

Quantitative Analysis of the Association Between Follow-Up Duration and Severity of Limbal Stem Cell Deficiency or Visual Acuity in Aniridia

Shimpei Komoto,¹ Yoshinori Oie,¹ Satoshi Kawasaki,¹ Ryo Kawasaki,^{1,2} Nozomi Nishida,¹ Takeshi Soma,¹ Shizuka Koh,¹ Kazuichi Maruyama,¹ Shinichi Usui,¹ Kenji Matsushita,¹ Motokazu Tsujikawa,¹ Naoyuki Maeda,¹ and Kohji Nishida¹

¹Department of Ophthalmology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

²Department of Vision Informatics, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

Correspondence: Yoshinori Oie, Department of Ophthalmology, Osaka University Graduate School of Medicine, Room E7, Yamadaoka 2-2, Suita, Osaka, 565-0871, Japan; yoie@ophthal.med.osaka-u.ac.jp.

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PURPOSE. This study aimed to quantitatively analyze the association between follow-up duration and the severity of limbal stem cell deficiency (LSCD) or visual acuity in patients with aniridia.

METHODS. A total of 52 eyes of 27 patients with aniridia were enrolled at Osaka University Hospital. Medical records were retrospectively reviewed to obtain information on the severity of LSCD and corrected distance visual acuity (CDVA). LSCD severity was based on a modified severity grading scale. We used an ordered logistic regression model to examine the association between follow-up duration and LSCD severity, and a linear regression model with a generalized linear mixed model for the association between follow-up duration and visual acuity.

RESULTS. The mean follow-up duration was 5.2 ± 6.3 years. The mean age at the last follow-up visit was 40.5 ± 18.9 years. The mean CDVA was 1.52 ± 1.09 logMAR. At the last follow-up, 1 examined eye (1.9%) was categorized as stage 0, 7 (13.5%) as Ia, 9 (17.3%) as Ib, 5 (9.6%) as Ic, 2 (3.8%) as IIb, 12 (23.1%) as IIc, and 11 (21.2%) as III. Five eyes (9.6%) were unclassifiable. There was a significant association between follow-up duration and LSCD severity (odds ratio per +1 year, 1.41; $P < 0.001$). CDVA significantly decreased as follow-up duration increased. Each increase of 1 year in the follow-up duration was associated with a mean difference of $+0.021$ logMAR (95% confidence interval [CI] 0.01–0.03; $P < 0.001$).

CONCLUSIONS. We quantitatively demonstrate that LSCD severity and visual impairment significantly progress as follow-up duration increases.

Keywords: aniridia, limbal stem cell deficiency, visual acuity, follow-up duration

Aniridia, first described by Barrara in 1821, is a disorder characterized by hypoplasia or the absence of an iris at birth. It is a rare disease, with a prevalence of 1:64,000 to 1:96,000,^{1,2} often caused by heterozygous mutations of the *PAX6* gene.^{3–5} It has been reported that although approximately 90% of aniridia cases have a genetic origin in the *PAX6* gene, some aniridia cases have no detectable intragenic mutations in *PAX6*.⁶

Approximately two-thirds of aniridia cases are familial with autosomal dominant inheritance, whereas the remaining one-third of cases are sporadic and result from de novo gene mutations.⁷ Aniridia is often complicated by several ocular abnormalities, including aniridia-related keratopathy (ARK), cataract, glaucoma, and foveal hypoplasia, which lead to decreased visual acuity beginning early in life and requiring continuous ophthalmological care.^{8,9} Additionally, ARK comprises limbal stem cell deficiency (LSCD) and stromal opacification.

LSCD can cause profound visual disturbance in severe cases and sometimes requires surgical management, such as

allogeneic limbal stem cell transplantation or keratoprosthesis.¹⁰ Although the ocular surface can stabilize after surgical intervention, the success rates of these procedures remain unsatisfactory.¹¹ Therefore, LSCD is considered a serious abnormality in patients with aniridia because its management is often difficult. However, a previously reported grading scale for ARK has not focused on LSCD and was, therefore, less objective.^{10,12,13} Recently, a staging system for LSCD was reported based on a global consensus by the International Limbal Stem Cell Deficiency Working Group.¹⁴ Thus, the purpose of the current study was to perform a quantitative analysis of the association between follow-up duration and the severity of LSCD or visual acuity in patients with aniridia.

MATERIALS AND METHODS

This retrospective, cross-sectional study was reviewed and approved by the institutional review board of Osaka University Hospital. All participants provided informed consent



TABLE 1. Diagnostic Criteria for Aniridia

A. Symptoms	1. Impaired bilateral visual acuity 2. Photophobia
B. Examination findings	1. Variable degree of iris hypoplasia 2. Foveal hypoplasia 3. Keratopathy, such as LSCD or corneal opacity 4. Cataract 5. Microphthalmia 6. Nystagmus 7. Glaucoma
C. Differential diagnosis	1. HSV infection 2. Trauma or ocular surgery 3. Coloboma 4. Rieger anomaly 5. Iridocorneal endothelial syndrome
D. Systemic abnormalities	Systemic abnormalities caused by <i>PAX6</i> mutations
E. Genetic analysis	<i>PAX6</i> mutations or deletions in the 11p13 region
F. Others	Existence of AD inheritance in the family

A patient was diagnosed as “Definite” when complicated with A + B1 + E + none of C, and “Probable” if associated with A + B1 + either B2 or B3 + none of C.

AD = autosomal dominant; HSV = Herpes simplex virus; LSCD = limbal stem cell deficiency.

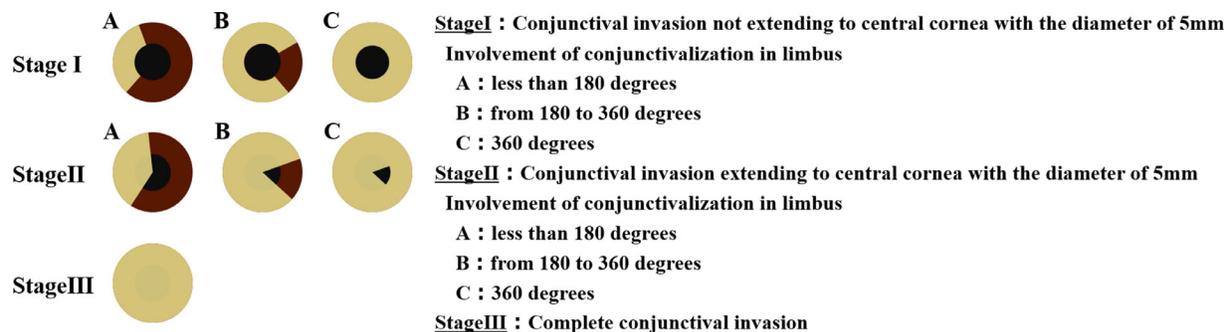


FIGURE 1. Staging system for LSCD. Eyes were graded according to the extent of conjunctival invasion of both the central cornea and limbus. LSCD = limbal stem cell deficiency in aniridia.

after the nature and risks/benefits of study participation were explained. The study was conducted in adherence to the tenets of the Declaration of Helsinki.

Patients diagnosed with aniridia at Osaka University Hospital Department of Ophthalmology between June 1995 and December 2017 were enrolled in this study. The inclusion criteria were patients diagnosed as “definite” or “probable” based on the diagnostic criteria (Table 1), which were defined by the Research on Rare and Intractable Diseases and Health, Labour, and Welfare Sciences Research Grant of the “Epidemiological Survey of Rare Intractable Corneal Diseases” Research Group in Japan.

Medical records were retrospectively reviewed for patient demographics (age, sex, *PAX6* mutation, and familial history), the severity of LSCD, corrected distance visual acuity (CDVA), and other ocular abnormalities (cataract, ocular hypertension, glaucoma, and foveal hypoplasia). The severity of LSCD was graded according to the modified staging system (Figure 1).¹⁴ The scale categorized stages I to III based on whether or not conjunctival invasion was expanded to the central 5 mm of the cornea. LSCD was cate-

gorized as stage 0 if there was no conjunctival invasion in the cornea; stage I, if conjunctivalization was observed to include up to the central 5 mm of the cornea; stage II, if there was conjunctival invasion in the central 5 mm area of the cornea; and stage III, if the entire corneal surface was covered by conjunctiva. Additionally, stages A to C were determined based on the degree of conjunctival invasion in the limbus. LSCD was classified as stage A, if the invasion affected less than 180 degrees of the limbus; stage B, if it affected 180 to 360 degrees of the limbus; and stage C, if it involved 360 degrees of the limbus. All staging was evaluated by three cornea specialists (S.K., T.S., and Y.O.). We determined that LSCD was complicated in cases of minimal conjunctival invasion of the corneal surface. We analyzed the severity of LSCD until limbal transplantation (LT) or cultivated oral mucosal epithelial transplantation (COMET) were performed.

Cataract was judged as positive when a patient had grade 1 or worse cataract in either nuclear, cortical, or posterior subcapsular cataract graded based on the Lens Opacities Classification System III,¹⁵ aphakia, or pseudophakia. Ocular

TABLE 2. Patient Characteristics at the Final Visit

Patients, cases, eyes	27, 52
Definite: Probable, cases, eyes	19, 36: 8, 16
FS: N: M: no mutations, cases	9: 7: 3: 2
Age, years, mean \pm SD	40.5 \pm 18.9
Male: Female, cases	15: 12
Familial: Sporadic: Unknown, cases	13: 11: 3
Follow-up duration, years, mean \pm SD	5.2 \pm 6.3
CDVA (logMAR), mean \pm SD	1.52 \pm 1.09
Limbic stem cell deficiency, eyes	51/52 (98.1%)
Stage 0: Ia: Ib: Ic: IIa: IIb: IIc: III: unclassifiable, eyes	1: 7: 9: 5: 0: 2: 12: 11: 5
Cataract, eyes	48/52 (92.3%)
Ocular hypertension, eyes	21/52 (40.4%)
Glaucoma, eyes	13/52 (25.0%)
Foveal hypoplasia, eyes	44/44 (100%)*
Interventions, cataract surgery: keratoplasty, eyes	30/52 (57.7%): 7/52 (13.5%)

CDVA = corrected distance decimal visual acuity; FS = frame-shift mutation; logMAR = logarithm of the minimum angle of resolution; M = missense mutation; N = nonsense mutation.

* Eight eyes were excluded because corneal opacity was too dense to examine the foveal status.

hypertension was defined when intraocular pressure determined using a noncontact or Icare (Icare, Helsinki, Finland) tonometer exceeded 21 mm Hg during 2 consecutive visits or in patients prescribed any antiglaucoma agent except for during peri-operative periods.

Glaucoma was defined as having both a funduscopy appearance of the optic nerve head and a corresponding visual field defect on Goldmann kinetic perimetry. Foveal hypoplasia was defined as a lack of foveal depression detected on optical coherence tomography (OCT).^{16,17} Patient abilities (i.e. finger counting, hand motions, light perception, and absence of light perception) were converted to visual acuities of 0.004, 0.002, 0.001, and 0.0005, respectively.¹⁸ We converted CDVA into a logarithm of the minimum angle of resolution (logMAR) value for statistical calculations.

We examined the association between follow-up duration and LSCD severity in an ordered logistic regression model using a generalized linear mixed model. We first estimated an unadjusted association and then adjusted for age at the first visit. We also examined the association between follow-up duration and visual acuity in a linear regression model using a generalized linear mixed model after adjusting for age at the first visit, LSCD severity (II or more vs. I or less), history of glaucoma (no glaucoma, ocular hypertension, or glaucoma), and lens status (no cataract, cataract, and pseudophakia/aphakia). A generalized linear mixed model is useful for evaluating one or more measurements obtained in the same patient and accounts for both within- and across-person variabilities. In the current study, we evaluated one or more repeated visits by one patient because of the retrospective design. We adopted a generalized linear mixed model to allow more flexibility in model selection (fixed effects or random effects) and examined repeated measures in our analysis.

A *P* value of < 0.05 was considered statistically significant. All models accounted for the high correlation caused by repeated measurements, including the right and left eyes in the same model. Statistical analyses were conducted by a biostatistician (R.K.) using Stata 16.0 (College Station, TX, USA).

RESULTS

A total of 52 eyes of 27 patients who met the inclusion criteria were enrolled. The mean follow-up duration was 5.2 ± 6.3 years. Two eyes of two patients were excluded from the evaluation of ocular abnormalities because of enucleation. Patient characteristics and ocular abnormalities at the final visit are summarized in Table 2. Two eyes that were treated with penetrating keratoplasty (PK) had conjunctival invasion into the central cornea within the diameter of trepanation of PK at the time of surgery. One eye treated with PK had severe bullous keratopathy at the time of surgery, and it was very hard to determine the LSCD severity in this eye due to severe epithelial edema. Four eyes were treated with ocular surface reconstruction for LSCD. Therefore, post-surgery visits were excluded from LSCD analyses because the postoperative analysis of LSCD severity can be affected by these surgeries. During follow-up, 30 of 52 eyes (57.7%) were treated with cataract surgery.

PAX6 mutations were detected in 19 of 21 patients (90.5%) whose DNA were sequenced, and 16 of these 21 patients (76.2%) had frame-shift or nonsense mutations that resulted in a premature termination codon (PTC). Thus, 36 eyes of 19 patients were diagnosed as "definite," 4 eyes in 2 patients as "probable" based on the diagnostic criteria A + B1 + B2 + none of C, 1 eye in 1 patient as "probable" based on A + B1 + B3 + none of C, and 11 eyes in 6 patients as "probable" based on A + B1 + both of B2 and B3 + none of C (Table 1).

The percentage of cases with LSCD at final follow-up was 98.1% (51 of 52 eyes). In five eyes, LSCD was obviously complicated based on slit-lamp photography, and exact staging was difficult due to band-shaped keratopathy or a lack of fluorescein photography. At the last follow-up, 1 eye (1/52, 1.9%) was categorized as stage 0, 7 (13.5%) as Ia, 9 (17.3%) as Ib, 5 (9.6%) as Ic, 2 (3.8%) as IIb, 12 (23.1%) as IIc, and 11 (21.2%) as III. The age of each stage was as follows (mean \pm SD / median, range): 32 in 0, 31.6 ± 16.8 / 32, 12–53 in Ia, 30.1 ± 15.0 / 25, 14–53 in Ib, 49.0 ± 7.0 / 50, 37–54 in Ic, 50.5 ± 20.5 / 50.5, 36–65 in IIb, 51.8 ± 13.8 / 47, 32–79 in IIc, 45.0 ± 17.5 / 41, and 23–69 in III.

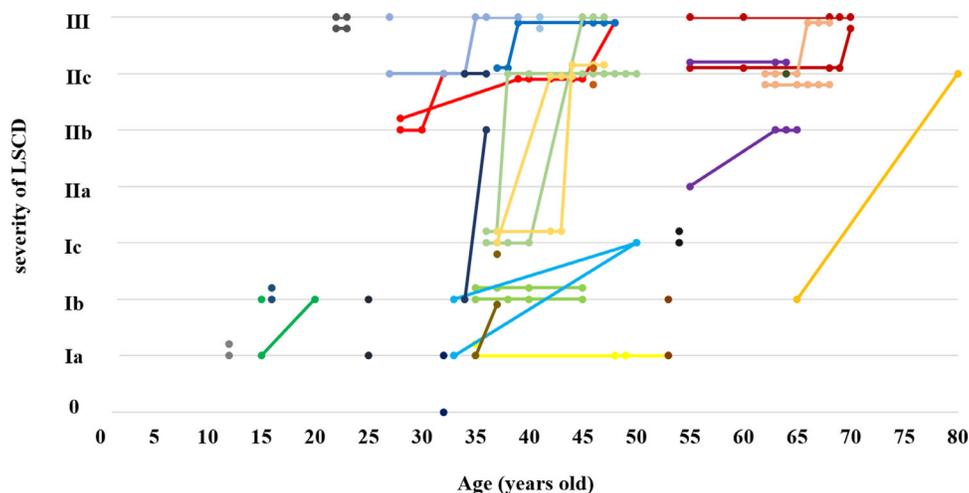


FIGURE 2. Relationship between the severity of LSCD and age. LSCD worsened as the follow-up duration increased in length, and the severity of one eye was similar to that of the contralateral fellow eye. Both eyes of each patient are represented by the same color. LSCD = limbal stem cell deficiency in aniridia.

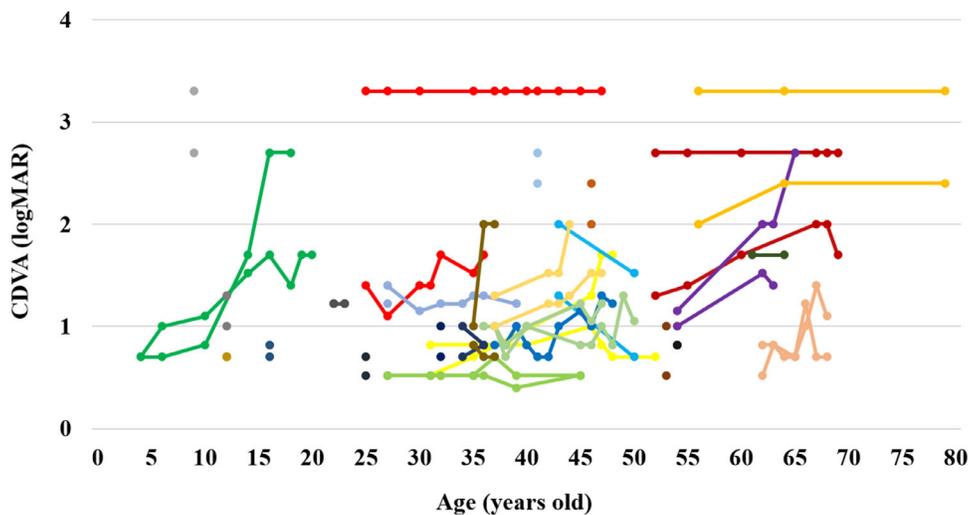


FIGURE 3. Relationship between CDVA value and age. CDVA worsened as the follow-up duration increased. Both eyes of each patient are shown in the same color. CDVA = corrected distance decimal visual acuity.

Figure 2 shows the relationship between the severity of LSCD and age. As indicated in the figure, LSCD tended to progress as the follow-up duration increased. Logistic regression showed that the estimated odds ratio for each progression in severity grade was associated with follow-up duration (odds ratio per 1 year of age = 1.45; 95% confidence interval [CI] = 1.24–1.70; $P < 0.001$), and this significant association remained consistent after adjusting for the age at first visit, glaucoma, and lens status (odds ratio per 1 year of age = 1.43; 95% CI = 1.22–1.69; $P < 0.001$).

CDVA at first visit was associated with the severity of LSCD (+0.48 in grade II or higher vs. grade I or lower, 95% CI = 0.12–0.83; $P = 0.008$) and glaucoma (+0.54; 95% CI = 0.03–1.04; $P = 0.038$) but not with the age at the first visit.

Figure 3 shows the relationship between CDVA and age. Each increase of 1 year in follow-up duration was associated with a mean difference of +0.025 logMAR (95% CI = 0.016–0.034; $P < 0.001$), and this trend remained

significant after adjusting for the age at first visit, the severity of LSCD, history of glaucoma, and lens status (+0.019 logMAR; 95% CI = 0.009–0.029; $P < 0.001$; Table 3).

The prevalence of LSCD, cataract, and foveal hypoplasia was high among ocular abnormalities. There were no cases with systemic abnormalities, including central nervous system disorders and Wilms tumor, aniridia, genitourinary anomalies, or retardation (WAGR) syndrome.

DISCUSSION

In the current study, the follow-up duration and the severity of LSCD were significantly associated, with an odds ratio of 1.41. It has been reported that ARK becomes more pronounced with age,^{12,19,20} and progressive corneal pathology has been attributed to multiple factors. Ramaesh et al. reported that corneal changes in aniridia may be related to an abnormality within the limbal stem cell niche, and its underlying mechanisms include an abnormal wound

TABLE 3. Associations of Corrected Distance Decimal Visual Acuity in LogMAR

Factors	Unadjusted Simple Regression Model				Multivariate Regression Model			
	Mean Difference	95% CI		P Value	Mean Difference	95% CI		P Value
Follow-up duration, per +1 yr	0.025	0.016	0.034	<0.001	0.023	0.014	0.033	<0.001
Age at first visit, per +1 yr of age	0.007	-0.009	0.023	0.388	0.014	-0.002	0.030	0.094
Severity of LSCD, II or higher vs. I or lower	0.275	0.115	0.435	0.001	0.098	-0.059	0.256	0.222
Glaucoma								
None	(reference)				(reference)			
Ocular hypertension	-0.073	-0.567	0.421	0.772	0.167	-0.251	0.583	0.434
Glaucoma	0.227	-0.361	0.816	0.449	0.695	0.156	1.234	0.012
Lens status								
No cataract	(reference)				(reference)			
Cataract	0.615	-0.658	1.888	0.344	-0.098	-1.248	1.051	0.867
Pseudophakia/aphakia	-0.177	-1.421	1.068	0.781	-1.000	-2.171	0.172	0.280

95% CI = 95% confidence interval; LSCD = limbal stem cell deficiency in aniridia.

healing response, defective corneal epithelial differentiation, and conjunctival changes caused by the downregulated expression of cytokeratin-12, gelatinase-B, and cell adhesion molecules.¹⁹ De la Paz et al. reported that LSCD was likely caused by a slow decline in the limbal stem cell population that occurs secondary to genetic defects in these cells and their mediators in the limbus.²¹ Ihnatko et al. observed progressive morphological degradation of the palisades of Vogt using in vivo confocal microscopy, and the sensitivity of this process to oxidative stress was implicated by the observation of transdifferentiation into a noncorneal phenotype in *PAX6*^{+/-} mice.²² Our findings are in agreement with the hypothesis that LSCD is a progressive condition that increases with age and is caused by many of the factors described above.

Visual acuity significantly declined as the follow-up duration increased, with two lines lost every 8 to 9 years. All progressive ocular abnormalities, such as ARK, cataract, and glaucoma, were related to the visual declines observed over increased follow-up times.^{8,23} Additionally, Mayer et al. reported that visual acuity continued to decrease even after successful cataract extraction in patients without glaucoma.²³ In this study, a significant association between visual acuity and follow-up duration was confirmed after adjustment for age at first visit, LSCD severity, history of glaucoma, and lens status. The results of our study are consistent with those presented in previous reports. However, stromal opacity could not be evaluated separate from limbal stem cell deficiency because of the retrospective nature of this clinical study.

The prevalence of LSCD, cataract, and foveal hypoplasia was as high as that of ocular abnormalities. Various reports have explored the frequency of ocular abnormalities. ARK occurs in 20 to 90% of patients, cataracts in 50 to 85%, glaucoma in 20 to 70%, and foveal hypoplasia in 10 to 95%.^{5,7,8,24} Hence, differences in the prevalence of ocular abnormalities might be due to the definition used. The high prevalence of LSCD and cataracts among the subjects in this study could be associated with the following strict definition: minimal conjunctival invasion of the corneal surface by LSCD and grade 1 or worse cataract. The differences in standardized diagnostic methods used across studies may be related to foveal hypoplasia. All our cases were diagnosed using OCT, a tool reported to be useful for diagnosis, except in cases with dense corneal opacity.^{16,17}

Although ARK includes LSCD and stromal opacification, the surgical procedures required by these conditions are different: stem cell transplantation is used for LSCD, whereas penetrating or anterior lamellar keratoplasty is needed for stromal opacification.^{22,23} Penetrating keratoplasty, when used in patients with LSCD, does not address stem cell deficiency and has a limited success rate of approximately 0 to 36%.^{8,21,23,25} Therefore, from the standpoint of surgical indications, each condition should be separately evaluated, and the modified staging system used in our study is potentially useful for evaluating LSCD because this scale objectively assesses both the extent of conjunctival invasion and limbal involvement.

In conclusion, we quantitatively demonstrate that LSCD severity and visual impairment significantly progress as the follow-up duration increases. This information is potentially useful for predicting the condition of patients with aniridia.

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References

- Nelson LB, Spaeth GL, Nowinski TS, Margo CE, Jackson L. Aniridia. A review. *Surv Ophthalmol*. 1984;28:621-642.
- Eden U, Iggman D, Riise R, Tornqvist K. Epidemiology of aniridia in Sweden and Norway. *Acta Ophthalmol*. 2008;86:727-729.
- Brauner SC, Walton DS, Chen TC. Aniridia. *Int Ophthalmol Clin*. 2008;48:79-85.
- Shaw MW, Falls HF, Neel JV. Congenital aniridia. *Am J Hum Genet*. 1960;12:389-415.
- Hingorani M, Hanson I, van Heyningen V. Aniridia. *Eur J Hum Genet*. 2012;20:1011-1017.
- Landsend ES, Utheim OA, Pedersen HR, Lagali N, Baraas RC, Utheim TP. The genetics of congenital aniridia—a guide for the ophthalmologist. *Surv Ophthalmol*. 2018;63:105-113.

7. Schanilec P, Biernacki R. Aniridia: a comparative overview. *Am Orthopt J*. 2014;64:98–104.
8. Lim HT, Kim DH, Kim H. PAX6 aniridia syndrome: clinics, genetics, and therapeutics. *Curr Opin Ophthalmol*. 2017;28:436–447.
9. Eden U, Lagali N, Dellby A, et al. Cataract development in Norwegian patients with congenital aniridia. *Acta Ophthalmol*. 2014;92:e165–167.
10. Lee H, Khan R, O'Keefe M. Aniridia: current pathology and management. *Acta Ophthalmol*. 2008;86:708–715.
11. Satake Y, Higa K, Tsubota K, Shimazaki J. Long-term outcome of cultivated oral mucosal epithelial sheet transplantation in treatment of total limbal stem cell deficiency. *Ophthalmol*. 2011;118:1524–1530.
12. Eden U, Riise R, Tornqvist K. Corneal involvement in congenital aniridia. *Cornea*. 2010;29:1096–1102.
13. Mackman G, Brightbill FS, Optiz JM. Corneal changes in aniridia. *Am J Ophthalmol*. 1979;87:497–502.
14. Deng SX, Borderie V, Chan CC, et al. Global consensus on definition, classification, diagnosis, and staging of limbal stem cell deficiency. *Cornea*. 2019;38:364–375.
15. Chylack LT, Jr, Wolfe JK, Singer DM, et al. The Lens Opacities Classification System III. The longitudinal study of cataract study group. *Arch Ophthalmol*. 1993;111:831–836.
16. Holmstrom G, Eriksson U, Hellgren K, Larsson E. Optical coherence tomography is helpful in the diagnosis of foveal hypoplasia. *Acta Ophthalmol*. 2010;88:439–442.
17. Karaca EE, Cubuk MO, Ekici F, Akcam HT, Waisbourd M, Hasanreisoglu M. Isolated foveal hypoplasia: clinical presentation and imaging findings. *Optom Vis Sci*. 2014;91:S61–65.
18. Tsubota K, Satake Y, Kaido M, et al. Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation. *N Engl J Med*. 1999;340:1697–1703.
19. Ramaesh K, Ramaesh T, Dutton GN, Dhillon B. Evolving concepts on the pathogenic mechanisms of aniridia related keratopathy. *Int J Biochem Cell Biol*. 2005;37:547–557.
20. Shiple D, Finklea B, Lauderdale JD, Netland PA. Keratopathy, cataract, and dry eye in a survey of aniridia subjects. *Clin Ophthalmol*. 2015;9:291–295.
21. de la Paz MF, Alvarez de Toledo J, Barraquer RI, Barraquer J. Long-term visual prognosis of corneal and ocular surface surgery in patients with congenital aniridia. *Acta Ophthalmol*. 2008;86:735–740.
22. Ihnatko R, Eden U, Fagerholm P, Lagali N. Congenital aniridia and the ocular surface. *Ocul Surf*. 2016;14:196–206.
23. Mayer KL, Nordlund ML, Schwartz GS, Holland EJ. Keratopathy in congenital aniridia. *Ocul Surf*. 2003;1:74–79.
24. Lee HK, Kim MK, Oh JY. Corneal abnormalities in congenital aniridia: congenital central corneal opacity versus aniridia-associated keratopathy. *Am J Ophthalmol*. 2018;185:75–80.
25. Kremer I, Rajpal RK, Rapuano CJ, Cohen EJ, Laibson PR. Results of penetrating keratoplasty in aniridia. *Am J Ophthalmol*. 1993;115:317–320.