phenotype Ontology
HPA	Human Protein Atlas
IL	interleukin
KEGG	Kyoto Encyclopedia of Genes and Genomes
MIRNA	miRTarBase
OSDI	Ocular Surface Disease Index
PGE2	prostaglandin E2
REAC	Reactome
RT-PCR	real-time PCR
SCLW	symptomatic contact lens wearer
TBUT	tear film breakup time
TF	Transfac TRANScription FACtor database
TNF	tumor necrosis factor
WP	WikiPathways

syndrome with aqueous tear deficiency [16], and dry eye disease (DED) [17]. These findings highlight the need to identify molecular biomarkers to determine the origin or presence of CLD.

In an exploratory study of our research group [18] we found differences in the expression of several pain-related genes in conjunctival epithelial cells between symptomatic CL wearers (SCLWs), asymptomatic CL wearers (ACLWs) and non-CL wearers (non-CLWs). We identified altered gene expression associated with hydrogel CL wear and CLD. CL wear modified the expression of several genes related to pain and inflammation, specifically those related to analgesia (*PDYN* and *PENK*) and inflammation or pain (*BDKRB1* and *PROK2*). Additionally, SCLWs showed decreased *PENK* and *PDYN* expression and increased *CCL2*, *IL1A*, *IL1B*, *IL2,* and *NGF* expression, all genes associated with pain and inflammation.

Although showing promising results, this study was limited by its small sample size (8 participants/group). An expanded study of ocular surface gene expression in CLWs is warranted to help understand CL-induced changes on the ocular surface and the molecular mechanisms responsible for CLD.

Therefore, the present study aimed to corroborate in a larger population the previous results regarding differences in neuropathic and inflammatory pain-related gene expression in conjunctival epithelial cells among SCLWs, ACLWs, and non-CLWs, thereby identifying alterations in gene expression associated with hydrogel CL wear and CLD.

2. Methods

This study was approved by the Institutional Review Board of the Institute of Applied Ophthalmobiology (Universidad de Valladolid) and the Ethics Committee of the Area de Salud Valladolid Este. This study complied with the principles of the Declaration of Helsinki. All participants received detailed information about the study before providing informed consent.

2.1. Participants

We enrolled non-CLWs and CLWs. The latter were categorized as ACLWs and SCLWs according to the Contact Lens Dry Eye Questionnaire (CLDEQ) short form© (copyright Begley & Chalmers 2016, with permission) [19,20].

2.2. Inclusion and exclusion criteria

The inclusion criteria were age ≥18 years, astigmatism ≤0.75 D, and, for the CLWs, use of hydrogel or silicone hydrogel CL for ≥6 months before study inclusion.

The exclusion criteria were overnight, extended, or continuous CL wear; any active ocular disease; any previous ocular surgery; any systemic disease that contraindicated CL wear; any topical treatment apart from artificial tears; and DED. DED was defined as an Ocular Surface Disease Index (OSDI) [21] score >12 and altered results in at least two of the following tests in at least one eye: a fluorescein tear film breakup time (TBUT) ≤7 s, an extent of fluorescein corneal staining ≥2 in any area of the cornea (graded 0–4 on the Cornea and Contact Lens Research Unit [CCLRU] scale) [22], and a Schirmer test without anesthesia ≤5 mm.

2.3. Study design

All participants were assessed regarding the inclusion and exclusion criteria. The CLWs were examined with their habitual CLs.

2.4. Clinical assessment

The participants wore their habitual correction (e.g., CL) to measure high-contrast visual acuity monocularly on a logMAR scale (Topcon Corporation, Ltd., Tokyo, Japan).

TBUT was measured using fluorescein strips (I-DEW FLO, Entod Research Cell UK, Ltd., Tottenham Lane, London, UK) wetted with 25 μL sodium chloride and applied into the inferior fornix, after shaking the excess fluid off the strip [23]. Participants were examined immediately after fluorescein instillation using a slit lamp (SL-D7, Topcon Corporation) with a blue filter (Topcon Corporation) and a Wratten #12 yellow filter (Eastman Kodak, Rochester, NY, USA). Three measurements of the time interval between the last of three blinks and the appearance of the first dry spot were obtained, and the average was recorded. Two minutes after fluorescein instillation, corneal staining was evaluated using the CCLRU scale [22], and the total mean score was determined.

Tear production was evaluated without topical anesthesia by placing a Schirmer sterile strip (Tearflo; Alcon Laboratories Inc., Fort Worth, TX, USA) in the external canthus of the inferior lid margin. The participants kept their eyes closed, and the strip wetting length was measured after 5 min.

2.5. Conjunctival impression cytology

Conjunctival impression cytology (CIC) under topical anesthesia (Colircusí Anestésico Doble; Alcon Laboratories) was used to collect conjunctival cells using one-half of a polyethersulfone filter (Supor 200, pore size: 0.20 μm, diameter: 13 mm; Gelman Laboratory, Ann Arbor, MI, USA). The filters were placed with moderate pressure on the upper temporal bulbar conjunctiva. The filters were carefully removed and transferred to 350 μL of RLT Buffer (Qiagen, Hilden, Germany) containing 1 % 2-mercaptoethanol. CIC samples were kept frozen at −80 °C

until analysis [15].

2.6. Gene expression analysis

Total RNA was obtained from each CIC using an RNeasy Micro Kit (Qiagen, Redwood City, CA, USA) following the manufacturer's instructions. DNase (Qiagen) digestion was performed to remove genomic (g)DNA traces. RNA concentrations were determined using the Quant-it RNA HS Assay kit (Life Technologies, Carlsbad, CA, USA) and a Qubit Fluorometer (Life Technologies). Total RNA was reverse-transcribed into cDNA using a commercial iScript cDNA Synthesis Kit (BioRad Laboratories Inc., Hercules, CA, USA) to synthesize 12 ng of cDNA from each sample.

Gene expression was analyzed using a commercial real-time PCR (RT-PCR) array (Pain, Neuropathic, and Inflammatory; SAB target list, BioRad Laboratories Inc.) that uses SYBR Green dye detection, according to the manufacturer's recommendations. Eighty-five human genes related to pain and inflammation were analyzed (Table 1) along with the six housekeeping genes beta-actin (*ACTB*), beta-2-microglobulin (*B2M*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), TATA-binding protein (*TBP*), hypoxanthine phosphoribosyltransferase 1 (*HPRT1*), and ribosomal protein lateral stalk subunit P0 (*RPLP0*). Moreover, the array included the following controls per sample: 1) one control to monitor gDNA contamination, 2) two RNA quality controls to monitor RNA integrity, 3) one reverse transcription control for first-strand synthesis, and 4) one positive PCR control for RT-PCR efficiency. The RT-PCR was performed using a 7500 Real-Time PCR System (Applied Biosystems, Madrid, Spain) as follows: 10 min at 95 °C, 40 cycles of 15 s at 95 °C, and 1 min at 60 °C.

Descriptions of the 85 analyzed genes were obtained from the NCBI gene database (https://www.ncbi.nlm.nih.gov/gene/; Appendix A).

2.7. Statistical analysis

Statistical analysis was performed by a licensed statistician (co-author IF) using R statistical software [24] and packages from the Bioconductor project [25,26].

The sample size was estimated based on data from our pilot study [18]. The effect sizes of all genes with significant differences between ACLW and SCLW groups were calculated, and the smallest one, corresponding to PTGS1 ($d = 1.245$), was used. Statistical power was established at 80%, and the level of significance was 0.00029 (0.05 divided by 170 to account for multiple testing of 85 genes with two comparisons per gene). Considering these data and the paired design, a minimum sample size of 20 participants per group was estimated.

The ACLW and SCLW groups were paired 1:1 for age, sex, and habitual number of CL-wearing hours, whereas non-CLWs were matched for age and sex. The propensity score [27] was calculated using the MatchIt package [28] in R.

2.7.1. Clinical and demographic characteristics

Clinical and demographic characteristics were compared between groups. For quantitative variables, comparisons between two groups were performed using paired Student's t-test or the Wilcoxon test, depending on whether the data were parametric or nonparametric (Shapiro-Wilk test), respectively. For the statistical comparison of three groups, repeated measures ANOVA or the Friedman test, depending on whether the data were parametric or nonparametric (Shapiro-Wilk test), respectively. Pairwise comparison of significant results was performed using paired Student's t-test or the Wilcoxon test, applying the Bonferroni correction. For qualitative variables, comparisons between groups were performed using the McNemar test (two groups) or the Cochran test (3 groups). Additionally, the characteristics of the pilot and newly enrolled samples were also compared. For quantitative variables, the unpaired Student's *t*-test or Mann-Whitney *U* test were used, depending on whether the data were parametric or nonparametric (Shapiro-Wilk test), respectively. For qualitative variables, the chi-square test was used. Statistical significance was set at $p \leq 0.05$.

2.7.2. Analysis of differentially expressed genes (DEGs)

mRNA expression was imputed for non-detected values ($Ct > 35$) using the method by McCall et al. [29] which is based on the expectation–maximization algorithm and implemented in the nondetects package of the Bioconductor project. Relative gene expression data were calculated using the Ct method after choosing reference genes (*ACTB*, *GAPDH*, and *RPLP0)* with the geNorm algorithm [30] using the ReadqPCR and NormqPCR packages [31].

Differences between non-CLW and CLW, as well as ACLW and SCLW, groups were compared using a mixed-effects ANOVA with partially nested fixed effects. Models were adjusted on a gene-by-gene basis with ΔCt as the dependent variable using the HTqPCR [32], lme4 [33], and lmerTest [34] packages. The family-wise error rate was controlled using the method by Westfall and Young [35]. Based on the mean estimates provided by our model, effects were quantified using the emmeans package [36]. Statistical significance was set at $p \leq 0.05$.

2.7.3. Gene set enrichment analysis

To assign a biological meaning to groups of DEGs differing between non-CLWs and CLWs or SCLWs and ACLWs and to facilitate the interpretation of our findings, gene set enrichment analysis was performed

Table 1
Genes analyzed by qPCR using the commercial PCR array profiler "Pain, Neuropathic, and Inflammatory (SAB Target List)".

Category		Genes
Synaptic transmission	Glutamate receptors	*GRIN1, GRIN2B, GRM1, GRM5*
	Serotonin receptors	*HTR1A, HTR2A*
	Calcium channel	*CACNA1B*
Pain conduction	Cannabinoid receptors	*CNR1, CNR2*
	Ion channel	*TRPA1, TRPV1, TRPV3*
	Opioid receptors	*OPRD1, OPRK1, OPRM1*
	Potassium channels	*KCNIP3, KCNJ6, KCNQ2, KCNQ3*
	Purinergic receptors	*ADORA1, P2RX3, P2RX4, P2RX7, P2RY1*
	Sodium channels	*SCN10A, SCN11A, SCN3A, SCN9A, SLC6A2*
Modulation of pain responses	Eicosanoid metabolism	*PLA2G1B, PTGER1, PTGER3, PTGER4, PTGES, PTGES2, PTGES3, PTGS1, PTGS2*
	Inflammation	*ACE, ALOX5, BDKRB1, CALCA, CCK, CCKBR, CCL2, CCR2, CD200, CD4, CHRNA4, CSF1, CX3CR1, DBH, EDN1, EDNRA, FAAH, GCH1, IL10, IL18, IL1A, IL1B, IL2, IL6, ITGAM, ITGB2, MAPK1, MAPK14, MAPK3, MAPK8, PROK2, TAC1, TACR1, TLR2, TLR4, TNF*
	Neurotransmitters	*ADRB2, COMT, DBH, MAOB, PDYN, PENK, PNOC*
	Neurotrophins	*BDNF, GDNF, NGF, NTRK1*

with the R package gProfileR [37]. Each gene was placed in the following biological annotations: Gene Ontology (GO biological process [GO:BP], molecular function [GO:MF], and cellular component [GO:CC]), Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome (REAC), WikiPathways (WP), Transfac: TRANScription FACtor database (TF), miRTarBase (MIRNA), Human Protein Atlas (HPA), CORUM protein complexes (CORUM), and Human Phenotype Ontology (HP). p-values were calculated for each biological category based on the hypergeometric distribution and adjusted for multiple comparisons using the method of Benjamini and Hochberg [38] where $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. Study population and clinical data

In total, 60 Caucasian participants (44 women, 16 men; mean age 26.1 ± 6.1 [range: 19–46] years) were included. In addition to 37 newly enrolled participants, data from 23 participants from the pilot study (one subject obtained an invalid result) were included [18]. The characteristics of both samples are presented in Table 2. The study population comprised 20 non-CLWs and 40 CLWs. The mean CL-wearing time of CLWs was 8.13 ± 4.36 years, and they used CLs for 4.85 ± 2.42 days/week and 8.28 ± 6.93 h/day. Conventional hydrogel and silicone hydrogel CLs were used by 15 and 25 CLWs, respectively (Table 3). CLWs were further divided into two groups (20 ACLWs and 20 SCLWs) based on the CLDEQ short form© [19,20]. The characteristics of the three groups are presented in Table 4.

3.2. Differentially expressed genes

The expression levels of the 85 analyzed genes in the four study groups (non-CLW, CLW, ACLW, and SCLW) are shown in Appendix B.

3.2.1. Effect of CL wear (non-CLW vs CLW)

Of the 85 examined genes, six (*GRIN1*, *P2RX7*, *PTGS1*, *CD200*, *EDN1,* and *TNF*) were significantly upregulated in CLWs compared to non-CLWs (Table 5). The fold changes for these genes ranged from 5.76 (*EDN1*) to 72.58 (*PTGS1*).

Additionally, in CLWs compared to non-CLWs, six DEG differed with borderline statistical significance of which five (*CACNA1B* [p = 0.054], *CCK* [p = 0.072], *HTR1A* [p = 0.057], *PROK2* [p = 0.059], and *P2RX3* [p = 0.065]) were upregulated and one (*PLA2G1B* [p = 0.058]) was downregulated (Table 5).

Table 2
Characteristics of the pilot study and newly enrolled samples.

	Pilot study	Newly enrolled	p-value
***Age** (years)*	25.8 ± 4.1	26.3 ± 7.2	0.380
***Sex** (male/female)*	7/16	9/28	0.826
***CL type** (conventional hydrogel/silicone hydrogel)*	6/9	9/16	1.000
***CL replacement schedule** (daily/frequent)*	7/8	6/19	0.257
***CL refraction** (diopters)*	−3.25 ± 2.00	−2.99 ± 1.55	0.768
***Visual acuity** (logMAR)*	−0.10 ± 0.05	−0.09 ± 0.08	0.457
***CL use** (years)*	8.2 ± 4.3	8.1 ± 4.5	0.963
***Weekly frequency of CL wear** (days/week)*	4.8 ± 2.1	4.9 ± 1.9	0.875
***Daily frequency of CL wear** (h/day)*	8.3 ± 2.6	8.3 ± 2.4	0.987
***CL wear on the day of the visit** (h)*	6.3 ± 1.9	5.7 ± 2.7	0.357
***OSDI questionnaire** (score 0–100)*	8.8 ± 5.8	7.6 ± 7.0	0.301
***Fluorescein tear film breakup time** (s)*	8.4 ± 7.9	9.7 ± 5.8	0.075
***Total corneal staining** (score 0–4)*	0.2 ± 0.4	0.2 ± 0.3	0.959
***Schirmer test without anesthesia** (mm)*	20.9 ± 12.1	20.8 ± 10.8	0.908

Data are presented as mean ± standard deviation or frequencies. CL: contact lens; OSDI: Ocular Surface Disease Index.

3.2.2. Effect of CLD (ACLW vs SCLW)

Regarding CLD effects, 11 out of the 85 genes (*GRIN1*, *GRM1, HTR1A, CACNA1B*, *ADORA1*, *P2RX3, PTGS1*, *BDKRB1*, *TNF, DBH,* and *PDYN*) were significantly downregulated in SCLWs compared to ACLWs (Table 5). Their fold change ranged from −4.81 (*TNF*) to −38.98 (*HTR1A*).

Furthermore, in SCLWs compared to ACLWs, four genes (*CALCA* [p = 0.095], *CNR1* [p = 0.063], *GDNF* [p = 0.089], and *KCNIP3* [p = 0.055]) were downregulated with borderline statistical significance. No gene was upregulated in SCLWs compared to ACLWs.

3.2.3. Gene set enrichment

More than a thousand terms were significantly overrepresented in the gene set enrichment analysis on significant or borderline significant DEGs in CLWs compared to non-CLWs. Table 6 shows the top 20 most significantly overrepresented terms which were principally found in the GO:BP annotation and were related to transport, concentration, and homeostasis of calcium ions.

Regarding significant or borderline significant DEGs in SCLWs compared to ACLWs, thousands of significantly overrepresented terms were found. The 20 most significantly overrepresented terms (Table 7) were also principally found in the GO:BP annotation. These terms involved synaptic signaling and transmission, sensory perception of pain, cell-cell signaling, and ion and cation transport.

Additionally, the term “neuroactive ligand-receptor interaction” was overrepresented in the KEGG annotation in both CLW and CLD groups (Tables 6 and 7, respectively).

4. Discussion

CLD is the main cause of CL cessation, but the causes of CLD remain unclear. Alterations in ocular surface neurobiology have been identified as important mechanisms contributing to dry eye symptoms and may play a considerable role in CLD [39]. In 2019, the findings of our pilot study [18] identified DEGs associated with CL wear and CLD. The results of this exploratory study required confirmation in a larger population. The current study confirmed some of the previously obtained results and described new genes altered in both CLWs and CLDs.

4.1. Effects of CL wear

In this study, *GRIN1, P2RX7,* and *PTGS1, CD200, EDN1,* and *TNF* were significantly upregulated in CLWs compared to non-CLWs (Fig. 1).

4.1.1. Synaptic transmission

Synaptic transmission and neurotransmission are involved in pain perception. *GRIN1* encodes a subunit of the glutamate receptor channel superfamily which plays an important role in synaptic plasticity [40]. *GRIN1* is expressed in the conjunctiva [41] however, the biological significance of *GRIN1* increases in the conjunctival epithelium of CLWs is unknown.

4.1.2. Pain conduction

Nociceptors transmit information to the central nervous system via action potentials, where various ion channels and receptors play important roles conducting pain signals. *P2RX7* encodes the purinergic receptor P2RX7, which is related to chronic inflammatory and neuropathic pain [42–44] and participates in the secretion of interleukins, cytokines (e.g., tumor necrosis factor [TNF]-α), chemokines, and prostaglandin E2 (PGE2) [45–47]. P2RX7 has been also related to chronic DED [48,49]. Furthermore, the complex P2RX7-NLRP3 inflammasome mediates the release of the pro-inflammatory cytokines IL-1β and IL-18, which may be involved in Sjögren’s-derived ocular dryness [50]. In the

Table 3
Contact lenses (CLs) used by the CL wearers group.

CL type	Replacement	Material	Diameter	Base curve	Laboratory	Number of participants
Conventional hydrogel	Monthly	Omafilcon B	14.2	8.6	CooperVision	3
		Hilafilcon B	14.2	8.6	Bausch & Lomb	2
	Daily	Nesofilcon A	14.2	8.6	Bausch & Lomb	3
		Nelfilcon A	14.0	8.7	Alcon	2
		Omafilcon A	14.2	8.7	CooperVision	2
		Etafilcon A	14.2	8.5	Johnson & Johnson	1
		Filcon IV 1	14.2	8.6	Servilens	1
		Ocufilcon D	14.2	8.6	CooperVision	1
Silicone hydrogel	Monthly	Comfilcon A	14.0	8.6	CooperVision	11
		Lotrafilcon B	14.2	8.6	Alcon	5
		Fanfilcon A	14.2	8.4	CooperVision	2
		Polymacon	14.2	8.6	Bausch & Lomb	1
	Biweekly	Senofilcon A	14.8	8.4	Johnson & Johnson	2
		Galyfilcon A	14.0	8.7	Johnson & Johnson	1
	Daily	Delefilcon A	14.1	8.5	Alcon	2
		Somofilcon A	14.1	8.6	CooperVision	1

Table 4
Characteristics of the study groups.

	Non-CLW	ACLW	SCLW	p-value
***Age** (years)*	25.9 ± 7.8	26.9 ± 5.1	25.5 ± 5.4	0.102
***Sex** (male/female)*	5/15	7/13	4/16	0.584
***CL type** (conventional hydrogel/silicone hydrogel)*	–	6/14	9/11	0.366
***CL replacement schedule** (daily/frequent)*	–	5/15	8/12	0.405
CL refraction (diopters)	–	−3.25 ± 1.50	−2.75 ± 2.00	0.267
Visual acuity (logMAR)	−0.11 ± 0.06	−0.08 ± 0.06	−0.11 ± 0.08	0.312
***CL use** (years)*	–	9.1 ± 4.8	7.1 ± 3.7	0.063
***Weekly frequency of CL wear** (days/week)*	–	5.8 ± 1.5	3.9 ± 1.9	**0.009**
***Daily frequency of CL wear** (h/day)*	–	8.8 ± 2.2	7.7 ± 2.5	0.134
***CL wear on the day of the visit** (h)*	–	5.8 ± 3.2	6.1 ± 1.3	0.721
***OSDI questionnaire** (score 0–100)*	4.1 ± 2.9	6.1 ± 4.1	13.2 ± 7.0	**<0.001***
***Fluorescein tear film breakup time** (s)*	11.1 ± 7.0	9.0 ± 7.6	9.0 ± 7.6	0.638
***Total corneal staining** (score 0–4)*	0.1 ± 0.2	0.3 ± 0.4	0.1 ± 0.2	0.500
***Schirmer test without anesthesia** (mm)*	22.1 ± 12.0	18.6 ± 9.8	22.2 ± 11.7	0.394

Data are presented as mean ± standard deviation or frequencies. ACLW: asymptomatic contact lens wearer; CL: contact lens; CLW: contact lens wearer; OSDI: Ocular Surface Disease Index; SCLW: symptomatic contact lens wearer. * pairwise comparisons with Bonferroni correction, p-value <0.05 in the symptomatic group compared with all other groups.

cornea, P2RX7 participates in wound healing by regulating corneal integrity and epithelial cell migration [51]. Therefore, purinergic receptors may participate in the regulation of inflammation in DED. Our results suggest that the upregulation of *P2RX7* in CLWs may indicate changes in the inflammatory state and increased pain sensitivity.

4.1.3. Modulation of pain responses

The transduction of pain signals can be modulated by different inflammatory and pain mediators (including growth factors, cytokines, and chemokines) released by neurons and immune cells, among others.

In this category, *PTGS1* expression was the highest with a 72.58-fold change. *PTGS1* encodes an enzyme that participates in the biosynthesis of prostaglandins which participate in the development of the inflammatory response and allodynia [52,53]. Prostaglandins potentiate the effects of other inflammatory mediators (e.g., bradykinin and histamine) which directly induce inflammation [52] and can be released with cytokines after stimulation of the ocular surface by tear hyperosmolarity [54,55]. The increased *PTGS1* expression in CLWs could be a consequence of the hyperosmolarity on the ocular surface provoked by CLs [56], similar to the increased tear PGE2 levels observed in DED subjects [54,57].

CD200 encodes the glycoprotein CD200. The interaction between CD200 and its inhibitory receptor CD200R modulates pro-inflammatory responses [58,59], and several studies have demonstrated the anti-inflammatory effects of CD200/CD200R interactions [60–62]. Therefore, the high *CD200* levels in CLWs could be a protective response to the pro-inflammatory state caused by CL wear, protecting cells against CL-induced tissue damage.

We also found higher *EDN1* expression in CLWs. This gene, associated with somatic pain, inflammation, and nerve injury [63–65] encodes endothelin 1, which is involved in nociceptive signaling [63,66]. *TNF* expression was also increased in CLWs. In addition to its well-known pro-inflammatory role, TNF-α induces neuropathic pain and hyperalgesia [67]. Gu et al. [68], proposed that *EDN1* and *TNF* participate linked in different pathways, including TNF signaling pathway. Thus, the increases in *EDN1* and *TNF* expression in CLWs may be related to upregulated TNF signaling and, therefore, may be involved in altered conjunctival inflammation and pain during CL wear. However, increased TNF-α levels in tear fluid have been described for reusable hydrogel CLWs [69] and also in CLWs exposed to adverse environmental conditions [7]. Moreover, the upregulation of both *PTGS1* and *TNF* in CLWs may indicate that the inflammatory state is further activated or that the presence of CLs modifies the basal conditions of the conjunctival epithelium. Additionally, the abovementioned increased P2RX7 receptor might lead to the observed increases in *PTGS1* and *TNF* expression [45,46].

4.2. Effects associated with CLD

Regarding CLD-associated changes in gene expression between SCLWs and ACLWs, *GRIN1*, *GRM1*, *HTR1A*, *CACNA1B*, *ADORA1*, *P2RX3*, *PTGS1*, *BDKRB1*, *TNF*, *DBH,* and *PDYN* were significantly downregulated in SCLWs (Fig. 2).

4.2.1. Synaptic transmission

All genes downregulated in SCLWs in this category (*GRIN1*, *GRM1, HTR1A,* and *CACNA1B)* encode proteins or receptors related to neuronal excitability or neurotransmitter release.

GRIN1 and *GRM1* encode G-protein-coupled glutamate receptors that mediate fast excitatory synaptic transmission (GRIN1) and modulate neurotransmission (GRM1) [70]. *GRM1* also appears to regulate inflammation, as its silencing is related to *CXCL1, IL6,* and *IL8/CXCL8* upregulation [71]. The downregulation of *GRIN1* and *GRM1* in SCLWs

Table 5
Differentially expressed genes in individuals with contact lens (CL) wear and CL discomfort (CLD).

Category		Gene	CL wear				CLD			
			Non-CLW (n=20) mean ΔCt (95% CI)	CLW (n=40) mean ΔCt (95% CI)	Fold change	Adjusted p-value	ACLW (n=20) mean ΔCt (95% CI)	SCLW (n=20) mean ΔCt (95% CI)	Fold change	Adjusted p-value
Synaptic transmission	Glutamate receptors	*GRIN1*	18.29 (8.446, 28.138)	15.05 (9.522, 20.583)	9.44	**0.006**	14.52 (9.270, 19.769)	18.83 (9.104, 28.546)	-19.77	**0.003**
		GRM1	16.49 (8.937, 24.039)	15.20 (10.815, 19.576)	2.45	0.194	14.67 (10.290, 19.040)	17.02 (9.442, 24.595)	-5.11	**0.037**
	Serotonin receptors	*HTR1A*	17.35 (7.082, 27.613)	14.79 (8.587, 20.986)	5.90	0.057	13.42 (7.428, 19.421)	18.71 (8.087, 29.331)	-38.98	**0.001**
	Calcium channel	*CACNA1B*	16.63 (8.736, 24.528)	14.65 (9.876, 19.418)	3.96	0.054	14.12 (9.507, 18.732)	17.16 (8.986, 25.333)	-8.23	**0.006**
Pain conduction	Purinergic receptors	*ADORA1*	15.07 (7.487, 22.658)	14.84 (10.463, 19.216)	1.18	0.824	13.41 (9.029, 17.785)	16.50 (8.923, 24.086)	-8.56	**0.007**
		P2RX3	16.71 (8.758, 24.656)	14.73 (9.974, 19.487)	3.94	0.065	14.41 (9.707, 19.122)	17.02 (8.871, 25.176)	-6.10	**0.035**
		P2RX7	12.61 (4.161, 21.058)	9.76 (4.919, 14.61)	7.19	**0.029**	11.50 (6.648, 16.359)	10.87 (2.461, 19.28)	1.55	0.548
Modulation of pain responses	Eicosanoid metabolism	*PTGS1*	17.98 (10.240, 25.729)	11.80 (7.323, 16.282)	72.58	**<0.0001**	12.39 (7.897, 16.884)	17.40 (9.646, 25.148)	-32.16	**<0.001**
	Inflammation	*BDKRB1*	14.46 (6.573, 22.343)	15.03 (10.307, 19.748)	-1.48	0.626	13.25 (8.325, 18.176)	16.24 (8.196, 24.274)	-7.92	**0.010**
		CD200	14.19 (5.069, 23.312)	11.33 (5.9, 16.755)	7.28	**0.020**	13.28 (8.309, 18.249)	12.24 (2.851, 21.627)	2.06	0.341
		EDN1	10.08 (2.497, 17.667)	7.56 (3.189, 11.924)	5.76	**0.025**	8.36 (3.993, 12.728)	9.28 (1.706, 16.849)	-1.89	0.359
		TNF	14.59 (6.010, 23.174)	10.63 (5.815, 15.455)	15.54	**<0.001**	11.48 (6.905, 16.055)	13.75 (5.275, 22.220)	-4.81	**0.044**
	Neurotransmitters	*DBH*	15.85 (5.046, 26.659)	14.55 (8.017, 21.075)	2.47	0.346	13.27 (6.955, 19.591)	17.13 (5.941, 28.310)	-14.45	**0.010**
		PDYN	17.42 (9.015, 25.819)	15.55 (10.525, 20.581)	3.16	0.108	14.88 (9.627, 20.124)	18.09 (9.531, 26.658)	-9.31	**0.011**

Data are presented as the estimated mean ΔCt (Ct gene of interest − Ct housekeeping) values (95% CI) for each gene; significant p-values are denoted in bold font. The up- and downregulated genes in these groups are highlighted in orange and green, respectively. Differences between groups were compared using a mixed-effects ANOVA with partially nested fixed effects. Statistical significance was set at p-value≤0.05. ACLW: asymptomatic contact lens wearer; CI: confidence interval; CLD: contact lens discomfort; CLW: contact lens wearer; SCLW: symptomatic contact lens wearer.

may disrupt neuronal or cellular excitability and could be involved in the upregulation of pro-inflammatory cytokines related to ocular discomfort. However, we did not find any significant increase in the expression of *IL6* or any other cytokine gene analyzed (*CCL2, IL1A, IL1B, IL2, IL10,* and *IL18*) in SCLWs compared to ACLWs. *CXCL1* or *IL8/CXCL8* expression might be altered in these patients; however, these cytokines were not included in our gene panel.

HTRA1 had the greatest negative fold change (−38.98). This gene encodes a serotonin receptor (5-hydroxytryptamine receptor 1 A) that is associated with pain sensitivity [72,73]. HTRA1 also regulates pain-related serotonin functions and plays a neuroprotective role [74, 75]. According to these results, the discomfort experienced by SCLWs might be partly due to altered neuroprotective mechanisms involving HTR1A receptors. Dysregulation of serotoninergic pathways in SCLWs is also possible, altering their pain sensitivity, resulting in discomfort.

CACNA1B encodes a subunit of the N-type voltage-gated calcium channel which is associated with chronic pain and controls neurotransmitter release [76,77]. Changes in *CACNA1B* expression might be linked to the observed downregulation of *HTRA1*, *GRIN1,* and *GRM1*, as the neuroprotective effects of HTRA1 involve a reduction in calcium influx and the release of glutamate [75,78,79].

4.2.2. Pain conduction

ADORA1 encodes the adenosine A1 receptor ADORA1, [80,81]. Adenosine receptors are involved in pain and inflammation [82–84] and T. Yang et al. [84], reported decreased TNF-α, IL-6, IL-1β, and IL-12 levels in aged *Adora1* knockout mice. The downregulated *ADORA1* expression in SCLWs compared with that in ACLWs could be a protective effect to decrease ADORA1 expression and the secretion of pro-inflammatory cytokines.

Like *P2RX7*, *P2RX3* encodes a purinergic receptor (P2RX3), which participates in the development of hyperalgesia owing to its activation by ATP released from damaged or inflamed tissues. De Oliveira Fusaro et al. [85], demonstrated in rats that activation of P2RX3 and P2RX2/3 receptors by ATP mediates bradykinin-induced mechanical hyperalgesia. According to our results, CLD observed in SCLWs may be related to hyperalgesia triggered by the upregulation of P2RX3 signaling. Thus, *P2RX3* downregulation in SCLWs compared to that in ACLWs may be a protective response to decrease P2RX3 levels and reduce the hyperalgesic state in SCLWs.

4.2.3. Modulation of pain responses

Modulation of pain responses was the most-altered CLD-related category, with five significantly downregulated genes in SCLWs compared to ACLWs.

PTGS1 is an enzyme involved in the biosynthesis of prostaglandins that can exhibit pro- and anti-inflammatory and immunomodulatory properties [53]. Some prostaglandins have concentration- and time-dependent cumulative effects causing pain even at low levels [52]. *PTGS1* may be downregulated in SCLWs via a feedback mechanism due to high prostaglandin levels, which cause discomfort. However, low *PTGS1* levels may be related to a decreased synthesis of anti-inflammatory prostaglandins, causing CLD. Although some studies found increased PGE2 levels in tears of individuals with DED [54,57,86], prostaglandin levels in tears of participants with and without CLD were not found significantly different [87].

The expression of bradykinin receptor gene *BDKRB1* was lower in SCLWs than in ACLWs. Bradykinin is an endogenous peptide associated with inflammation [88]. The bradykinin receptors BDKRB1 and BDKRB2 are involved in the initiation and maintenance of inflammatory processes [89]. *BDRKB1* can be highly and rapidly upregulated following inflammatory stimuli [90]. As mentioned above, ATP-induced

Table 6
Gene set enrichment analysis of the top 20 differentially expressed genes in contact lens wearers (CLWs).

			CLW	
Category	Term ID	Term description	Count	p-value
GO:BP	GO:0043269	Regulation of ion transport	7	<0.001
GO:BP	GO:0007204	Positive regulation of cytosolic calcium ion concentration	6	<0.001
GO:BP	GO:0051480	Regulation of cytosolic calcium ion concentration	6	<0.001
GO:BP	GO:0006816	Calcium ion transport	6	<0.001
GO:BP	GO:0010524	Positive regulation of calcium ion transport into cytosol	4	<0.001
GO:BP	GO:0055074	Calcium ion homeostasis	6	<0.001
GO:BP	GO:0006874	Cellular calcium ion homeostasis	6	<0.001
GO:BP	GO:0072503	Cellular divalent inorganic cation homeostasis	6	<0.001
GO:BP	GO:0051049	Regulation of transport	8	<0.001
GO:BP	GO:0010243	Response to organonitrogen compound	7	<0.001
GO:BP	GO:0072507	Divalent inorganic cation homeostasis	6	<0.001
GO:BP	GO:1901698	Response to nitrogen compound	7	<0.001
GO:BP	GO:0006875	Cellular metal ion homeostasis	6	<0.001
GO:BP	GO:0043270	Positive regulation of ion transport	5	<0.001
GO:BP	GO:0006812	Cation transport	7	<0.001
GO:BP	GO:0030003	Cellular cation homeostasis	6	<0.001
GO:BP	GO:0006873	Cellular ion homeostasis	6	<0.001
GO:BP	GO:0055065	Metal ion homeostasis	6	<0.001
GO:BP	GO:0019233	Sensory perception of pain	4	<0.001
KEGG	KEGG:04080	Neuroactive ligand-receptor interaction	6	<0.001

The 20 most significantly overrepresented terms are shown. p-values were calculated based on the hypergeometric distribution and adjusted for multiple comparisons using the method of Benjamini and Hochberg [38] where $p \leq 0.05$ was considered statistically significant. GO:BP: Gene Ontology biological process; KEGG: Kyoto Encyclopedia of Genes and Genomes; Count: number of altered genes present in the term.

Table 7
Gene set enrichment analysis of the top 20 differentially expressed genes in symptomatic contact lens wearers (SCLWs).

			CLD	
Category	Term ID	Term description	Count	p-value
GO:BP	GO:0043269	Regulation of ion transport	11	<0.001
GO:BP	GO:0098916	Anterograde *trans*-synaptic signaling	10	<0.001
GO:BP	GO:0007268	Chemical synaptic transmission	10	<0.001
GO:BP	GO:0099537	*Trans*-synaptic signaling	10	<0.001
GO:BP	GO:0099536	Synaptic signaling	10	<0.001
GO:BP	GO:0019233	Sensory perception of pain	7	<0.001
GO:BP	GO:0007267	Cell-cell signaling	11	<0.001
GO:BP	GO:0006811	Ion transport	12	<0.001
GO:BP	GO:0007610	Behavior	9	<0.001
GO:BP	GO:0045776	Negative regulation of blood pressure	5	<0.001
GO:BP	GO:0003008	System process	12	<0.001
GO:BP	GO:0006812	Cation transport	10	<0.001
GO:BP	GO:0051049	Regulation of transport	11	<0.001
GO:BP	GO:0044057	Regulation of system process	8	<0.001
GO:CC	GO:0045202	Synapse	10	<0.001
KEGG	KEGG:04080	Neuroactive ligand-receptor interaction	9	<0.001
GO:BP	GO:0051480	Regulation of cytosolic calcium ion concentration	7	<0.001
GO:BP	GO:0050877	Nervous system process	10	<0.001
GO:CC	GO:0036477	Somatodendritic compartment	8	<0.001
GO:CC	GO:0150034	Distal axon	6	<0.001

The 20 most significantly overrepresented terms are shown. p-values were calculated based on the hypergeometric distribution and adjusted for multiple comparisons using the method of Benjamini and Hochberg [38] where $p \leq 0.05$ was considered statistically significant. GO:BP: Gene Ontology biological process; GO:CC: Gene Ontology cellular component; KEGG: Kyoto Encyclopedia of Genes and Genomes; Count: number of altered genes present in the term; CLD: contact lens discomfort.

P2RX3 and P2RX2/3 activation mediates bradykinin-induced mechanical hyperalgesia [85]. In SCLWs, decreased expression of *BDKRB1*, as well as that of *P2RX3*, might be a protective mechanism to reduce P2RX3 levels and alleviate the hyperalgesic state in SCLWs. Kininogen (the precursor of bradykinin) had increased levels in the tears of CLWs [91]; however, bradykinin levels in tears were not significantly different between CLWs and non-CLWs [92].

TNF was significantly downregulated in SCLWs compared to ACLWs and upregulated in CLWs compared to non-CLW. TNF-α levels are increased in CLWs under adverse environmental conditions [93] and in the tears of patients with DED [94]. On one side, the increased *TNF* expression in ACLWs might reflect their higher frequency of CL wear reported. And, on the other, the decreased *TNF* expression in SCLWs might be due to a protective feedback mechanism in which, due to high TNF-α levels, the expression of this gene is downregulated.

Lastly, *DBH* and *PDYN* expression was decreased in SCLWs compared with ACLWs. *DBH* encodes dopamine β-hydroxylase, that converts dopamine to norepinephrine. The sympathetic system plays an important role in tear production [95,96], and dopamine levels affect the spontaneous blink rate [97]. Downregulation of *DBH* in SCLWs may lead to an alteration in dopaminergic metabolism, which may reduce the blink rate, interfering tear dynamics [98] and leading to CLD experienced by these individuals. This hypothesis agrees with the lower blink rate reported in dry eye subjects during the use of visual display terminals [99].

PDYN encodes a precursor protein for three opioid neuropeptides [100] being *PDYN* expression critical for pain regulation [101]. The decreased *PDYN* levels in SCLWs could be related to CLD; conversely, the

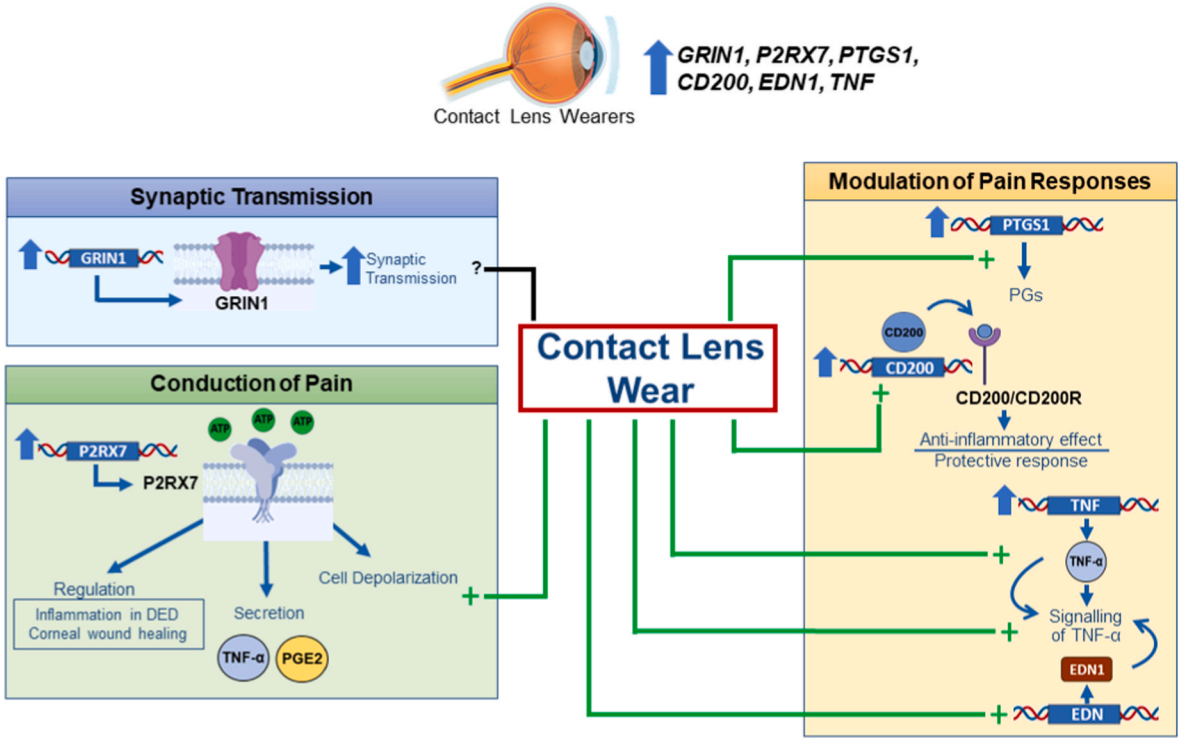

Fig. 1. Summary of differentially expressed genes in conjunctival epithelial cells from contact lens wearers (CLWs) compared to non-CLWs.
Six genes were upregulated in CLWs compared to non-CLWs and were involved in three different categories related to pain or inflammation. **Synaptic transmission**: The glutamate receptor **GRIN1** participates in synaptic transmission. Currently, the role of this gene in the development of pain and inflammation in CLWs remains unknown. **Pain conduction**: **P2RX7** is associated with chronic inflammatory and neuropathic pain. P2RX7 also contributes to TNF-α and prostaglandin E2 secretion, increases pain sensitivity, and induces an inflammatory state on the ocular surface. **Modulation of pain responses**: **PTGS1** participates in the biosynthesis of prostaglandins (PGs), which are involved in the development of inflammatory responses. The increase in the expression of ***CD200*** might be a protective response against the pro-inflammatory state caused by CL wear. ***EDN1*** and ***TNF*** might participate together in TNF-α signaling. Therefore, increased *EDN1* and *TNF* expression in CLWs could be involved in the alteration of conjunctival inflammation and pain states. ATP: adenosine triphosphate; CD200R: CD200 receptor; DED: dry eye disease; *EDN1*: endothelin 1; *GRIN1*: glutamate ionotropic receptor NMDA type subunit 1; *P2RX7*: purinergic receptor P2X, ligand-gated ion channel 7; PGE2: prostaglandin E2; PGs: prostaglandins; *PTGS1*: prostaglandin-endoperoxide synthase 1; *TNF*: tumor necrosis factor; TNF-α: tumor necrosis factor α.
This figure has been created using Biorender.com and PowerPoint.

higher *PDYN* levels in ACLWs could be responsible for the lack of symptomatology in these participants.

4.3. Biological meaning

To assess the biological significance of the altered genes, we performed enrichment analysis in both CLW and CLD groups. This analysis revealed that thousands of terms were significantly overrepresented. The 20 top terms that were significantly overrepresented in the CLW and CLD groups were related to calcium and ion transport, synaptic processes sensory pain perception, and behavior (understood as internally coordinated responses of organisms to internal/external stimuli, which involve nervous system activity). This enrichment analysis reinforces our hypothesis regarding molecular changes and alterations in the transmission of pain associated with CL use and the presence of CLD.

4.4. Final considerations

CL wear increased the expression of genes involved in synaptic transmission (*GRIN1*), pain conduction (*P2RX7*), and modulation of pain response (*PTGS1, CD200, EDN1,* and *TNF*), suggesting that CL use modifies the basal conditions of the ocular surface, with the potential to induce a para-/pro-inflammatory state of the conjunctiva.

In contrast, the expression of genes involved in synaptic transmission (*GRIN1*, *GRM1*, *HTR1A*, and *CACNA1B*), pain conduction (*ADORA1* and *P2RX3*), and pain response modulation (*PTGS1*, *BDKRB1*, *TNF*, *DBH,* and *PDYN*) was downregulated in SCLWs compared to ACLWs. This downregulation of various receptors and ion channels disrupts neuronal excitability and pain perception, leading to the occurrence of CLD. Additionally, some of these gene alterations have been described in relation to DED, suggesting a common pathological mechanism.

Some authors have suggested that CLD is caused by an imbalance in mechanical adaptation mechanisms [102]. The pattern found in this study comparing CLWs with non-CLWs behaves in a way opposite to that when comparing SCLWs and ACLWs. This suggests that ACLWs are protected by adaptive mechanical mechanisms on the ocular surface, alleviating symptom severity. It is possible that these adaptive mechanisms fail in SCLWs, which is why some CLWs develop CLD. As such, this study establishes a starting point for determining the molecular mechanisms underlying adaptation to CL wear in ACLWs and its failure in SCLWs.

A major objective of this study was to corroborate the results of a pilot study [18]. Based on the differences between non-CLWs and CLWs, increased *PTGS1* expression was observed in CLWs in both studies. *CACNA1B* and *PROK2*, which were previously found to be significantly increased, were also increased in this study but only bordered on statistical significance. *CD200* and *GRIN1* also showed significantly higher expression levels in CLWs; however, they were significantly downregulated in our previous study. Moreover, in this study, we found increased expression of *EDN1*, *P2RX7,* and *TNF* in CLWs, genes that were previously unaltered. Regarding CLD effects, the current results for *ADORA1*, *BDKRB1, CACNA1B*, *HTR1A*, *PDYN*, and *PTGS1* confirmed the changes found in the pilot study, whereas *P2RX3* was upregulated in SCLWs in our previous study. The other four genes, *DBH, GRIN1*, *GRM1,*

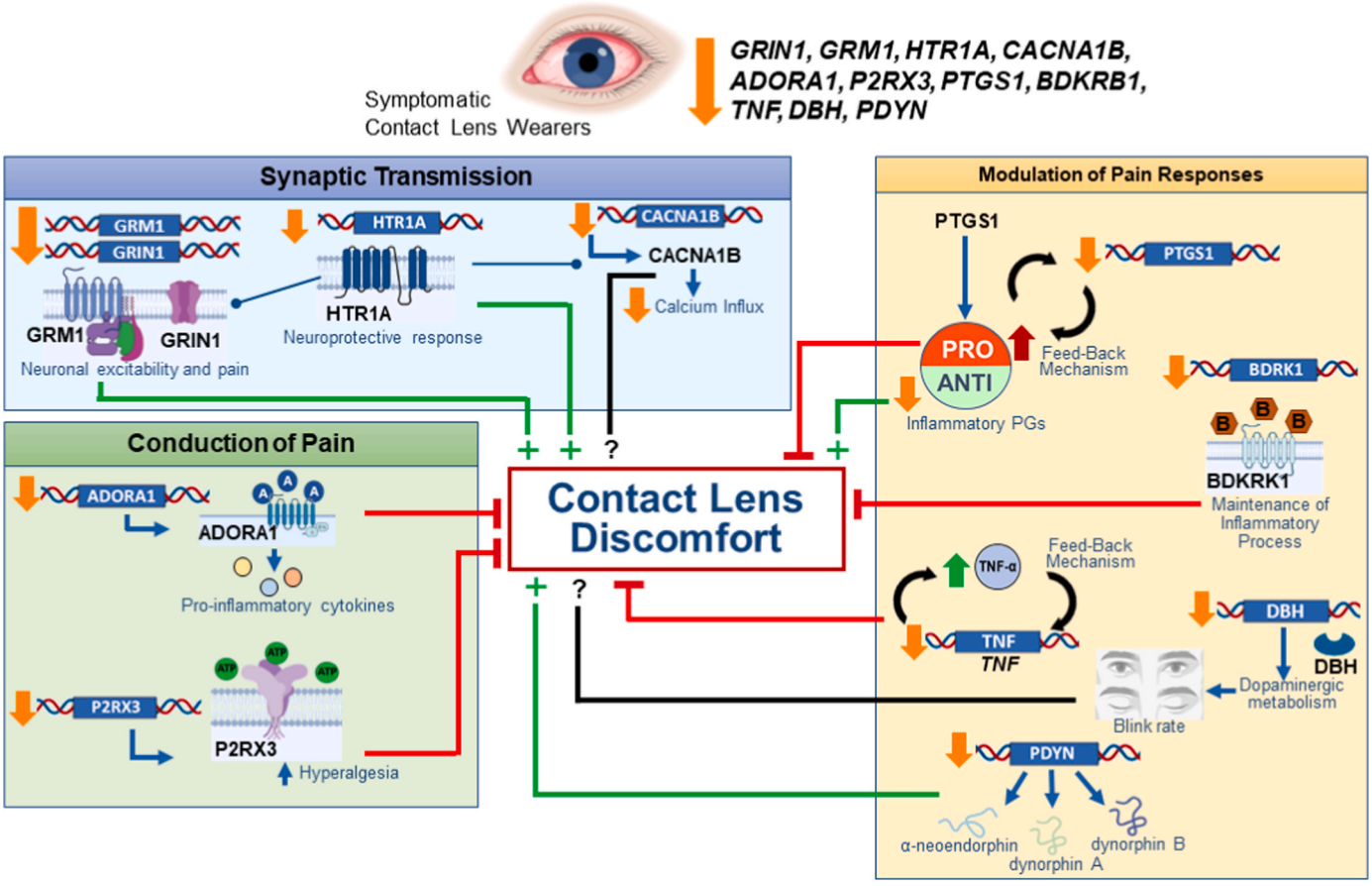

Fig. 2. Summary of differentially expressed genes in conjunctival epithelial cells from symptomatic contact lens wearers (SCLWs) compared to asymptomatic contact lens wearers (ACLWs).

Eleven genes were downregulated in SCLWs compared to ACLWs. These genes belong to three categories related to pain or inflammation. **Synaptic transmission**: The decrease in ***GRIN1*** and ***GRM1*** expression in SCLWs could alter their neuronal or cellular excitability, increasing CLD. Downregulation of ***HTR1A*** may attenuate the neuroprotective response of this receptor, which is related to CLD observed in SCLWs. In addition, the downregulation of *HTRA1* could also be associated with the downregulation of ***CACNA1B***, *GRIN1,* and *GRM1*, as the neuroprotective effects of HTRA1 involve a reduction in calcium influx and the release of glutamate. **Pain conduction**: The decrease in the expression of ***ADORA1*** could be a protective response to reduce the production of pro-inflammatory cytokines associated with ADORA1 receptor activation. Downregulation of ***P2RX3*** may be a protective mechanism that decreases mechanical hyperalgesia mediated by the activation of this receptor. **Modulation of pain responses**: The downregulation of ***PTGS1*** in SCLW could be either a protective feedback mechanism in response to pro-inflammatory prostaglandins or a cause of the CLD observed due to a decrease in the synthesis of anti-inflammatory prostaglandins. The decrease in both ***BDKRB1*** and P2RX3 might be a protective mechanism to reduce the inflammatory state present in SCLWs. The downregulation of ***TNF*** may be also a protective feedback mechanism in response to high levels of the pro-inflammatory cytokine TNF-α. The reduction in ***DBH*** expression might alter the dopaminergic metabolism, which could alter the blink rate in these individuals. Finally, the decrease in the expression of ***PDYN*** could be one of the causes of the occurrence of CLD in SCLWs, since this gene encodes a precursor protein for different opioid neuropeptides, which are essential for pain regulation. A: adenosine; *ADORA1*: adenosine A1 receptor; ATP: adenosine triphosphate; B: bradykinin; BDKRB1: bradykinin receptor B1; CACNA1B: calcium voltage-gated channel subunit alpha 1 B; DBH: dopamine β-hydroxylase; *GRIN1*: glutamate ionotropic receptor NMDA type subunit 1; *GRM1*: glutamate metabotropic receptor 1; *HTR1A*: 5-hydroxytryptamine (serotonin) receptor 1 A; *P2RX3*: purinergic receptor P2X, ligand-gated ion channel 3; *PDYN*: prodynorphin; PGs: prostaglandins; *PTGS1*: prostaglandin-endoperoxide synthase 1; *TNF*: tumor necrosis factor; TNF-α: tumor necrosis factor α.

This figure has been created using Biorender.com and PowerPoint.

and *TNF,* were significantly downregulated in this study but were not DEGs in the pilot study. The increased sample size in the current study allowed us to obtain results that were statistically more consistent than those obtained in the pilot study.

This study has some limitations. We excluded subjects with DED based on the criteria established in the pilot study [18]; which, although similar, differs from the one proposed by TFOS DEWS II [103]. We only analyzed the effects of hydrogel or silicone hydrogel CLs. Nevertheless, soft CLs represent 86% of CL fittings, and silicone hydrogel was the most commonly fitted CL (76%) in 2022 [104]. We assumed that cells collected by CIC are mainly epithelial, but we cannot completely exclude the presence of a small amount of other cell types present on the ocular surface (e.g., immune cells); therefore, we cannot dismiss the possibility that some of the observed gene expression changes are related to these cells. It would be interesting to further analyze gene expression in other cell types on the ocular surface to complete our study. Finally, we analyzed gene expression but not the corresponding protein levels. Due to post-translational mechanisms, these changes in mRNA expression might not correspond to the final amount of the expressed protein. To corroborate these results, it would be interesting to analyze the expression of proteins encoded by the genes we found to be altered because, to our knowledge, most of these proteins have not been studied in CLD.

In conclusion, the present study confirms that changes in pain-related gene expression in conjunctival epithelial cells in CLWs may account for or contribute to the occurrence of CLD in SCLWs or its absence in ACLWs.

Author contributions

AACG participated in the interpretation of data, analysis, and drafting of the manuscript. LVN, CVS, ALdlR, MBV, and CAdA participated in patient recruitment and clinical data collection. CGV performed laboratory experiments. IF performed the statistical analyses. AEdS and MJGG participated in the study design, data collection, data analysis and interpretation, financial support, and manuscript writing. All authors approved the final version of the manuscript.

Funding

This work was supported by CooperVision Inc., Ministry of Universities and European Social Fund (grant FPU19/01109), and Junta de Castilla y León and European Social Fund (grant EDU/556/2019). The funders played no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtos.2023.12.004.

References

[1] Richdale K, Sinnott LT, Skadahl E, Nichols JJ. Frequency of and factors associated with contact lens dissatisfaction and discontinuation. Cornea 2007;26:168–74. https://doi.org/10.1097/01.ico.0000248382.32143.86.
[2] Nichols KK, Redfern RL, Jacob JT, Nelson JD, Fonn D, Forstot SL, et al. The TFOS international workshop on contact lens discomfort: report of the definition and classification subcommittee. Invest Ophthalmol Vis Sci 2013;54:TFOS14–T19. https://doi.org/10.1167/iovs.13-13074.
[3] Craig JP, Willcox MDP, Argüeso P, Maissa C, Stahl U, Tomlinson A, et al. The TFOS International Workshop on Contact Lens Discomfort: report of the contact lens interactions with the tear film subcommittee. Invest Ophthalmol Vis Sci 2013;54:TFOS123–T156. https://doi.org/10.1167/iovs.13-13235.
[4] Efron N, Jones L, Bron AJ, Knop E, Arita R, Barabino S, et al. The TFOS International Workshop on Contact Lens Discomfort: report of the contact lens interactions with the ocular surface and adnexa subcommittee. Invest Ophthalmol Vis Sci 2013;54:TFOS98–122. https://doi.org/10.1167/iovs.13-13187.
[5] Dogru M, Ward SK, Wakamatsu T, Ibrahim O, Schnider C, Kojima T, et al. The effects of 2 week senofilcon—a silicone hydrogel contact lens daily wear on tear functions and ocular surface health status. Cont Lens Anterior Eye 2011;34:77–82. https://doi.org/10.1016/j.clae.2010.12.001.
[6] González-Pérez J, Villa-Collar C, Moreiras TS, Gesto IL, González-Méijome JM, Rodríguez-Ares MT, et al. Tear film inflammatory mediators during continuous wear of contact lenses and corneal refractive therapy. Br J Ophthalmol 2012;96:1092–8. https://doi.org/10.1136/bjophthalmol-2012-301527.
[7] López-de la Rosa A, González-García MJ, Calonge M, Enríquez-de-Salamanca A. Tear inflammatory molecules in contact lens wearers: a literature review. Curr Med Chem 2019;27:523–48. https://doi.org/10.2174/0929867326666190409152921.
[8] Poyraz C, Irkec M, Mocan MC. Elevated tear interleukin-6 and interleukin-8 levels associated with silicone hydrogel and conventional hydrogel contact lens wear. Eye Contact Lens 2012;38:146–9. https://doi.org/10.1097/ICL.0b013e3182482910.
[9] Yüksel Elgin C, İskeleli G, Talaz S, Akyol S. Comparative analysis of tear film levels of inflammatory mediators in contact lens users. Curr Eye Res 2016;41:441–7. https://doi.org/10.3109/02713683.2015.1037001.
[10] Willcox MD, Zhao Z, Naduvilath T, Lazon de la Jara P. Cytokine changes in tears and relationship to contact lens discomfort. Mol Vis 2015;21:293–305.
[11] Liu Q, McDermott AM, Miller WL. Elevated nerve growth factor in dry eye associated with established contact lens wear. Eye Contact Lens 2009;35:232–7. https://doi.org/10.1097/ICL.0b013e3181b3e87f.
[12] Masoudi S, Stapleton FJ, WillcoxMDP. Differences in tear film biochemistry of symptomatic and asymptomatic lens wearers. Optom Vis Sci 2017;94:914–8. https://doi.org/10.1097/OPX.0000000000001110.
[13] Gad A, Vingrys AJ, Wong CY, Jackson DC, Downie LE. Tear film inflammatory cytokine upregulation in contact lens discomfort. Ocul Surf 2019;17:89–97. https://doi.org/10.1016/j.jtos.2018.10.004.
[14] López-de la Rosa A, Martín-Montañez V, López-Miguel A, Calonge M, Enríquez-de-Salamanca A, González-García MJ. Corneal sensitivity and inflammatory biomarkers in contact lens discomfort. Opt Vis Sci 2016;93:892–900. https://doi.org/10.1097/OPX.0000000000000784.
[15] Cocho L, Fernández I, Calonge M, Martínez V, González-García MJ, Caballero D, et al. Gene expression-based predictive models of graft versus host disease-associated dry eye. Invest Ophthalmol Vis Sci 2015;56:4570–81. https://doi.org/10.1167/iovs.15-16736.
[16] Pflugfelder SC, de Paiva CS, Moore QL, Volpe EA, Li DQ, Gumus K, et al. Aqueous tear deficiency increases conjunctival interferon-γ (IFN-γ) expression and goblet cell loss. Invest Ophthalmol Vis Sci 2015;56:7545–50. https://doi.org/10.1167/iovs.15-17627.
[17] de Paiva CS, Chotikavanich S, Pangelinan SB, Pitcher 3rd JD, Fang B, Zheng X, et al. IL-17 disrupts corneal barrier following desiccating stress. Mucosal Immunol 2009;2:243–53. https://doi.org/10.1038/mi.2009.5.
[18] López-de la Rosa A, Fernández I, García-Vázquez C, Arroyo-del Arroyo C, González-García MJ, Enríquez-de-Salamanca A. Conjunctival neuropathic and inflammatory pain-related gene expression with contact lens wear and discomfort. Ocul Immunol Inflamm 2021;29:587–606. https://doi.org/10.1080/09273948.2019.1690005.
[19] Nichols JJ, Mitchell GL, Nichols KK, Chalmers R, Begley C. The performance of the contact lens dry eye questionnaire as a screening survey for contact lens-related dry eye. Cornea 2002;21:469–75. https://doi.org/10.1097/00003226-200207000-00007.
[20] Nichols JJ, Mitchell GL, Nichols KK. Assessment of the reliability of self–reported dry eye in contact lens wearers: the contact lens dry eye questionnaire (CLDEQ) short–form (abstract). Invest Ophthalmol Vis Sci 2004;45. ARVO E-Abstract 3453.
[21] Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the ocular surface disease Index. Arch Ophthalmol 2000;118:615–21. https://doi.org/10.1001/archopht.118.5.615.
[22] Terry RL, Schnider CM, Holden BA, Cornish R, Grant T, Sweeney D, et al. CCLRU standards for success of daily and extended wear contact lenses. Optom Vis Sci 1993;70:234–43. https://doi.org/10.1097/00006324-199303000-00011.
[23] Mooi JK, Wang MTM, Lim J, Müller A, Craig JP. Minimising instilled volume reduces the impact of fluorescein on clinical measurements of tear film stability. Cont Lens Anterior Eye 2017;40:170–4. https://doi.org/10.1016/j.clae.2017.01.004.
[24] R Core Team. R. A language and environment for statistical computing. R Foundation for Statistical Computing; 2021. https://www.r-project.org/. [Accessed 7 June 2022].
[25] Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 2004;5:R80. https://doi.org/10.1186/gb-2004-5-10-r80.
[26] Huber W, Carey VJ, Gentleman R, Anders S, Carlson M, Carvalho BS, et al. Orchestrating high-throughput genomic analysis with Bioconductor. Nat Methods 2015;12:115–21. https://doi.org/10.1038/nmeth.3252.
[27] Rosenbaum PR, Rubin DB. The central role of the propensity score in observational studies for causal effects. Biometrika 1983;70:41–55. https://doi.org/10.1093/biomet/70.1.41.
[28] Ho DE, Imai K, King G, Stuart EA. MatchIt: nonparametric preprocessing for parametric causal inference. J Stat Softw 2011;42:1–28. https://doi.org/10.18637/jss.v042.i08.
[29] McCall MN, McMurray HR, Land H, Almudevar A. On non-detects in qPCR data. Bioinformatics 2014;30:2310–6. https://doi.org/10.1093/bioinformatics/btu239.
[30] Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 2002;3. https://doi.org/10.1186/gb-2002-3-7-research0034. RESEARCH0034.
[31] Perkins JR, Dawes JM, McMahon SB, Bennett DLH, Orengo C, Kohl M. ReadqPCR and NormqPCR: R packages for the reading, quality checking and normalisation of RT-qPCR quantification cycle (Cq) data. BMC Genom 2012;13:296. https://doi.org/10.1186/1471-2164-13-296.
[32] Dvinge H, Bertone P. HTqPCR: high-throughput analysis and visualization of quantitative real-time PCR data in R. Bioinformatics 2009;25:3325–6. https://doi.org/10.1093/bioinformatics/btp578.
[33] Bates D, Mächler M, Bolker BM, Walker SC. Fitting linear mixed-effects models using lme4. J Stat Softw 2015;67:1–48. https://doi.org/10.18637/jss.v067.i01.
[34] Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest Package: tests in linear mixed effects models. J Stat Softw 2017;82:1–26. https://doi.org/10.18637/jss.v082.i13.
[35] Westfall PH, Young SS. Resampling-based multiple testing: examples and methods for P-value adjustment. New York: Wiley; 1993.
[36] Length RV. emmeans: estimated marginal means, aka least-squares means. R package version 1.6.2-1. 2021. https://CRAN.R-project.org/package=emmeans. [Accessed 7 June 2022].
[37] Kolberg L, Raudvere U, Kuzmin I, Vilo J, Peterson H. gprofiler2 – an R package for gene list functional enrichment analysis and namespace conversion toolset g: Profiler. F1000Res, vol. 9; 2020. p. 709. https://doi.org/10.12688/f1000research.24956.1.
[38] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol 1995;57:289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

[39] Stapleton F, Marfurt C, Golebiowski B, Rosenblatt M, Bereiter D, Begley C, et al. The TFOS international workshop on contact lens discomfort: report of the subcommittee on neurobiology. Invest Ophthalmol Vis Sci 2013;54:TFOS71–97. https://doi.org/10.1167/iovs.13-13226.
[40] Paoletti P, Bellone C, Zhou Q. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nat Rev Neurosci 2013;14:383–400. https://doi.org/10.1038/nrn3504.
[41] Wolf J, Boneva S, Schlecht A, Lapp T, Auw-Haedrich C, Lagrèze W, et al. The Human Eye Transcriptome Atlas: a searchable comparative transcriptome database for healthy and diseased human eye tissue. Genomics 2022;114:110286. https://doi.org/10.1016/j.ygeno.2022.110286.
[42] Burnstock G. P2X ion channel receptors and inflammation. Purinergic Signal 2016;12:59–67. https://doi.org/10.1007/s11302-015-9493-0.
[43] Kambur O, Kaunisto MA, Winsvold BS, Wilsgaard T, Stubhaug A, Zwart JA, et al. Genetic variation in P2RX7 and pain tolerance. Pain 2018;159:1064–73. https://doi.org/10.1097/j.pain.0000000000001188.
[44] Arulkumaran N, Unwin RJ, Tam FWK. A potential therapeutic role for P2X7 receptor (P2X7R) antagonists in the treatment of inflammatory diseases. Expet Opin Invest Drugs 2011;20:897–915. https://doi.org/10.1517/13543784.2011.578068.
[45] Savio LEB, Mello P de A, da Silva CG, Coutinho-Silva R. The P2X7 receptor in inflammatory diseases: angel or demon? Front Pharmacol 2018;9:52. https://doi.org/10.3389/fphar.2018.00052.
[46] Lara R, Adinolfi E, Harwood CA, Philpott M, Barden JA, Di Virgilio F, et al. P2X7 in cancer: from molecular mechanisms to therapeutics. Front Pharmacol 2020;11:793. https://doi.org/10.3389/fphar.2020.00793.
[47] Solle M, Labasi J, Perregaux DG, Stam E, Petrushova N, Koller BH, et al. Altered cytokine production in mice lacking P2X7 receptors. J Biol Chem 2001;276:125–32. https://doi.org/10.1074/jbc.M006781200.
[48] Wang JN, Fan H, Song JT. Targeting purinergic receptors to attenuate inflammation of dry eye. Purinergic Signal 2023;19:199–206. https://doi.org/10.1007/s11302-022-09851-9.
[49] Puro DG. Impact of P2X7 purinoceptors on goblet cell function: implications for dry eye. Int J Mol Sci 2021;22:6935. https://doi.org/10.3390/ijms22136935.
[50] Baldini C, Rossi C, Ferro F, Santini E, Seccia V, Donati V, et al. The P2X7 receptor-inflammasome complex has a role in modulating the inflammatory response in primary Sjögren's syndrome. J Intern Med 2013;274:480–9. https://doi.org/10.1111/joim.12115.
[51] Mayo C, Ren R, Rich C, Stepp MA, Trinkaus-Randall V. Regulation by P2X7: epithelial migration and stromal organization in the cornea. Invest Ophthalmol Vis Sci 2008;49:4384–91. https://doi.org/10.1167/iovs.08-1688.
[52] Zurier RB. Prostaglandins, leukotrienes, and related compounds. In: Firestein GS, Budd RC, Gabriel SE, McInnes IB, O'Dell JR, editors. Kelley and Firestein's textbook of rheumatology. Elsevier; 2017. p. 366–83. https://doi.org/10.1016/b978-0-323-31696-5.00024-3.
[53] Zurier RB. Prostaglandins: then and now and next. Semin Arthritis Rheum 2003;33:137–9. https://doi.org/10.1016/j.semarthrit.2003.09.001.
[54] Walter SD, Gronert K, McClellan AL, Levitt RC, Sarantopoulos KD, Galor A. ω-3 tear film lipids correlate with clinical measures of dry eye. Invest Ophthalmol Vis Sci 2016;57:2472–8. https://doi.org/10.1167/iovs.16-19131.
[55] Woo Ji Y, Seo Y, Choi W, Yeo A, Noh H, Kweon Kim E, et al. Dry eye-induced CCR7+ CD11b+ cell lymph node homing is induced by COX-2 activities. Invest Ophthalmol Vis Sci 2014;55:6829–38. https://doi.org/10.1167/iovs.14-14744.
[56] López-de la Rosa A, Arroyo-del Arroyo C, Enríquez-de-Salamanca A, Pinto-Fraga J, López-Miguel A, González-García MJ. The ability of the Contact Lens Dry Eye Questionnaire (CLDEQ)-8 to detect ocular surface alterations in contact lens wearers. Cont Lens Anterior Eye 2019;42:273–7. https://doi.org/10.1016/j.clae.2018.11.012.
[57] Shim J, Park C, Lee HS, Park MS, Lim HT, Chauhan S, et al. Change in prostaglandin expression levels and synthesizing activities in dry eye disease. Ophthalmology 2012;119:2211–9. https://doi.org/10.1016/j.ophtha.2012.05.038.
[58] Jenmalm MC, Cherwinski H, Bowman EP, Phillips JH, Sedgwick JD. Regulation of myeloid cell function through the CD200 receptor. J Immunol 2006;176:191–9. https://doi.org/10.4049/jimmunol.176.1.191.
[59] Hoek RH, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, et al. Down-regulation of the macrophage lineage through interaction with OX2 (CD200). Science 2000;290:1768–71. https://doi.org/10.1126/science.290.5497.1768.
[60] Walker DG, Dalsing-Hernandez JE, Campbell NA, Lue LF. Decreased expression of CD200 and CD200 receptor in Alzheimer's disease: a potential mechanism leading to chronic inflammation. Exp Neurol 2009;215:5–19. https://doi.org/10.1016/j.expneurol.2008.09.003.
[61] Chitnis T, Imitola J, Wang Y, Elyaman W, Chawla P, Sharuk M, et al. Elevated neuronal expression of CD200 protects Wlds mice from inflammation-mediated neurodegeneration. Am J Pathol 2007;170:1695–712. https://doi.org/10.2353/ajpath.2007.060677.
[62] Wang XJ, Ye M, Zhang YH, Chen SD. CD200-CD200R regulation of microglia activation in the pathogenesis of Parkinson's disease. J Neuroimmune Pharmacol 2007;2:259–64. https://doi.org/10.1007/s11481-007-9075-1.
[63] Takahashi K, Jones PM, Kanse SM, Lam HC, Spokes RA, Ghatei MA, et al. Endothelin in the gastrointestinal tract. Presence of endothelinlike immunoreactivity, endothelin-1 messenger RNA, endothelin receptors, and pharmacological effect. Gastroenterology 1990;99:1660–7. https://doi.org/10.1016/0016-5085(90)90472-D.
[64] Whittle BJR, Esplugues JV. Induction of rat gastric damage by the endothelium-derived peptide, endothelin. Br J Pharmacol 1988;95:1011–3. https://doi.org/10.1111/j.1476-5381.1988.tb11733.x.
[65] Mokha JS, Hyams JS, Glidden NC, Balarezo F, Young E. Characterizing clinical features and location-specific gene expression profiles associated with pain burden in children with functional dyspepsia. Neuro Gastroenterol Motil 2021;33:e14185. https://doi.org/10.1111/nmo.14185.
[66] Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411–5. https://doi.org/10.1038/332411a0.
[67] Yang R, Liu C, Yu D, Ma L, Zhang Y, Zhao S. Correlation between hyperalgesia and upregulation of TNF-α and IL-1β in aqueous humor and blood in second eye phacoemulsification: clinical and experimental investigation. J Immunol Res 2021;2021:7377685. https://doi.org/10.1155/2021/7377685.
[68] Gu C, Lhamo T, Zou C, Zhou C, Su T, Draga D, et al. Comprehensive analysis of angiogenesis-related genes and pathways in early diabetic retinopathy. BMC Med Genom 2020;13:142. https://doi.org/10.1186/s12920-020-00799-6.
[69] Chao C, Stapleton F, Willcox MDP, Golebiowski B, Richdale K. Preinflammatory Signs in established reusable and disposable contact lens wearers. Optom Vis Sci 2017;94:1003–8. https://doi.org/10.1097/OPX.0000000000001129.
[70] Alvarez FJ, Villalba RM, Carr PA, Grandes P, Somohano PM. Differential distribution of metabotropic glutamate receptors 1a, 1b, and 5 in the rat spinal cord. J Comp Neurol 2000;422:464–87. https://doi.org/10.1002/1096-9861(20000703)422:3%3C464::AID-CNE11%3E3.0.CO;2-%23.
[71] Sexton RE, Hachem AH, Assi AA, Bukhsh MA, Gorski DH, Speyer CL. Metabotropic glutamate receptor-1 regulates inflammation in triple negative breast cancer. Sci Rep 2018;8:16008. https://doi.org/10.1038/s41598-018-34502-8.
[72] Tour J, Löfgren M, Mannerkorpi K, Gerdle B, Larsson A, Palstam A, et al. Gene-to-gene interactions regulate endogenous pain modulation in fibromyalgia patients and healthy controls-antagonistic effects between opioid and serotonin-related genes. Pain 2017;158:1194–203. https://doi.org/10.1097/j.pain.0000000000000896.
[73] Lindstedt F, Karshikoff B, Schalling M, Olgart Höglund C, Ingvar M, Lekander M, et al. Serotonin-1A receptor polymorphism (rs6295) associated with thermal pain perception. PLoS One 2012;7:e43221. https://doi.org/10.1371/journal.pone.0043221.
[74] Polter AM, Li X. 5-HT1A receptor-regulated signal transduction pathways in brain. Cell Signal 2010;22:1406–12. https://doi.org/10.1016/j.cellsig.2010.03.019.
[75] Madhavan L, Freed WJ, Anantharam V, Kanthasamy AG. 5-Hydroxytryptamine 1A receptor activation protects against N-methyl-D-aspartate-induced apoptotic cell death in striatal and mesencephalic cultures. J Pharmacol Exp Ther 2003;304:913–23. https://doi.org/10.1124/jpet.102.044370.
[76] Smith AB, Cunnane TC. Multiple calcium channels control neurotransmitter release from rat postganglionic sympathetic nerve terminals. J Physiol 1997;499:341–9. https://doi.org/10.1113/jphysiol.1997.sp021931.
[77] Lee MS. Recent progress in the discovery and development of N-type calcium channel modulators for the treatment of pain. Prog Med Chem 2014;53:147–86. https://doi.org/10.1016/B978-0-444-63380-4.00004-4.
[78] Mauler F, Fahrig T, Horváth E, Jork R. Inhibition of evoked glutamate release by the neuroprotective 5-HT1A receptor agonist BAY x 3702 in vitro and in vivo. Brain Res 2001;888:150–7. https://doi.org/10.1016/s0006-8993(00)03074-2.
[79] Lee HJ, Ban JY, Cho SO, Seong YH. Stimulation of 5-HT1A receptor with 8-OH-DPAT inhibits hydrogen peroxide-induced neurotoxicity in cultured rat cortical cells. Pharmacol Res 2005;51:261–8. https://doi.org/10.1016/j.phrs.2004.09.003.
[80] Haskó G, Linden J, Cronstein B, Pacher P. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. Nat Rev Drug Discov 2008;7:759–70. https://doi.org/10.1038/nrd2638.
[81] Cronstein BN, Sitkovsky M. Adenosine and adenosine receptors in the pathogenesis and treatment of rheumatic diseases. Nat Rev Rheumatol 2017;13:41–51. https://doi.org/10.1038/nrrheum.2016.178.
[82] Kreckler LM, Wan TC, Ge ZD, Auchampach JA. Adenosine inhibits tumor necrosis factor-α release from mouse peritoneal macrophages via A2A and A2B but not the A3 adenosine receptor. J Pharmacol Exp Ther 2006;317:172–80. https://doi.org/10.1124/jpet.105.096016.
[83] Ryzhov S, Zaynagetdinov R, Goldstein AE, Novitskiy Sv, Blackburn MR, Biaggioni I, et al. Effect of A2B adenosine receptor gene ablation on adenosine-dependent regulation of proinflammatory cytokines. J Pharmacol Exp Ther 2008;324:694–700. https://doi.org/10.1124/jpet.107.131540.
[84] Yang T, Gao X, Sandberg M, Zollbrecht C, Zhang XM, Hezel M, et al. Abrogation of adenosine A1 receptor signalling improves metabolic regulation in mice by modulating oxidative stress and inflammatory responses. Diabetologia 2015;58:1610–20. https://doi.org/10.1007/s00125-015-3570-3.
[85] de Oliveira Fusaro MCG, Pelegrini-da-Silva A, Araldi D, Parada CA, Tambeli CH. P2X3 and P2X2/3 receptors mediate mechanical hyperalgesia induced by bradykinin, but not by pro-inflammatory cytokines, PGE2 or dopamine. Eur J Pharmacol 2010;649:177–82. https://doi.org/10.1016/j.ejphar.2010.09.037.
[86] Lekhanont K, Sathianvichitr K, Pisitpayat P, Anothaisintawee T, Soontrapa K, Udomsubpayakul U. Association between the levels of prostaglandin E2 in tears and severity of dry eye. Int J Ophthalmol 2019;12:1127–33. https://doi.org/10.18240/ijo.2019.07.12.
[87] Panthi S, Chen J, Wilson L, Nichols JJ. Detection of lipid mediators of inflammation in the human tear film. Eye Contact Lens 2019;45:171–81. https://doi.org/10.1097/ICL.0000000000000551.

[88] Marceau F, Bachelard H, Bouthillier J, Fortin JP, Morissette G, Bawolak MT, et al. Bradykinin receptors: agonists, antagonists, expression, signaling, and adaptation to sustained stimulation. Int Immunopharm 2020;82:106305. https://doi.org/10.1016/j.intimp.2020.106305.
[89] Couture R, Harrisson M, Vianna RM, Cloutier FC. Kinin receptors in pain and inflammation. Eur J Pharmacol 2001;429:161–76. https://doi.org/10.1016/s0014-2999(01)01318-8.
[90] Calixto JB, Medeiros R, Fernandes ES, Ferreira J, Cabrini DA, Campos MM. Kinin B1 receptors: key G-protein-coupled receptors and their role in inflammatory and painful processes. Br J Pharmacol 2004;143:803–18. https://doi.org/10.1038/sj.bjp.0706012.
[91] Mann AM, Tighe BJ. The detection of kinin activity in contact lens wear. Adv Exp Med Biol 2002;506:961–6. https://doi.org/10.1007/978-1-4615-0717-8_136.
[92] Masoudi S, Zhao Z, Stapleton F, Willcox M. Contact lens-induced discomfort and inflammatory mediator changes in tears. Eye Contact Lens 2017;43:40–5. https://doi.org/10.1097/ICL.0000000000000237.
[93] Martín-Montañez V, Enríquez-De-Salamanca A, López-De La Rosa A, López-Miguel A, Fernández I, Calonge M, et al. Effect of environmental conditions on the concentration of tear inflammatory mediators during contact lens wear. Cornea 2016;35:1192–8. https://doi.org/10.1097/ico.0000000000000960.
[94] Massingale ML, Li X, Vallabhajosyula M, Chen D, Wei Y, Asbell PA. Analysis of inflammatory cytokines in the tears of dry eye patients. Cornea 2009;28:1023–7. https://doi.org/10.1097/ICO.0b013e3181a16578.
[95] Dartt DA. Neural regulation of lacrimal gland secretory processes: relevance in dry eye diseases. Prog Retin Eye Res 2009;28:155–77. https://doi.org/10.1016/j.preteyeres.2009.04.003.
[96] Ding C, Walcott B, Keyser KT. The α1- and β1-adrenergic modulation of lacrimal gland function in the mouse. Invest Ophthalmol Vis Sci 2007;48:1504–10. https://doi.org/10.1167/iovs.05-1634.
[97] Jongkees BJ, Colzato LS. Spontaneous eye blink rate as predictor of dopamine-related cognitive function—a review. Neurosci Biobehav Rev 2016;71:58–82. https://doi.org/10.1016/j.neubiorev.2016.08.020.
[98] Tsubota K, Nakamori K. Effects of ocular surface area and blink rate on tear dynamics. Arch Ophthalmol 1995;113:155–8. https://doi.org/10.1001/archopht.1995.01100020037025.
[99] Srivastav S, Basu S, Singh S. Tear film changes in symptomatic versus asymptomatic video display terminal users following computer challenge test. Ocul Surf 2023;30:53–6. https://doi.org/10.1016/j.jtos.2023.08.003.
[100] Lieb A, Thaler G, Fogli B, Trovato O, Posch MA, Kaserer T, et al. Functional characterization of spinocerebellar ataxia associated dynorphin A mutant peptides. Biomedicines 2021;9:1882. https://doi.org/10.3390/biomedicines9121882.
[101] Nikoshkov A, Hurd YL, Yakovleva T, Bazov I, Marinova Z, Cebers G, et al. Prodynorphin transcripts and proteins differentially expressed and regulated in the adult human brain. Faseb J 2005;19:1543–5. https://doi.org/10.1096/fj.05-3743fje.
[102] Chen J, Simpson TL. A role of corneal mechanical adaptation in contact lens-related dry eye symptoms. Invest Ophthalmol Vis Sci 2011;52:1200–5. https://doi.org/10.1167/iovs.10-5349.
[103] Wolffsohn JS, Arita R, Chalmers R, Djalilian A, Dogru M, Dumbleton K, et al. TFOS DEWS II diagnostic methodology report. Ocul Surf 2017;15:539–74. https://doi.org/10.1016/j.jtos.2017.05.001.
[104] Morgan PB, Woods CA, Tranoudis IG, Efron N, Jones L, Faccia P, et al. International contact lens prescribing in 2022. Contact Lens Spectr 2023;38:28–35.