

Improvement of Evaporative Dry Eye With Meibomian Gland Dysfunction in Model Mice by Treatment With Ophthalmic Solution Containing Mineral Oil

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Purpose: Meibomian gland dysfunction (MGD) is a major cause of evaporative dry eye. The purpose of this study is to assess the efficacy of a mineral oil-containing ophthalmic solution (MO) in mitigating the evaporative dry eye phenotypes in a mouse model in which fatty acid elongase *Elovl1* is disrupted.

Methods: *Elovl1*-deficient mice were assessed in terms of number of plugged meibomian gland orifices, tear film breakup time (BUT), corneal fluorescein staining (CFS) score, tear quantity, and histology. The effects of the MO on the dry eye phenotypes were compared with those in groups not treated or treated with blank ophthalmic solution (BL).

Results: Untreated *Elovl1*-deficient mice exhibited dry eye phenotypes with MGD symptoms such as plugging of meibomian gland orifices ($P = 0.002$ compared with control mice), high CFS scores ($P = 0.002$), and shortened BUT ($P < 0.001$). Among three groups of *Elovl1*-deficient mice (MO treated, BL treated, and untreated), the MO-treated group exhibited fewer plugged orifices (MO treated, 7.6; BL treated, 10.5 [$P = 0.033$]; untreated, 13.0 [$P < 0.001$]), lower CFS scores (MO treated, 1.1; BL treated, 2.7 [$P = 0.013$]; untreated, 2.5 [$P = 0.050$]), and improved BUT (MO treated, 19.4 seconds; BL treated, 8.3 seconds [$P = 0.098$]; untreated, 1.5 seconds [$P = 0.008$]).

Conclusions: *Elovl1*-deficient mice exhibited multiple MGD symptoms, which were improved by MO.

Translational Relevance: Our findings reveal the usefulness of *Elovl1*-deficient mice as a model for dry eye with MGD and suggest the potential of mineral oil eye drops as a treatment for this condition.

Introduction

The tear film that covers the ocular surface is composed of a mucin layer, an aqueous layer (these two are collectively referred to as the muco-aqueous layer), and a lipid layer, in order from the cornea outward.^{1–3} Dry eye disease is a multifactorial ocular surface disease caused by destabilization of the tear film, and it is accompanied by various ocular symptoms.⁴ The causes of dry eye include increasing age,^{5,6} abnormalities in sex hormone secretion,^{6–8} video display terminal work,^{6,9} and environmental factors.^{6,10,11}

Most dry eye is classified into two types—aqueous-deficient dry eye (ADDE) and evaporative dry eye (EDE)—and both types often occur together.¹² In ADDE, decreased lacrimal gland function caused by factors such as aging or immunologic disorders leads to reduced tear secretion (the aqueous layer component) from the lacrimal glands.^{12,13} EDE is caused by abnormalities in the tear film lipid layer (TFLL), which prevents evaporation of water from the aqueous layer,^{1,14} reduces the surface tension of the tear film,^{1,15} increases tear viscoelasticity,¹⁶ and lubricates the corneal surface.¹ The meibomian glands, which are located behind the upper and lower eyelids,

are specialized sebaceous glands.^{1,14} The lipids secreted by these glands are called meibum lipids and are the primary components of the TFLL.¹⁷ The predominant cause of EDE is meibomian gland dysfunction (MGD), the pathology of which includes obstruction of the meibomian glands (mainly due to hyperkeratinization of the duct epithelium) and meibomian gland atrophy or dropout.^{12,14} In some cases of MGD, the quantity and/or composition of the meibum lipids is altered by factors such as aging,^{18,19} hormone imbalance,^{20,21} and contact lens use.²²

A number of dry eye treatments that depend on the cause of the pathology or the type of dry eye disease have been developed.²³ For example, for dry eye accompanied by inflammation, anti-inflammatory or immunosuppressive ophthalmic solutions are administered.^{24,25} For ADDE, ophthalmic solutions containing aqueous supplements such as viscosity-enhancing agents or punctal plugs are used.²³ For mucin deficiency, drugs stimulating mucin production such as diquafosol tetrasodium^{23,26} and rebamipide^{23,27} are approved for use in some countries, including Japan. For EDE/MGD, the lipids obstructing the meibomian glands are removed by warm compression while maintaining good hygiene of the eyelid.^{23,28–30} The application of ophthalmic solutions containing lipids such as mineral oil,^{23,31–35} castor oil,^{23,35–39} or a mixture of several lipids^{40,41} has also been reported. However, in most of those studies, the ophthalmic solutions used contained other substances, such as polymers, in addition to the lipids, and no control experiments (i.e., using blank ophthalmic solutions) were conducted. Therefore, it remains unclear whether the lipid components themselves are actually responsible for the reported effects.

Animal models have been used in dry eye research to reveal, in particular, the efficacy of treatment and the mechanisms of pathogenesis. One of these, the *Elov11*-deficient mouse, exhibits the EDE phenotype.⁴² *Elov11* encodes a fatty acid (FA) elongase involved in the production of saturated or monounsaturated very long-chain (VLC) FAs (FAs with a carbon chain length of ≥ 21).^{43–45} These VLCFAs and their derivative VLC fatty alcohols are used as the components of the major meibum lipids, the cholesteryl esters and wax monoesters, respectively.^{17,42} In *Elov11*-deficient mice, the chain lengths of the cholesteryl esters and wax monoesters are reduced.⁴² In other words, the quality of the TFLL is altered. However, no detailed dry eye phenotype tests, such as assessment of the obstruction of meibomian gland orifices, tear film stability, or corneal damage score, have been performed on *Elov11*-deficient mice. In the present study, we examined whether *Elov11*-deficient mice exhibit a typical MGD-

type dry eye phenotype and whether mineral oil-containing ophthalmic solution (MO) improves the dry eye phenotype in these mice.

Methods

Ethics Statement

This study was approved by the institutional review board of Lion Corporation. All experiments followed the guidelines in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Mice

Since whole-body *Elov11* disruption causes neonatal lethality due to skin barrier abnormalities,⁴⁶ we used *Tg(IVL-Elov11) Elov11^{-/-}* mice (*Tg-Elov11^{-/-}*), in which *Elov11* is disrupted in all tissues except for the epidermis.⁴⁷ In the epidermis of *Tg-Elov11^{-/-}* mice, *Elov11* is expressed under the control of the involucrin (*IVL*) promoter. *Tg(IVL-Elov11) Elov11^{+/+}* mice were used as controls. Both lines of mice were produced as described previously,⁴⁷ and 9-week-old females were used. The mice were kept in an environment with a temperature of $22.0 \pm 1^\circ\text{C}$, humidity of $53 \pm 2\%$, and a 12-hour light/dark cycle. They were fed with CE-2 solid diet (CLEA Japan, Tokyo, Japan) and given water *ad libitum*.

Preparation and Treatment of Ophthalmic Solutions and Experimental Schedule

The MO was prepared as follows. First, 0.1% mineral oil (Sonneborn, Petrolia, PA, USA) was emulsified with 0.75% polyoxyethylene hydrogenated castor oil 60 (Nippon Surfactant Industries, Tokyo, Japan) and 0.1% polysorbate 80 (Kao, Tokyo, Japan). These were then mixed with 0.4% sodium chloride (Tomita Pharmaceutical, Naruto, Japan), 0.1% potassium chloride (Ako Kasei, Ako, Japan), 0.8% boric acid (Kanto Chemical, Tokyo, Japan), 0.24% borax (Kozakai Pharmaceutical, Tokyo, Japan), 0.05% sodium hydrogen sulfite (FUJIFILM Wako Pure Chemical, Osaka, Japan), and 0.01% sodium edetate (Nagase ChemteX, Osaka, Japan). The composition of the blank ophthalmic solution (BL) was identical to the MO except that it did not contain the mineral oil. *Tg-Elov11^{-/-}* mice were divided into three groups (untreated, $n = 4$; BL treated, $n = 6$; MO treated, $n = 8$) at 9 weeks of age. Each mouse in the BL-treated and MO-treated groups received the corresponding ophthalmic solution in

both eyes for 14 weeks at a dose of 5 μ L per eye, four times per day, 5 days per week. During this period, we took the following measurements at the indicated ages: the number of plugged meibomian gland orifices (9 weeks and 13 weeks), corneal fluorescein staining (CFS) score (9 weeks and 13 weeks), tear film breakup time (BUT) (20 weeks), and tear quantity (23 weeks). Two BL-treated mice died at 11 and 14 weeks of age, and two MO-treated mice died at 22 weeks of age. Following the completion of these analyses, the mice were euthanized at 23 weeks of age, and their eyelids were subjected to histologic analyses.

Evaluation of Dry Eye Phenotypes

Mice were anesthetized via inhalation of isoflurane (Pfizer, New York, NY, USA). The number of plugged meibomian gland orifices in the upper and lower eyelids of control and *Tg-Elov11*^{-/-} mice (control mice, *n* = 4; *Tg-Elov11*^{-/-} mice: untreated, *n* = 4; BL treated, *n* = 6; MO treated, *n* = 8) at 9 to 13 weeks of age was counted under an SL-17 slit-lamp microscope (Kowa, Nagoya, Japan). The slit lamp was aimed downward, and each mouse was placed on its side on the laboratory bench under anesthesia. The eyelids were carefully opened using fingers and tweezers so as not to injure the eyelids or eyeball, and the eye was observed under the microscope while illuminated by the slit lamp.

To evaluate corneal damage, the CFS score was determined in control and *Tg-Elov11*^{-/-} mice (control mice, *n* = 4; *Tg-Elov11*^{-/-} mice: untreated, *n* = 4; BL treated, *n* = 6; MO treated, *n* = 8) at 13 weeks of age. First, 2 μ L of 0.5% fluorescein sodium solution (FUJIFILM Wako Pure Chemical) was applied to the eye of an anesthetized mouse using a micropipette (FUKAEKASEI, Kobe, Japan). Two minutes later, the eye was washed with saline, and the excess saline after washing was removed with paper wipes. The cornea was then observed under the slit-lamp microscope with a cobalt blue filter. In CFS, wounds on the surface of the cornea were stained. The CFS was scored from grade 0 to 4, according to a previous report,³⁴ as follows: 0, no fluorescein staining; 1, slightly punctate staining with <30 spots; 2, punctate (but not diffuse) staining with >30 spots; 3, severe diffuse staining but no positive plaque; and 4, severe diffuse staining with positive fluorescein plaque.

Tear film BUT was assessed in control and *Tg-Elov11*^{-/-} mice (control mice, *n* = 4; *Tg-Elov11*^{-/-} mice: untreated, *n* = 4; BL treated, *n* = 4; MO treated, *n* = 8) at 20 weeks of age. The protocol was developed based on previously reported methods^{48,49} and our preliminary experiments. First, 1 μ L of 1% fluorescein sodium solution was loaded onto the eye of an

anesthetized mouse using a micropipette. The quantity of 1% fluorescein sodium solution (1 μ L) suitable for the staining was determined in preliminary experiments. Next, the eye was manually opened and closed, and the time until the uniform distribution of the fluorescein on the eye surface was lost was measured under the slit-lamp microscope with a cobalt blue filter.

The tear quantity was determined in control and *Tg-Elov11*^{-/-} mice (control mice, *n* = 4; *Tg-Elov11*^{-/-} mice: untreated, *n* = 4; BL treated, *n* = 4; MO treated, *n* = 4) at 23 weeks of age. One end of a Zone-Quick cotton thread (Ayumi Pharmaceutical, Tokyo, Japan) was inserted into the lower eyelid for 15 seconds. The thread was removed and the length of the wet portion of the thread was measured using a caliper.

Histologic Analyses

The eyelids of 23-week-old mice were fixed at 4°C for at least 48 hours in Super Fix KY-500 (Kurabo, Osaka, Japan). The fixative solution was then replaced with 30% sucrose dissolved in water, and the samples were stored at 4°C overnight. The samples were embedded in OCT Compound (Sakura Finetek Japan, Tokyo, Japan) and then frozen in cooled isopentane. Meibomian gland sections 6 μ m thick were prepared using a cryostat (Leica CM1850; Leica, Wetzlar, Germany) and stained with hematoxylin and eosin (HE) or oil red O (ORO) as follows. For HE staining, sections were washed with water, stained with hematoxylin solution (FUJIFILM Wako Pure Chemical; 0.1% hematoxylin, 5% aluminum potassium sulfate, 5% chloral hydrate, and 0.1% citric acid monohydrate), washed with 0.1 M Tris-HCl (pH 7.6) and 80% ethanol, stained with 0.5% eosin (FUJIFILM Wako Pure Chemical) dissolved in ethanol, and dehydrated in ethanol. For ORO staining, sections were washed with water, treated with 60% isopropyl alcohol, stained with ORO solution (equal mixture of water and saturated ORO solution [FUJIFILM Wako Pure Chemical] dissolved in 2-propanol), washed with 60% isopropyl alcohol and water, stained with hematoxylin solution, and washed with 0.1 M Tris-HCl (pH 7.6). The bright-field images were observed and captured using an upright microscope (BX53; Olympus, Tokyo, Japan).

Statistical Analyses

All data are presented as means \pm SDs. Statistical analyses were performed using JMP 14 software (SAS Institute, Cary, NC, USA) with the significance of differences evaluated using Student's *t*-test, paired *t*-test, or parametric Tukey's test.

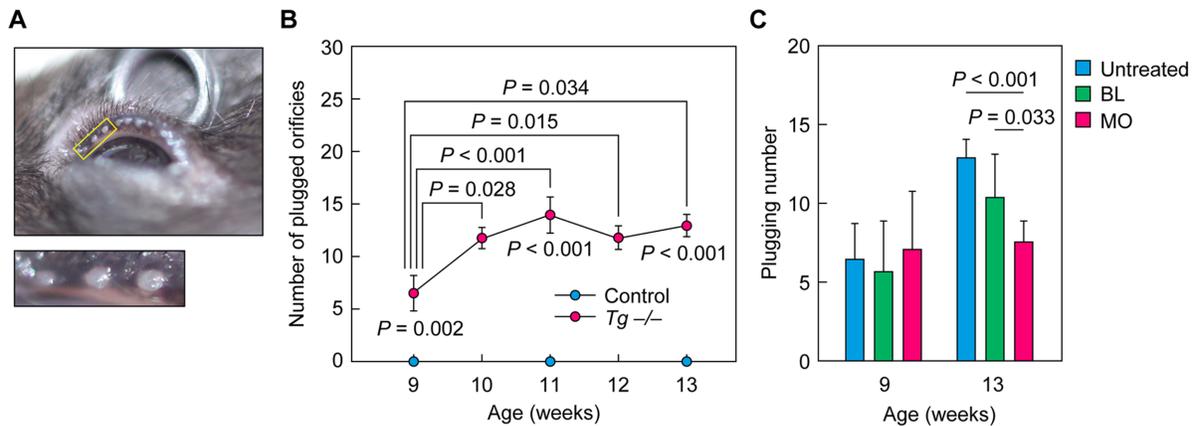


Figure 1. Plugging of meibomian gland orifices in *Elov1*-deficient mice reduced by mineral oil. (A) Plugged meibomian gland orifices in a 22-week-old *Tg-Elov1*^{-/-} mouse. The lower panel is an enlarged view of the area outlined by the yellow rectangle in the upper panel. (B) Number of plugged meibomian gland orifices in the upper and lower eyelids of control and *Tg-Elov1*^{-/-} (*Tg*^{-/-}) mice at 9 to 13 weeks of age ($n = 4$). Values presented are means \pm SDs, and P values for statistical tests versus control mice of the same age (Student's t -test) and 9-week-old mice of the same genotype (paired t -test) are indicated. (C) Nine-week-old *Tg-Elov1*^{-/-} mice were untreated or treated with BL or MO for 4 weeks, and the number of plugged meibomian gland orifices in the upper and lower eyelids was counted (untreated, $n = 4$; BL, $n = 6$; MO, $n = 8$). Values presented are means \pm SDs, and P values of Tukey's test are indicated.

Results

Mineral Oil Reduces Plugging in *Elov1*-Deficient Mice

In many dry eye patients with MGD, plugging is observed in the meibomian gland orifices.⁵⁰ We found raised white masses in the meibomian gland orifices in the *Elov1*-deficient (*Tg-Elov1*^{-/-}) mice (Fig. 1A), and when the eyelids were squeezed with fingers or tweezers, toothpaste-like secretions were extruded from the orifices. These features are similar to those of the plugging in human MGD,⁵⁰ so this condition in *Tg-Elov1*^{-/-} mice is hereafter referred to as plugging. It is unclear whether this plugging completely or partially interferes with meibum lipid secretion. The plugging was already present in *Tg-Elov1*^{-/-} mice at 9 weeks of age, when observation began, and the number of glands that were plugged increased with age (Fig. 1B). There was no plugging observed in the control mice.

Next, we investigated the effect of mineral oil on the plugging. We treated 9-week-old *Tg-Elov1*^{-/-} mice with BL or MO for 4 weeks (i.e., up to 13 weeks of age), four times per day, 5 days per week. At 9 weeks of age, there was no significant difference in the number of plugged orifices among groups. By 13 weeks of age, in the untreated group, the number of plugged orifices doubled from 6.5 at the start to 13 at the end and increased from 5.8 to 10.5 in the BL-treated group (Fig. 1C). In contrast, the number of plugged orifices in the MO-treated group was 7.3 at the start and 7.6 at the

end, which was significantly lower than in the other two groups. These results indicate that mineral oil is effective in suppressing the progression of plugging.

Mineral Oil Inhibits Lipid Aggregate Production in the Meibomian Glands of *Elov1*-Deficient Mice

Plugging in dry eye patients with MGD is often caused by hyperkeratinization of the epithelial cells in the meibomian gland ducts.¹⁴ We therefore examined the morphology of the meibomian glands, especially the orifice and duct portions, of *Tg-Elov1*^{-/-} mice at 23 weeks of age, via HE staining. There were no obvious abnormalities in the gland orifices or ducts, and their morphology was similar to that of the control mice (Fig. 2A, 2B). The morphology of the meibomian gland acini of *Tg-Elov1*^{-/-} mice was also normal, which was consistent with our previous report.⁴² These findings suggest that the plugging observed in *Tg-Elov1*^{-/-} mice was not the result of hyperkeratinization of the duct epithelium but was formed by the meibum lipids themselves.

Next, we stained the meibomian glands with ORO, which stains lipids. In untreated control mice, the ducts and acini of the meibomian glands were uniformly stained with ORO (Fig. 2C). In contrast, in untreated *Tg-Elov1*^{-/-} mice, the staining pattern was not uniform, and we observed some roughly circular, darkly stained areas (referred to hereafter as lipid aggregates) partly surrounded by less-stained areas in the ducts and acini

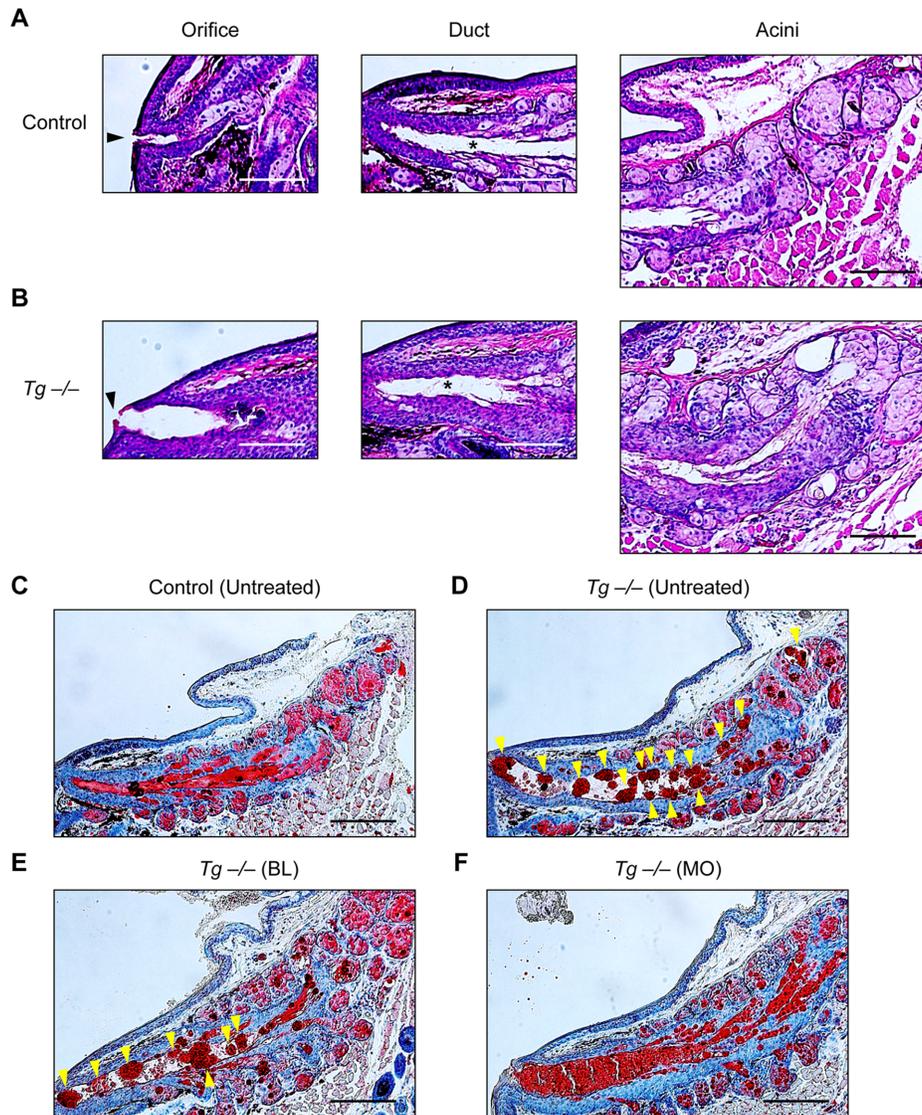


Figure 2. Lipid aggregation in the meibomian glands of *Elovl1*-deficient mice reduced by mineral oil. (A, B) The meibomian glands of 23-week-old control (A) and *Tg-Elovl1*^{-/-} (*Tg*^{-/-}) mice (B) were subjected to HE staining, and the gland orifices, ducts, and acini are shown. *Black arrowheads* and *asterisks* indicate gland orifices and ducts, respectively. (C–F) Nine-week-old control and *Tg-Elovl1*^{-/-} mice were untreated or treated with BL or MO for 14 weeks, and the meibomian glands were subjected to ORO staining. *Yellow arrowheads* indicate lipid aggregation. Scale bars: 200 μ m.

(Fig. 2D). These lipid aggregates were reduced in the BL-treated *Tg-Elovl1*^{-/-} mice (Fig. 2E), and few were observed in the MO-treated group (Fig. 2F). These results indicate that mineral oil inhibits the production of lipid aggregates.

Suppression of Corneal Damage in *Elovl1*-Deficient Mice by Mineral Oil

We have previously reported that many *Tg-Elovl1*^{-/-} mice older than 5 months old exhibit

corneal abnormalities with turbidity, brown to red coloring, or protrusions.⁴² In the present study, we investigated corneal damage in younger *Tg-Elovl1*^{-/-} mice, using CFS. There was no significant difference in CFS score between the control and untreated *Tg-Elovl1*^{-/-} mice at 9 weeks of age (Fig. 3). However, by 13 weeks of age, the score of the untreated *Tg-Elovl1*^{-/-} mice had increased and was significantly higher than that of the control mice. Next, we examined the effect of mineral oil on the progression of corneal damage in *Tg-Elovl1*^{-/-} mice. Although treatment with BL for 4 weeks did not affect

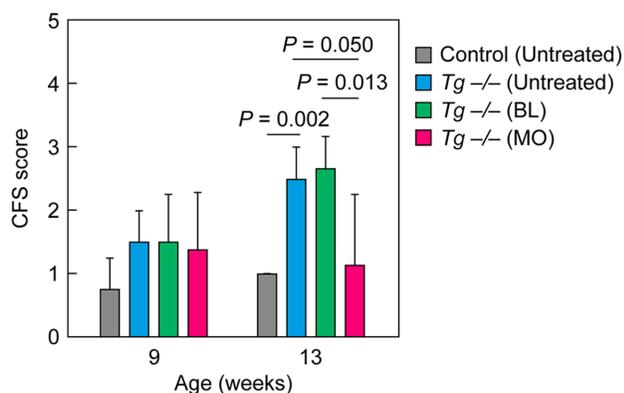


Figure 3. CFS score in *Elov1*-deficient mice improved by mineral oil. Control and *Tg-Elov1*^{-/-} (*Tg*^{-/-}) mice at 9 weeks of age were untreated or treated with BL or MO for 4 weeks, and the CFS score was determined (control mice, $n = 4$; *Tg-Elov1*^{-/-} mice untreated, $n = 4$; treated with BL, $n = 6$; treated with MO, $n = 8$). Values presented are means \pm SDs, and P values for Tukey's test are indicated.

the CFS score, MO treatment reduced it to the levels of the untreated control mice. These results indicate that mineral oil suppresses the progression of corneal damage.

MO Improves Tear Film Stability in *Elov1*-Deficient Mice

BUT is an indicator of tear film stability, with a shorter BUT implying less stability.⁵¹ The BUT of untreated *Tg-Elov1*^{-/-} mice at 20 weeks of age was 1.5 seconds, which was significantly shorter than that of untreated control mice (18.5 seconds; Fig. 4A). Treatment of *Tg-Elov1*^{-/-} mice with BL for 11 weeks, from the ages of 9 until 20 weeks, resulted in a moderate increase in BUT, to 8.3 seconds. Treatment with the MO resulted in a further increase in BUT (to 19.4 seconds), which was significantly longer than for the untreated group. The tear amount was similar in all groups (Fig. 4B).

Discussion

We have previously reported the EDE dry eye phenotypes of *Tg-Elov1*^{-/-} mice.⁴² The reported phenotypes included increases in eye-blink frequency and water evaporation from the ocular surface and the development of corneal opacity in older animals. However, we had not yet analyzed tear film stability or corneal damage in younger animals. In the present study, we performed more detailed analyses of dry eye in *Tg-Elov1*^{-/-} mice, including observation of the meibomian gland orifices, examination of the meibo-

mian gland histology, and determination of CFS score and BUT. We found that these mice exhibit MGD-type EDE phenotypes, with plugging of the meibomian gland orifices, lipid aggregation in the meibomian glands, corneal damage, and reduced BUT compared with control mice (Figs. 1–4). In addition, we have shown that mineral oil can suppress many of these dry eye phenotypes (Figs. 1–3).

The two major meibum lipids are cholesteryl esters and wax monoesters, which together account for 60% to 92% of total meibum lipids (depending on the study).^{17,52,53} These lipids are the least polar among mammalian lipids.⁵⁴ The meibum lipids also include lipids with higher polarity than the cholesteryl esters and wax monoesters, such as (*O*-acyl)- ω -hydroxy FAs (OAHFAs), wax diesters, cholesteryl OAHFAs, and triglycerides, albeit in small quantities.^{17,54} The lipid polarity gradient formed by these diverse meibum lipids may be what allows the TFL to be stably retained on the aqueous layer.⁵⁴ The FA elongase ELOVL1 is highly active toward C20 to C24 saturated acyl-CoAs but shows rather weaker activity toward monounsaturated C20 to C24 acyl-CoAs.^{42–45,55} The FA chain lengths of cholesteryl esters and the fatty alcohol chain lengths of wax monoesters in the meibum lipids are mainly C20 to C34, and their FA/fatty alcohol portions, especially the saturated ones, are shortened in *Tg-Elov1*^{-/-} mice.^{42,56} Since OAHFAs contain a mostly monounsaturated C30 to C34 ω -hydroxy FA, their quantities are not greatly affected by the *Elov1* disruption.⁴² Although the composition of other meibum lipids, including wax diesters, has not yet been investigated, it is possible that *Elov1* disruption causes shortening of their chain lengths and/or reduction of their quantities. Here, we observed plugging of the meibomian gland orifices and lipid aggregation in the meibomian glands in *Tg-Elov1*^{-/-} mice (Figs. 1 and 2). Although the exact causes of these phenomena are unclear, a change in the meibum lipid composition due to *Elov1* disruption may lead to impaired meibum-lipid mixing or lipid-polarity gradient formation, which would prevent the meibum lipids from spreading over the aqueous layer.

It is unclear whether plugging completely or partially interferes with meibum lipid secretion in *Tg-Elov1*^{-/-} mice. In either case, plugging of the meibomian gland orifices may reduce the quantities of meibum lipids supplied to the ocular surface. We observed corneal damage in these mice (Fig. 3). It is likely that the decrease in lipid quantities or instability of the TFL (Fig. 4A) causes increased blinking and friction between the eyelids and the ocular surface, causing this corneal damage. In addition, the increases in the number of plugged orifices and CFS

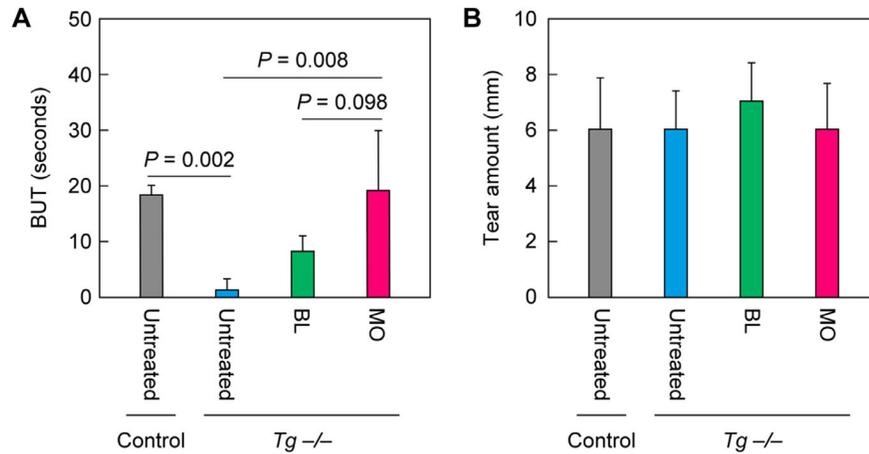


Figure 4. Tear film stability in *Elov11*-deficient mice increased by MO. (A) Nine-week-old control and *Tg-Elov11*^{-/-} (*Tg*^{-/-}) mice were untreated or treated with BL or MO for 11 weeks, and BUT was examined (control mice, $n = 4$; *Tg-Elov11*^{-/-} mice untreated, $n = 4$; treated with BL, $n = 4$; treated with MO, $n = 8$). Values presented are means \pm SDs, and P values for Tukey's test are indicated. (B) Nine-week-old control and *Tg-Elov11*^{-/-} mice were untreated or treated with BL or MO for 13 weeks, and tear amount was measured ($n = 4$). Values presented are means \pm SDs.

score at 13 weeks of age compared with 9 weeks of age suggest that these symptoms progressed with age. In our previous report, *Tg-Elov11*^{-/-} mice developed obvious corneal abnormalities with turbidity, brown to red coloring, or protrusions from 5 months of age onward.⁴² In the present study, however, no such abnormalities were observed at the end of our analyses (23 weeks of age). Since we used different facilities for breeding the animals in the two studies, differences in the local environment such as temperature, humidity, and airflow may have caused this discrepancy.

In this study, we evaluated the effectiveness of mineral oil for treating MGD-type dry eye and found that it resulted in significant differences in the number of plugged orifices and extent of corneal damage (Figs. 1 and 3). In addition, BUT tended to be longer in the MO group than in the BL group (Fig. 4A). From these results, we speculate that the MO is effective in improving tear film stability in *Tg-Elov11*^{-/-} mice. Mineral oil may dissolve the meibum lipids plugging the gland orifice. Alternatively, it is possible that the mineral oil is directly incorporated into the TFLL and functions as the nonpolar lipid component of the TFLL, thus improving tear film stability.⁵⁷ Treatment with MO also suppressed lipid aggregation in the meibomian glands of *Tg-Elov11*^{-/-} mice (Fig. 2F). It is unlikely that mineral oil alters the properties of the meibum lipids in the gland ducts or acini. Rather, we speculate that it stimulates the secretion of meibum lipids from the meibomian gland ducts by dissolving the plugging, which in turn suppresses the growth of aggregates. The blank solution also had some weak

effects on the dry eye phenotypes of *Tg-Elov11*^{-/-} mice. Although the differences were not statistically significant, the number of plugged orifices (Fig. 1C) and the BUT (Fig. 4A) were somewhat lower and longer, respectively, in the BL group than in the untreated group. In addition, the lipid aggregation in the meibomian glands was suppressed by BL treatment (Fig. 2E). The periodic washing of the ocular surface with the BL solution or the emulsifying effect of the surfactant in the BL solution on the meibum lipids may explain these effects.

In conclusion, here we show that *Tg-Elov11*^{-/-} mice are a useful model of EDE with MGD. Furthermore, our results indicate that mineral oil is effective in improving EDE dry eye symptoms. We expect that these findings will lead to the use of ophthalmic treatments containing mineral oil for patients with EDE, especially those with MGD.

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