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LOXL1 genetic polymorphisms are associated with exfoliation glaucoma in the Japanese population

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Purpose: We performed genetic association studies using a native Japanese population to examine the reproducibility of results of *lysyl oxidase-like 1* (*LOXL1*) genetic association studies for exfoliation glaucoma (XFG) beyond the differences of ethnicity. We also quantified *LOXL1* mRNA expression in the human lens capsule to examine the possible correlation between *LOXL1* expression and XFG pathogenesis.

Methods: We performed a case-control study using 95 Japanese XFG patients and 190 controls. Real-time polymerase chain reaction (PCR) analysis was performed using lens capsules obtained during surgery.

Results: The TT genotype in the single nucleotide polymorphism (SNP) rs1048661 and the GG genotype in the SNP rs3825942 in exon 1 of *LOXL1* were significantly associated with an increased risk of XFG under recessive models (χ^2 test, $p=5.34\times10^{-34}$ and $p=2.1\times10^{-8}$, respectively). Quantification of *LOXL1* mRNA expression demonstrated no significant difference between XFG and senile cataract samples.

Conclusions: Although the functional effects of the *LOXL1* SNP appear to be qualitative rather than quantitative, the amino acid substitution (R141L) caused by SNP rs1048661 is not a simple decisive factor for XFG due to the inverted allele frequency between Japanese XFG and Caucasian XFG patients. Further genetic and functional studies are essential for clarifying XFG pathogenesis.

Exfoliation glaucoma (XFG) is an age-related disorder associated with exfoliation syndrome (XFS), manifested by abnormal fibrillar deposits on the lens and iris epithelium [1]. Recently, a genome wide association study performed for the Caucasian population revealed a strong association between the genotype of single genetic polymorphisms (SNPs) in the *lysyl oxidase-like 1* (*LOXL1*) gene and the occurrence of XFS/XFG [2]. It was reported that the rate of XFG occurrence significantly differed from one ethnic population to another [3], therefore it is logically important to perform a case-control study using another ethnic population such as the Japanese. In this study, we found a strong genetic association between the occurrence of XFG and the *LOXL1* single nucleotide polymorphism (SNP) genotype. One of the nonsynonymous SNP (rs1048661) showed a very strong association with XFG. However, the risk allele was inverse compared to the Caucasian study. To gain further insight into the role of *LOXL1* for XFG, it is important to compare the expression levels of *LOXL1* mRNA in XFG eyes and non-glaucomatous eyes. We obtained anterior lens capsules during combined glaucoma-cataract surgery or during cataract surgery alone from XFG patients and non-glaucomatous senile cataract patients, respectively. We then performed a quantitative analysis of *LOXL1* mRNA expression using these anterior lens capsules.

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METHODS

Subjects: All XFG patients were diagnosed by slit-lamp examination for the existence of exfoliation material on the anterior lens capsule with maximal dilation of the pupils and with glaucomatous optic neuropathy as well as visual field defect. Peripheral blood was obtained from 95 XFG patients 47–93 years of age (mean age: 75.7±8.1 years). The controls were 190 randomly-selected, population-based individuals 54–83 years of age (mean age: 65.0±6.8 years) with no glaucomatous changes or existence of exfoliation materials (Table 1). All of the XFG patients and normal volunteers were recruited at Kyoto Prefectural University Hospital (Kyoto,

Table 1. Clinical characters of the exfoliation glaucoma patients and control.

	XFG	Control
Total number of subjects	95	190
Mean age (range)	75.7 (47–93 years)	65.0 (54–83 years)

Ninety-five exfoliation glaucoma patients 47 to 93 years of age (mean age: 75.7 years), and 190 randomly-selected population-based individuals 54 to 83 years of age (mean age: 65.0 years) with no glaucomatous changes or existence of exfoliation materials were involved in this study.

TABLE 2. CASE-CONTROL STUDY OF TWO NONSYNONYMOUS SINGLE NUCLEOTIDE POLYMORPHISMS IN *LOXL1*.

rs1048661 (R141L)* Genotype	Control	XFG	rs3825942 (G153D)** Genotype	Control	XFG
GG	33	0	GG	135	94
GT	114	1	GA	53	1
TT	43	94	AA	2	0

The rs1048661 TT genotype and the rs3825942 GG genotype were significantly associated with an increased risk of XFG under recessive models ($\chi 2$ test). The single asterisk indicates TT versus GT+GG, P value=5.34x10–34, OR=321.3, and 95% CI=43.5–2373.2, and the double asterisk indicates GG versus GA+AA, p=2.1x10–8, OR=38.3, and 95% CI=5.2–281.6.

Japan) and examined by glaucoma specialists using slit-lamp microscopy and an automated visual field analyzer. All study subjects were ethnically Japanese. According to the rules of the process committee at Kyoto Prefectural University of Medicine, written informed consent was obtained from all participants before participation in this genetic association study. The study was conducted in accordance with the tenets of the Declaration of Helsinki.

Genotyping: We genotyped two nonsynonymous single nucleotide polymorphisms (SNPs; rs1048661 and rs3825942) in the *LOXL1* gene region according to the previous report [2]. We genotyped the SNPs with both direct sequencing and the TaqMan genotyping assay (Applied Biosystems, Foster City, CA). We used a set of primers (5′-GAT CCA GTG GGA GAA CAA CG-3′ and 5′-GGT ACT CGG GCA GCT CTT C-3′) for direct sequencing. Genotyping was performed using on a 3130xl Sequence Detection System or with 7500 Realtime-time polymerase chain reaction (PCR) system (Applied Biosystems). The TaqMan genotyping assay was performed according to the manufacturer's protocol. All the genotyping procedures were approved by the ethics committee of Kyoto Prefectural University of Medicine.

Statistical analysis: The frequencies of the genotypes were compared between XFG patients and controls in the recessive model. In this model, frequencies of the homozygous genotype for major alleles were compared using a 2x2 contingency table. Here, the association was evaluated using the χ^2 test for the contingency table. A p-value of less than 0.01 was considered to be statistically significant. Odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated.

Real-time polymerase chain reaction analysis: Anterior capsules that were obtained during glaucoma/cataract or cataract surgery with written informed consent were immediately stored with RNAlater reagent (Ambion, Austin, TX) to protect the RNA. All procedures were approved by the ethics committee of Kyoto Prefectural University of Medicine. Total RNA was isolated with the Micro RNA extraction kit (Qiagen Japan, Tokyo, Japan) from the anterior capsules, and then cDNA was prepared as described previously [4]. The anterior capsules were obtained from 10 XFG patients undergoing combined (cataract+glaucoma) surgery (mean age 74.9±8.0 years; male:female=6:4) and 10 non-glaucomatous, senile cataract patients (mean age: 76.5±10.6 years; male:female=4:6). To avoid contamination of blood, anterior lens capsulotomy was performed before glaucoma surgery in cases of combined procedures. We used TaqMan real-time PCR probes and primers specific for human *LOXL1* (Hs00173746_m1), and 18S rRNA from Applied Biosystems (Assay-on-Demand gene expression products). Real-time PCR analysis was performed on a 7500 real-time PCR system. The relative expression of *LOXL1* mRNA in the anterior lens capsule was quantified by the standard curve method using 18S rRNA expression in the same cDNA as the control.

RESULTS

Case-control association study in the LOXL1 gene region: We genotyped 95 XFG cases and 190 control subjects by direct sequencing methods. The rs1048661 TT genotype and the rs3825942 GG genotype in the first exon of *LOXL1* were significantly associated with an increased risk of XFG under recessive models (χ^2 test, raw p value=$5.34x10^{-34}$, OR=321.3, 95% CI=43.5–2373.2 and p=$2.1x10^{-8}$, OR=38.3, 95% CI=5.2–281.6, respectively; Table 2). We further tested reproducibility of the genotyping by TaqMan genotyping assay and confirmed the genotyping results because the results of the rs1048661 genotyping was not concordant with the Hardy–Weinberg equilibrium (HWE). The results obtained by the two methods were identical.

Haplotype analysis of LOXL1 single nucleotide polymorphisms: First, we checked the state of linkage disequilibrium (LD) between rs1048661 and rs3825942 and found that those two SNPs are in a state of strong LD (Table 3). Next, we examined the distribution of two-locus haplotypes in the XFG and control samples (Table 4) using Haploview [5] and PENHAPLO [6] software. Among the two-locus haplotypes of SNPs in exon 1 (rs1048661 G/T and rs3825942 G/A), the TG haplotype showed an increased risk for XFG (TG/TG versus others; p=$6.87x10^{-7}$, OR=312.162) in recessive models. We checked HWE among control subjects using this two-locus haplotype and found that the

two-locus haplotype was concordant with HWE (p=0.0125, χ^2 test).

LOXL1 mRNA quantification using anterior lens capsules: cDNA was synthesized from total RNA isolated from the anterior lens capsules of patients undergoing cataract surgery. We analyzed both XFG (n=10) and senile cataract (n=10) specimens. Figure 1 is a representative result of two independent results of experiments run in duplicate. There was no statistically significant difference between the expression levels of *LOXL1* mRNA in the lens epithelium obtained from XFG patients and senile cataract patients. (Figure 1, p=0.529 by the Mann–Whitney U-test)

DISCUSSION

Similar to previous studies among Caucasian [2,7,8], we found a strong genetic association between the *LOXL1* SNP and XFG patients. The lysyl oxidase protein family has multiple functions including specific oxidative deamination of lysine residues and the cross-linking of elastin [9]. Phenotypic analysis of *LOXL1* knockout mice [10] showed that the LOXL1 protein has an essential role for the homeostasis of elastic fibers, which also contribute to the trabecular meshwork structures [11]. Therefore, it is functionally reasonable to assume that *LOXL1* is one of the causative genes for XFG.

For the rs3825942 genotype, our results are consistent with those of Thorleifsson et al. [2]. The allele frequency of rs3825942 G is consistently higher among Caucasian and Japanese XFG patients so it is reasonable to conclude that rs3825942 A is a protective allele against the occurrence of XFG.

On the other hand, the risk allele for XFG occurrence is rs1048661-"T" among the Japanese population and rs1048661-"G" among the Caucasian population. We double-checked these genotype results (rs3825942 and rs1048661) by both direct sequencing and the TaqMan genotyping assay because the genotype results of rs1048661 within control subjects were not concordant with HWE. The results obtained from two different methods were in complete agreement. Therefore non-concordance with HWE is not due to genotyping errors. We further checked the two-locus haplotype and found that the haplotype was concordant with HWE. Since the two-locus, rs1048661 and rs3825942, was in the state of linkage disequilibrium (D'=1.0), it was appropriate to check the HWE by haplotype.

Inverted genotypes of XFG patients between the Japanese and Caucasian population suggests the following possibilities: (1) The 141st amino acid substitution (R141L) does not have a dominant role for the pathophysiology of XFG, (2) the heterozygote for the rs1048661 G/T genotype may have a protective role against XFG occurrence, or (3) there might be another causative polymorphism in a state of linkage disequilibrium with rs1048661 G/T. If the last possibility is the case, there might be a historical recombination between the SNP rs1048661 and the causative polymorphism.

As a next step, we quantified relative *LOXL1* mRNA expression in anterior lens capsules and found that there was no significant difference between XFG and senile cataract controls. These results are different from the previous study by Thorleifsson et al. [2], which showed a significantly higher *LOXL1* mRNA expression with the TT genotype (the protective genotype against XFG in the Caucasian population) than those with the TG or GG genotype using adipose tissues.

Table 3. Linkage disequilibrium state of rs1048661 and rs3825942.

SNP	D'	r2
Control+XFG	1	0.243
Control only	1	0.196
XFG only	1	1

We calculated the state of linkage disequilibrium between rs1048661 and rs3825942 among the control population, XFG population, and total population. Linkage disequilibrium coefficients were expressed as D' or r2.

Table 4. Structures and frequency of two locus haplotype.

	Frequency	
Haplotype	Case	Control
TG	0.9947	0.5263
GG	0	0.3237
GA	0.0053	0.15

We examined the frequency of two-locus haplotypes in the XFG and control samples using Haploview and PENHAPLO software. The TG haplotype means rs1048661-T and rs3825942-G, the GG haplotype means rs1048661-G and rs3825942-G, and the GA haplotype means rs1048661-G and rs3825942-A.

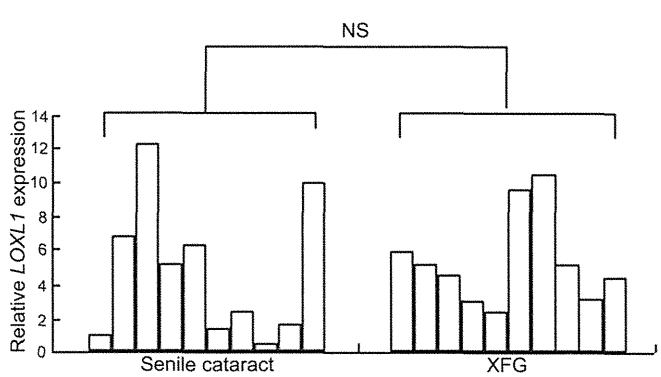

Figure 1. Real-time polymerase chain reaction analysis of *LOXL1* mRNA expression in anterior lens capsules. Total RNA was extracted from the anterior lens capsule of XFG/senile cataracts. Real-time PCR analysis was performed with expression assay probes. The amount of relative expression was normalized to that of 18Sr RNA. (N.S.: Not statistically significant, p=0.529; Mann–Whitney's U-test).

We hypothesize that the difference might be due to the mixed *LOXL1* SNP genotype of our senile cataract surgery samples, which are not able to clarify genotype due to ethical procedure reasons. However, our *LOXL1* mRNA expression analysis reflects a direct pathological site within the ocular tissue and better represents *LOXL1* expression status in the affected eyes than that of adipose tissue [2]. Therefore, we deduced that it is likely that the quantitative difference of *LOXL1* mRNA is not a direct pathogenetic cause of XFG.

To further clarify the pathophysiological role of *LOXL1* for XFG, we are now performing extensive SNP discovery around *LOXL1* to find out other possible causative polymorphisms. In addition, functional analysis of LOXL1-fibulin5 protein interaction [12] using two types of recombinant LOXL1 precursor protein (141R and 141L) is ongoing. Since LOXL1-fibulin5 interaction is essential for mature cross-linked elastin formation [10,12], we are focused on determining the role of the two variants of *LOXL1* and its mixture to gain further insight into our genetic association results showing that the rs1048661 G/T heterozygote is protective against XFG. It is also important to investigate the protective role of LOXL1 protein variants that possess the 153D amino acid, which is indicated by multiple genetic association studies including our own [2,7,8] as well as to analyze the possible role of the pairs of 141/153 amino acid variants in consideration of our haplotype analysis result.

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Effects of Switching from Topical β-Blockers to Latanoprost on Intraocular Pressure in Patients with Normal-Tension Glaucoma

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ABSTRACT

Aims: **The effects of switching from topical β-blockers (β) to latanoprost (LA) on intraocular pressure (IOP) and IOP-reduction rate (IOP-RR) in patients with normal-tension glaucoma (NTG) were investigated.**

Subjects and Methods: **Sixty (60) NTG patients (60 eyes) were divided into three equal groups receiving carteolol hydrochloride (group A), nipradilol (group B), and betaxolol hydrochloride (group C) twice-daily for 3 months. The drugs were changed to topical LA administered once-daily for the next 3 months.**

Results: **Baseline IOP was 14.4 ± 0.9, 14.6 ± 0.6, and 14.6 ± 0.9 mmHg in groups A, B, and C, respectively. At 3 months, IOP was 12.4 ± 0.6, 13.4 ± 0.6, and 12.9 ± 0.8 mmHg and 10.5 ± 0.5, 11.1 ± 0.8, and 11.7 ± 0.8 mmHg at 6 months in groups A, B, and C, respectively. At 3 months, IOP-RR was 10.4 ± 5.5, 9.5 ± 2.6, and 10.8 ± 4.7% and 24.1 ± 4.3, 22.9 ± 5.9, and 19.4 ± 3.8% at 6 months in groups A, B, and C, respectively. The groups did not significantly differ in the first 3 months regarding IOP and IOP-RR. Switching to LA significantly decreased IOP and increased IOP-RR in all groups.**

Conclusion: **In NTG patients, LA reduced IOP more effectively than the β tested.**

INTRODUCTION

GLAUCOMA AFFECTS MORE THAN 66 million people in the world and is the leading cause of blindness.[1] The Tajimi study,[2,3] which was one of the largest glaucoma epidemiology studies in Japan, showed that the glaucoma prevalence rate in Japanese older than 40 years of age is 5.0%, and the rate of open-angle glaucoma is 3.9%. That study also reported that almost 90% of the open-angle glaucoma cases consisted of normal-tension glaucoma (NTG). Thus, NTG is the most prevalent glaucoma type in Japan, and is one where the difficulty of managing treatment needs to be addressed.

In the treatment of NTG, reducing intraocular pressure (IOP) is the only evidence-based therapy,[4–8] though in some NTG patients, trabeculectomy is one of the effective treatments to prevent the progression of visual-field defects.[5,7,9] However, there are some patients whose glaucomatous damages progress while their IOP is kept in the low teens. In such patients, topical beta (β)-blockers with neuroprotective effects[10–14]

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TABLE 1. GROUP BACKGROUNDS

Group	n (M/F)	Age (years)	Baseline intraocular pressure (mm Hg)
A	16 (7/9)	59.5 ± 3.2	14.4 ± 0.9
B	14 (4/10)	60.8 ± 3.7	14.6 ± 0.6
C	13 (2/11)	59.2 ± 4.1	14.6 ± 0.9

Note. In these 3 groups, there was no significant difference among age and baseline intraocular pressure.

are frequently chosen. In this prospective study, we compared the IOP-reduction effects of three types of topical β-blockers (carteolol hydrochloride, nipradilol, and betaxolol hydrochloride), which were supposed to have neuroprotective effects and latanoprost (LA) in the same NTG subjects.

As for nipradilol, it is a nonselective alpha-1 beta-adrenergic antagonist that reduces IOP, and it is as potent as timolol for primary open-angle glaucoma (POAG)[15] because it increases uveoscleral outflow by the alpha-1 blocking effects and decreases aqueous production through the β-blocking effect.[16]

METHODS

At the outpatient clinic of Kyoto Prefectural University of Medicine and the Baptist Eye Clinic (Kyoto, Japan), 60 patients with NTG (60 eyes: 21 males and 39 females; mean age, 61.7 ± 1.9 years), who were newly diagnosed or who used only one antiglaucoma eye drop, were enrolled. This study was carried out between June 2001 and April 2003. They had enough informed consent by a glaucoma specialist and did not suffer from asthma, arrhythmia, or heart disease. In patients who had been treated with other topical medications, 4 weeks were allowed for wash-out before starting topical β-blockers. The diagnostic criteria for NTG were (1) normal iridocorneal open angle, (2) no evidence of IOP higher than 21 mmHg, (3) glaucomatous changes in the visual field with optic-nerve cupping, and (4) absence of other optic neuropathies. The 60 patients were divided into three equally sized groups, and each group received one type of β-blocker (group A, carteolol hydrochloride; group B, nipradilol; group C, betaxolol hydrochloride) twice a day for 3 months. During the subsequent 3 months, each group was treated once a day with topical LA only. Their IOP was measured once a month during this 6-month period between the hours of 9:30 AM and 12:00 PM by glaucoma specialists with a Goldmann applanation tonometer (Haag-Streit, Bern, Switzerland). The IOP-reduction rate (IOP-RR) was then calculated and the effect of switching from the β-blocker to LA was statistically analyzed by using the formula (baseline IOP-current IOP)/baseline IOP × 100. Statistical analysis was performed with the Tukey-Kramer test, the paired- and unpaired *t* test, and the chi-square test.

We defined nonresponders as patients whose IOP-RR was 10%[17,18] or less in each 3-month period and calculated the rate of nonresponse to β-blockers and LA. To analyze IOP, right-eye data was used when data from both eyes was available.

RESULTS

In the course of this study, 17 patients dropped out; 6 (35.3%) owing to LA side effects (a reddish conjunctive state, or irritated conjunctiva), 3 (17.6%) due to β-blocker side effects, and 8 (47.1%) owing to other reasons, such as failure to continue visits. All drop-out patients were subsequently excluded from our statistical analysis. Subjects analyzed after exclusion of the dropouts are summarized in Table 1. Baseline IOP was 14.4 ± 0.9, 14.6 ± 0.6, and 14.6 ± 0.9 mmHg in groups A, B, and C, respectively. There was no

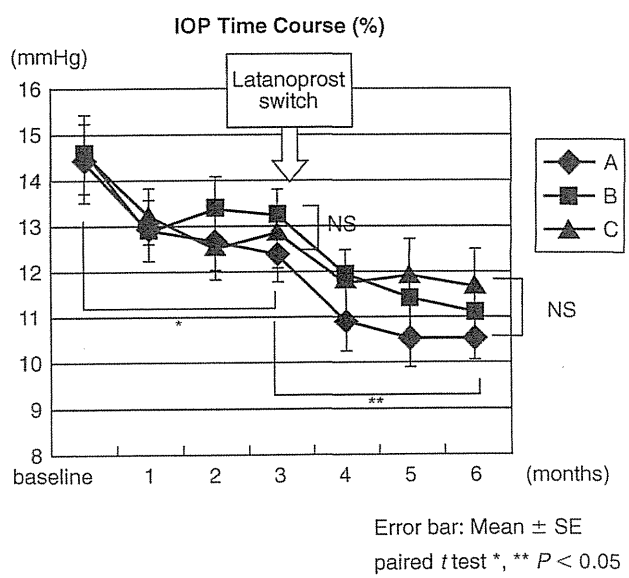

FIG. 1. By switching from β-blocker to latanoprost, intraocular pressure was significantly decreased in all groups.

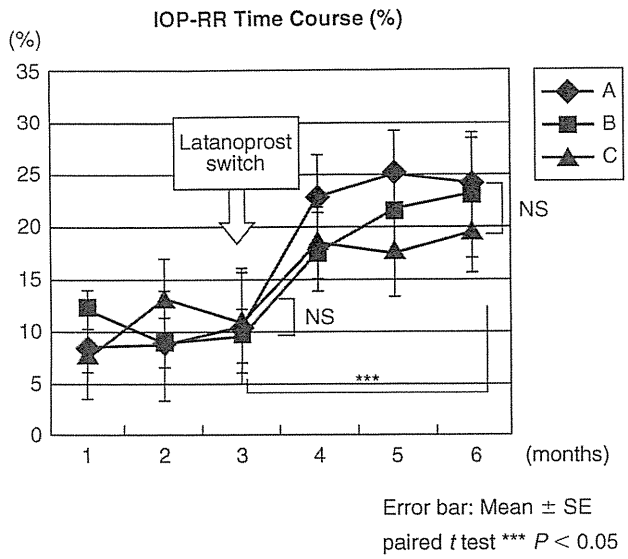

FIG. 2. There were no significant intergroup differences throughout 6 months (Tukey-Kramer test). In all three groups, the intraocular pressure reduction rate was significantly higher at 6 than at 3 months after the inception of the study (paired *t* test).

TABLE 2. THE CHANGE OF INTRAOCULAR PRESSURE REDUCTION RATE (IOP-RR) AND NONRESPONDERS AT EACH 3-MONTH PERIOD

	β-blockers		*Latanoprost*	
IOP-RR	n	%	n	%
30%	4	9.3	14	32.6
20%	9	20.9	12	27.9
10%<	7	16.3	8	18.6
Nonresponders	23	53.5*	9	20.9*

Asterisk indicates a significant difference in nonresponders between β-blocker and latanoprost (chi-square test, Yates $P = 0.0257$).

significant difference with respect to age or baseline IOP among the three groups. The IOP time courses are shown in Figure 1. At 3 months, IOP was 12.4 ± 0.6, 13.4 ± 0.6, and 12.9 ± 0.8 mmHg, and at 6 months, IOP was 10.5 ± 0.5, 11.1 ± 0.8, and 11.7 ± 0.8 mmHg in groups A, B, and C, respectively. Irrespective of the type of β-blocker administered during the first 3 months, IOP became significantly lower than the baseline; there

TABLE 3. PREVIOUS RESULTS OF MONOTHERAPY SWITCHING β-BLOCKER TO LATANOPROST

Authors	*Glaucoma type*	*Race*	*Before switching eye drop*	n	*pre latanoprost IOP*	*post latanoprost IOP*	*RR (%) from β-blocker to latanoprost*	*Evaluation period*
Zimmerman et al.	POAG	Caucasian	Betaxolol	236	19.9 ± 4.0	16.3 ± 3.3	18.1	6 months
	OH	Black	Carteolol	80	20.1 ± 3.4	15.8 ± 3.3	21.4	
	PE	Hispanic	Levobunolol	177	19.9 ± 3.5	16.9 ± 3.3	15.1	
	PD	Asian	Timolol hemihydrate	173	19.9 ± 3.9	17.6 ± 3.7	11.6	
			Timolol	397	20.6 ± 3.6	17.7 ± 3.3	14.1	
			Timolol gel	816	20.3 ± 3.8	17.4 ± 3.7	14.3	
Bayer et al.	POAG	n/a	Timolol	462	21.1 ± 4.1	17.4 ± 3.1	17.5	24 months
	OH		Levobunolol	79	20.9 ± 3.5	17.4 ± 2.1	16.7	
	PE		Metipranolol	60	20.5 ± 3.5	17.2 ± 2.7	16.1	
	CACG		Betaxolol	40	21.6 ± 4.0	17.1 ± 3.6	20.8	
			Carteolol	33	22.3 ± 2.8	18.0 ± 2.9	19.3	
Haverkamp et al.	POAG OH PE CACG	n/a	Beta-blocker	451	21.3 ± 4.0	17.3 ± 2.7	18.8	3 months
Bron et al.	POAG OH PE	n/a	Timolol	17	26.3 ± 1.2	19.6 ± 1.1	25.5	6 weeks
Ikeda	NTG	Japanese	Carteolol	16	12.4 ± 0.6	10.5 ± 0.5	15.3	3 months
			Betaxolol	14	13.4 ± 0.6	11.1 ± 0.8	17.2	
			Nipradilol	13	12.9 ± 0.8	11.7 ± 0.8	9.3	

POAG, primary open-angle glaucoma; OH, ocular hypertension; PE, pseudoexofoliation; PD, pigment dispersion; CACG, chronic angle-closure glaucoma; NTG, normal-tension glaucoma; carteolol, carteolol hydrochloride; betaxolol, betaxolol hydrochloride.

was no significant intergroup difference. During the 3 months of LA administration, a significant decrease in IOP was again revealed in all groups, irrespective of the type of β-blocker administered before switching. Again, there was no intergroup difference. At 3 months, IOP-RR was 10.4 ± 5.5, 9.5 ± 2.6, and 10.8 ± 4.7%, and at 6 months, IOP-RR was 24.1 ± 4.3, 22.9 ± 5.9, and 19.4 ± 3.8% in groups A, B, and C, respectively. In all three groups, the IOP-RR was significantly higher at 6 than at 3 months after the inception of the study (Fig. 2). All data are presented as the mean ± standard error.

Table 2 shows the change of IOP-RR and nonresponders rate from each β-blocker to LA. These results confirm that the IOP-reduction effect of LA is significantly greater than that of the β-blockers tested. For the rate of nonresponders, LA is significantly lower than β-blockers.

DISCUSSION

As for POAG and ocular hypertension, there have previously been similar studies regarding switching from β-blocker to LA.[19–22] However, and to the best of our knowledge, our study is the first to compare LA and the three types of β-blockers in the switching condition, while previous reports compared between the two discrete NTG groups.[23–25] The IOP-RR from β-blocker to LA in the NTG patients shown in our data was smaller (9.3%–17.2%) than that in the POAG patients (11.6%–25.5%) presented in previous studies (Table 3).

When discussing drug effectiveness, it is very important to compare that effectiveness in the same patients, not in different patients, because each patient has a possibility to react differently to each drug. However, a switching study is very difficult to perform because of the high dropout rate owing to the long study period or drug side effects. Our study also suffered the relatively high dropout rate, mainly because the patients could not come regularly owing to the long study period. Other reasons why the patients dropped out were the side effects of the medications; LA is twice as much as β-blockers.

We chose three topical β-blockers, carteolol hydrochloride, nipradilol, and betaxolol hydrochloride, because they were frequently used to treat NTG patients in Japan, considering the neuroprotecitve effects and the increase of ocular blood flow.[26,27] However, from our clinical impression, these drugs seem to be less effective for IOP reduction, when compared to LA, even in the NTG patients. Therefore, in this current study, we just focused on the IOP reduction effects of the three β-blockers and LA administered, and found that latanoprost is more effective than any of the three β-blockers, which showed the same IOP reduction during the administration period, and that the types of β-blockers administered prior to switching had no effect on patients' response to LA.

Recently, attention has been paid to LA nonresponders who show little or no IOP reduction effects by latanoprost. However, few reports mentioned the β-blocker nonresponders. In our study, the nonresponder rate of β-blockers among NTG patients was higher than that of LA, and more than half of the β-blocker nonresponders (53.5%) responded to LA after switching, while only 1 patient did not respond to LA but responded to β-blockers. There were 7 double nonresponders for both β-blockers and LA, each of whose baseline IOP was significantly lower (12.0 ± 0.8 mmHg) than that of the responders (15.7 ± 0.4 mmHg) ($P < 0.001$; unpaired t test). This result leads to the facts that the NTG patients whose baseline IOP is in the low teens, or lower, are more difficult to reduce IOP than those of mid-teens or higher.

In cases where a NTG patient was found to be a double nonresponder, the available treatment options for managing and controlling that patient's IOP are quite limited. One possible option is to use other types of drugs, such as carbonic anhydrase inhibitors; however, that treatment might prove to be inadequate in many cases. Another option is a combination therapy, although the IOP-reduction power of each individual portion of the therapy might be weak.

CONCLUSION

This study suggests that LA provides a much greater IOP-RR and lower nonresponder rate than that of β-blockers in Japanese NTG patients.

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Anterior Segment Optical Coherence Tomography Findings of Acute Angle-Closure Glaucoma in Vogt-Koyanagi-Harada Disease

Ultrasound biomicroscopy (UBM) is reported to be useful for the evaluation of the anterior chamber, chamber angle, and ciliary body. However, a UBM examination is difficult and invasive for the patient. The new Visante (Carl Zeiss Meditec, Oberkochen, Germany) imaging system using anterior segment optical coherence tomography (AS-OCT) makes it possible to visualize the anterior segment noninvasively to obtain quantitative information. We report a patient with acute angle-closure glaucoma associated with Vogt-Koyanagi-Harada (VKH) disease in whom imaging with AS-OCT was carried out to evaluate the anterior segment.

Case Report

A 50-year-old woman presented with a 2-day history of headache and blurred vision in both eyes. Her visual acuity in the right eye was 1.0 when corrected by –4.25 diopters, and 0.9 corrected by –3.50 diopters in the left eye. Intraocular pressure was 44 mmHg in the right eye and 42 mmHg in the left eye. Slit-lamp examination disclosed a shallow anterior chamber and narrow chamber angle in both eyes. Signs of iridocyclitis were absent. Fundus examination disclosed serous retinal detachment, edema around the optic disc, and circumferential detachment of the ciliary body in both eyes. (Fig.1A). She was diagnosed as having acute angle-closure glaucoma in VKH disease. Fluorescein angiography (Fig. 1B) and the human leukocyte antigen pattern of positive DR-4 (DRB1*04) also supported the diagnosis. AS-OCT images showed a shallow anterior chamber, narrow chamber angle, and supraciliary fluid in both eyes. The iris showed anterior bowing consistent with a pupillary block in both eyes (Fig. 2A–C).

These findings improved after two separate pulsed treatments with methylprednisolone at the initial daily dosage of 1000 mg followed by oral prednisolone. After the initial pulsed treatment, intraocular pressure was 13 mmHg in the right eye and 15 mmHg in the left. Three weeks after corticosteroid treatment, the patient's visual acuity was 0.9 in both eyes, and the refraction state became –2.00 diopters in the right eye and –1.00 diopters in the left.

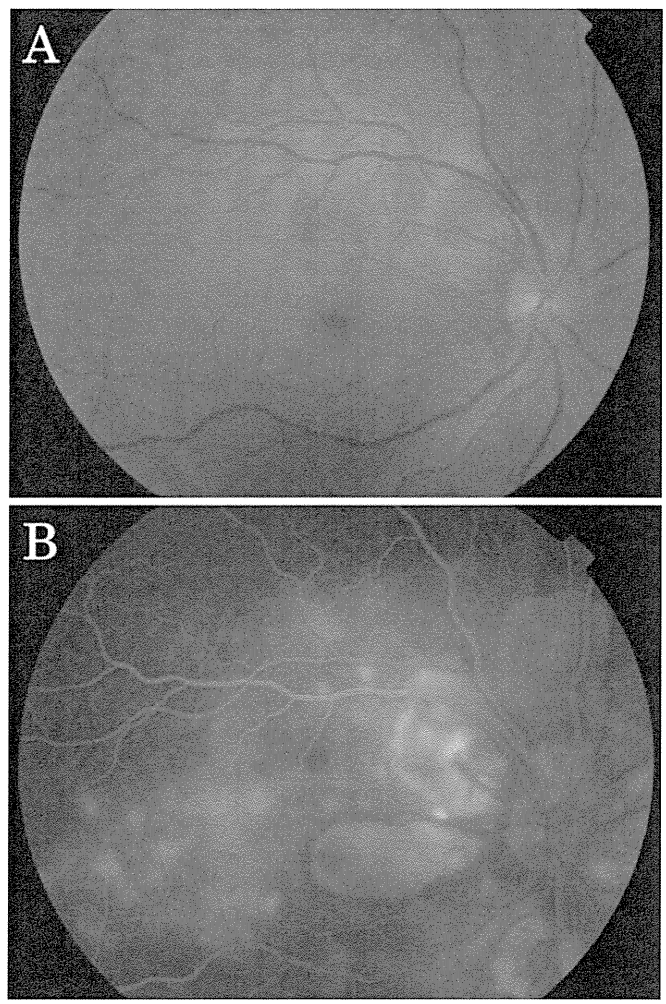

Figure 1. A Fundus photograph showing serous retinal detachment in the right eye of a Vogt-Koyanagi-Harada patient. **B** Fluorescein angiogram showing hyperfluorescent spots consistent with dye leakage and dye pooling in the subretinal space.

Comments

VKH disease is a bilateral panuveitis with serous retinal detachment and symptoms of meningeal irritation, dysacusia, and poliosis.[1] The disease is accompanied by various degrees of inflammation of the iris, ciliary body, and choroid, but changes in the anterior segment of the eyeball have been examined only by means of slit-lamp microscopy. UBM examination is a useful method for evaluating these changes of the anterior segment, and the diagnostic utility of UBM has been reported in various studies of many kinds of disease.

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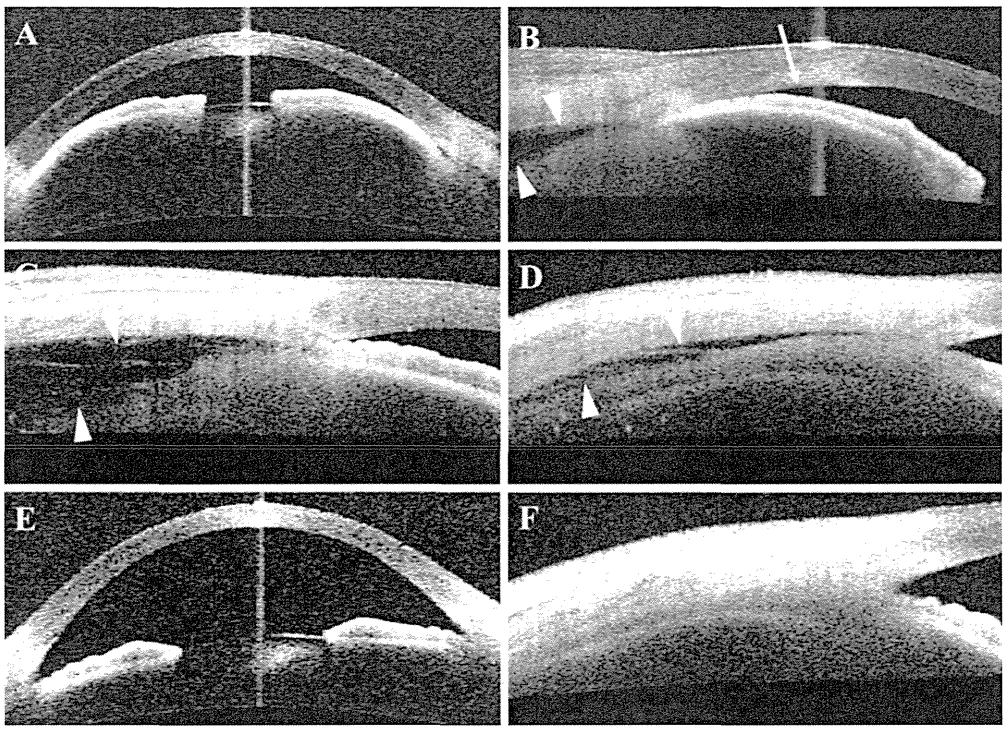

Figure 2A–F. Right eye. **A, B** Anterior segment optical coherence tomography images showing shallow anterior chamber, narrow chamber angle (*arrow*), and supraciliary fluid (*arrowheads*). **C** The supraciliary fluid (*arrowheads*) was evident before steroid treatment. **D** Ten days after steroid treatment, the supraciliary fluid (*arrowheads*) decreased. **E, F** Fourteen days after steroid treatment, the anterior chamber returned to the normal depth and supraciliary fluid had disappeared.

Anterior segment imaging is a rapidly advancing field in ophthalmology.[2] AS-OCT is a new imaging system that provides quantitative information and qualitative imaging of the cornea and anterior chamber with a short measurement time. This system does not require contact with the eye to obtain measurements, so it is a noninvasive examination. AS-OCT can measure corneal thickness, anterior chamber depth, and chamber angle. AS-OCT has also been used for measurements of corneal flap depth following laser in situ keratomileusis (LASIK), of anterior chamber width prior to phakic intraocular lens implantation, and of morphologic changes occurring in eyes after glaucoma surgery.[2]

Many studies have reported UBM findings in VKH disease,[3–5] yet to the best of our knowledge this is the first report about AS-OCT findings of acute angle-closure glaucoma in VKH disease. After systemic corticosteroid treatment, AS-OCT images showed that the anterior chamber returned to the normal depth, the ciliary body reverted to its normal position, and the supraciliary fluid disappeared in both eyes (Fig. 2D–F). Supraciliary fluid secondary to inflammation of the uvea is considered to be related to the development of a shallow anterior chamber.[3–5] This study shows that AS-OCT is a simple and noninvasive imaging technique with high resolution that is useful for observing the response to corticosteroid treatment in a VKH patient with acute angle-closure glaucoma.

Key Words: acute angle-closure glaucoma, anterior segment optical coherence tomography, supraciliary fluid, Vogt-Koyanagi-Harada disease

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Stat6-Independent Tissue Inflammation Occurs Selectively on the Ocular Surface and Perioral Skin of IκBζ$^{-/-}$ Mice

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Purpose. IκBζ$^{-/-}$ mice have been reported to be affected by allergic dermatitis. This study was conducted to analyze the pathophysiological role of IκBζ and to address the functional relevance of Th2-mediated immune responses in the development of ocular surface inflammation and dermatitis by IκBζ$^{-/-}$ mice.

Methods. BALB/c background IκBζ$^{-/-}$ mice were established without individual differences; IκBζ/Stat6 double-knockout (WKO) mice unable to produce Th2 cytokine were created; and microscopic-, histologic-, and immunochemical studies were performed. In IκBζ$^{-/-}$ mice the serum IgE levels were examined by ELISA, and quantitative PCR was used to study the gene expression of IFN-γ, IL4, IL10, TNFα, IL6, IL17α, and CCL11 in eyelid tissue.

Results. IκBζ$^{-/-}$ mice exhibited a severe inflammatory phenotype on the ocular surface and perioral skin. The inflammatory infiltrates in the perioral skin consisted primarily of CD4$^+$ and CD8$^+$ cells; CD4$^+$ and CD45R/B220$^+$ cells were mainly detected in the conjunctiva. In eyelid and perioral skin tissue, the expression of IL-17α and of Th1 and Th2 cytokines, but not of CCL11, was augmented. IκBζ$^{-/-}$ and IκBζ$^{+/-}$ mice did not differ significantly in their serum total IgE levels before, 0 to 4 weeks, and 5 to 9 weeks after disease onset. IκBζ/Stat6 WKO mice showed the same or slightly more severe inflammation than did IκBζ$^{-/-}$ mice.

Conclusions. IgE and Stat6 are not responsible for the immune pathologic response leading to the development of ocular surface and perioral skin inflammation in IκBζ$^{-/-}$ mice. IκBζ$^{-/-}$ mice may be a suitable model for Stevens-Johnson syndrome, but not for atopic dermatitis. (*Invest Ophthalmol Vis Sci.* 2008;49:3387–3394) DOI:10.1167/iovs.08-1691

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IκBζ (also known as MAIL and INAP) is an ankyrin-repeat containing nuclear protein that is highly homologous to the IκB family member Bcl-3.[1–3] IκBζ was originally reported to be a regulator of transcription factor NF-κB, which is strongly induced by interleukin (IL)-1 and lipopolysaccharide (LPS).[1–4] IκBζ, induced by diverse PAMPs (pathogen-associated microbial products), such as peptidoglycan (PGN), bacterial lipoprotein, flagellin, MALP-2, R-848, and CpG DNA,[5] regulates NF-κB activity, possibly to prevent the excessive inflammation caused by bacterial components.[3,6]

We have reported that IκBζ$^{-/-}$ mice with a 129/Ola×C57BL/6 background expressly exhibit severe, spontaneous ocular surface inflammation accompanied by the eventual loss of almost all goblet cells and suggested that IκBζ participates in the negative regulation of ocular surface inflammation.[6] We also proposed IκBζ$^{-/-}$ mice as a suitable model for Stevens-Johnson syndrome (SJS), an ocular surface inflammatory disease, because they manifest the loss of goblet cells that occurs in human SJS.[6]

Another group reported that MAIL (molecular-possessing ankyrin repeats induced by LPS, equal to IκBζ)$^{-/-}$ mice, also from a 129/Ola×C57BL/6 background, represent a valuable new animal model for research on atopic dermatitis, because these animals were affected by allergic dermatitis.[7]

The inflammatory phenotypes of previously reported 129/Ola×C57BL/6 background IκBζ$^{-/-}$ mice were not uniform, and there were individual variations. For example, although all IκBζ$^{-/-}$ mice manifested ocular surface and periocular skin inflammation, only some developed dermatitis in the perioral area, neck, or ventral trunk.[5,7] To analyze the pathophysiological role of IκBζ, we established BALB/c background IκBζ$^{-/-}$ mice.

STAT6 is a critical transcriptional factor that regulates IL-4-mediated Th2 immune responses.[8,9] It is phosphorylated and activated through an IL-4R-mediated signal. It translocates as a phosphorylated homodimer and subsequently regulates IL-4-mediated transcriptional events, including Th2 differentiation and Ig class switching to IgE. IL-4-mediated STAT6 activation is an efficient cascade for the generation of Th2 cells during primary T-cell activation. The disruption of the STAT6 gene in mice has revealed its requirement for the development of Th2 cells and Th2-specific immune responses, such as IgE hyperproduction and atopic bronchial asthma.[10,11]

To address the functional relevance of Th2-mediated immune responses in the development of ocular surface inflammation and dermatitis in IκBζ$^{-/-}$ mice, we created mice lacking both IκBζ and Stat6 (IκBζ/Stat6 WKO) that are not able to produce Th2 cytokines, such as IL-4.[9]

Materials and Methods

BALB/c Background IκBζ KO Mice and IκBζ/Stat6 WKO Mice

BALB/c background IκBζ knockout (KO) mice were produced by back-crossing 129/Ola×C57BL/6 IκBζ KO mice with BALB/c mice for six generations. For genotyping we used genomic DNA isolated from the tail of 2- to 3-week-old heterozygous parents (DNeasy kit; Qiagen, Valencia, CA); PCR amplification was as previously reported.[6] Briefly, PCR amplification on a thermal cycler (GeneAmp; Applied Biosystems, Foster City, CA), with the IκBζ gene primer pair for wild IκBζ, IκBζ-wild (GCTCATCCAGCTAACCTGAACAGTGTT), and IκBζ-ex03 (GTTTAAGGTGGCGGTTCTGCTCTTTG) resulted in an ~1200-bp fragment from wild-type ($I\kappa B\zeta^{+/+}$) mice. The gene primer pair for the inserted neomycin gene, IκBζ-ex03, and PKG-rc2 (CTAAAGCGCATGCTCCAGACTGCCTTG) yielded an ~1200-bp fragment from the homozygotes ($I\kappa B\zeta^{-/-}$). Both fragments were obtained from the heterozygotes ($I\kappa B\zeta^{+/-}$).

BALB/c background IκBζ/Stat6 WKO mice were produced by mating BALB/c background $I\kappa B\zeta^{+/-}$ Stat6 KO mice obtained from BALB/c background $I\kappa B\zeta^{+/-}$ mice and BALB/c background Stat6 KO mice. To genotype the Stat6 gene, we performed PCR amplification with the Stat6 gene primer pair for wild Stat6, as previously reported.[9] The use of primer A, specific for the targeted Stat6 gene (TCACTGGGGGACCGGATCCGGGATCTT), and primer B, specific for the Stat6 gene downstream of the targeting construct (GCGCTCAGCTCGGGCCTACACACATTA), resulted in an ~1300-bp fragment from wild-type ($Stat6^{+/+}$) mice. The gene primer pair for the inserted neomycin gene, primer B and primer C, which is specific for the neo resistance gene (ATCGCCTTCTATCGCCTTCTTGACGAG), yielded an ~1300-bp fragment from homozygotes ($Stat6^{-/-}$). Both fragments were obtained from heterozygotes ($Stat6^{+/-}$). All studies were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Histologic Analysis

The whole eyeball, together with the eyelids, conjunctiva, and perioral skin of the mice, were fixed in 10% neutral buffered formalin. Fixed tissues were then embedded in paraffin, 6-μm sections were cut, and representative sections from each sample were stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) reagent.

Immunohistologic Analysis

The whole eyeball, together with the eyelids, conjunctiva, and perioral skin of the mice, was embedded in OCT compound (Sakura Finetek, Torrance, CA) and then flash frozen in liquid nitrogen. Sections (6 μm thick) were cut and fixed with 100% acetone at 4°C for 10 minutes and blocked (30 minutes) with 10% normal donkey serum in phosphate-buffered saline (PBS). The rat monoclonal antibody was reactive with mouse CD45R/B220 (BD Biosciences, San Diego, CA), mouse CD4 (BD Biosciences), and mouse CD8 (eBioscience, San Diego, CA). The rat IgG2a isotype (BD Biosciences) was used for the negative control. The secondary antibody (biotin-SP-conjugated AffiniPure $F(ab')_2$ fragment donkey anti-rat IgG(H+L), 1:500 dilution; Jackson ImmunoResearch, West Grove, PA) was applied for 30 minutes. The ABC reagent (Vectastain; Vector Laboratories, Inc., Burlingame, CA), for increased sensitivity, and peroxidase substrate solution (DAB substrate kit; Vector Laboratories) were used as chromogenic substrates.

Measurement of Total IgE in Serum

Serum from collected blood was separated by centrifugation at 1000*g* for 10 minutes. Serum total IgE levels were assessed by ELISA using a mouse IgE ELISA (OptEIA; BD Bioscience) according to the manufacturer's recommendation.

Quantitative RT-PCR

The upper and lower eyelids were collected and homogenized in liquid nitrogen. Total RNA was extracted (RNeasy mini kit; Qiagen, Tokyo, Japan) and treated with DNase1 (DNase1 kit; Qiagen) to remove any residual genomic DNA. Reverse transcription (SuperScript Preamplification kit; Invitrogen) and real-time quantitative PCR were performed (Prism 7700; Applied Biosystems [ABI], Foster City, CA), according to published procedures.[9] The primers and probes for mouse IκBζ, CCL11, TNF-α, IFN-γ, IL-4, IL-10, IL-6, IL-17α, and mouse GAPDH were from ABI. The quantification data were normalized to the expression of the housekeeping gene GAPDH.

Results

Macroscopic Observations on $I\kappa B\zeta^{-/-}$ Mice

To analyze the pathophysiological role of IκBζ we established BALB/c background $I\kappa B\zeta^{-/-}$ mice. The phenotype of these mice is uniform, and there are no individual differences, although the phenotype of 129/Ola×C57BL/6 background $I\kappa B\zeta^{-/-}$ mice varies, and there are individual differences. The ratio of wild-type (+/+) to heterozygous (+/−) to homozygous (−/−) mutant mice born from heterozygous intercrosses was 107:220:33 (1:2:0.3), indicating that 70% of $I\kappa B\zeta^{-/-}$ embryos died in utero. The birth ratio of $I\kappa B\zeta^{-/-}$ mice is higher than of 129/Ola×C57BL/6 background mice (1:1.7:0.1).[7] None of the $I\kappa B\zeta^{+/+}$ or $I\kappa B\zeta^{+/-}$ mice exhibited symptoms of ocular surface- or skin inflammation until the age of 32 weeks (data not shown).

$I\kappa B\zeta^{-/-}$ mice manifested a severe inflammatory phenotype on the ocular surface, especially along the eyelids, and on the perioral skin. Kinetic monitoring of the inflammatory phenotype in the eyes and perioral skin revealed that the phenotype was absent at the time of birth. When these mice were between 4 and 6 weeks of age, the inflammatory phenotype of the eyelids became evident. Its appearance was followed by inflammatory symptoms in the perioral skin and became more severe as the animals grew older (Fig. 1A). Their severe eyelid inflammation was characterized by eyelid swelling, alopecia, and abnormal hair growth. No inflammation appeared on the abdominal and dorsal skin (data not shown). Severe perioral skin inflammation in these mice was characterized by erythema with excoriation and partial hair loss. Their dermatitis gradually progressed during the observation period until 32 weeks and resulted in lichenified chronic dermatitis. No morphologic or behavioral abnormalities were evident.

Histologic Analysis of IκBζ KO Mice

Histologic analysis of the perioral skin of $I\kappa B\zeta^{-/-}$ mice at 6 weeks of age, 2 weeks after symptom onset, revealed hyperplasia and spongiosis in the epidermis, including the hair follicles, inter- and intracellular edema in the epidermis, and heavy infiltration of the dermis by inflammatory cells (Fig. 1B). Histologically, the abdominal and dorsal skin of $I\kappa B\zeta^{-/-}$ mice did not manifest this inflammatory phenotype (data not shown). In the perioral skin of $I\kappa B\zeta^{+/-}$ mice of the same age, we detected neither obvious pathologic changes nor infiltrated inflammatory cells (Fig. 1B). Before the manifestation of inflammation, the perioral skin of $I\kappa B\zeta^{-/-}$ mice exhibited no distinct histologic changes (data not shown).

Histologic analysis of the eyes of the same $I\kappa B\zeta^{-/-}$ mice, performed 2 weeks after symptom onset, showed heavy infiltration by inflammatory cells into the submucosal area of the conjunctiva. Moreover, there was a severe decrease in the number of goblet cells in conjunctival epithelia (Fig. 1C). Similar to the perioral skin, histologic analysis of the periocular skin (eyelids) of the same $I\kappa B\zeta^{-/-}$ mice revealed hyperplasia