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

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Received: June 23, 2019
Accepted: March 13, 2020
Published: June 26, 2020

Citation: Komoto S, Oie Y, Kawasaki S, et al. Quantitative analysis of the association between follow-up duration and severity of limbal stem cell deficiency or visual acuity in aniridia. Invest Ophthalmol Vis Sci. 2020;61(6):57. https://doi.org/10.1167/iovs.61.6.57

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 stage 0 and 5 (9.6%) as stage 1a, 9 (17.3%) as stage 1b, 5 (9.6%) as stage 1c, 2 (3.8%) as stage 1b, 12 (23.1%) as stage 1c, and 11 (21.2%) as stage 1 d. 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, often caused by heterozygous mutations of the PAX6 gene. 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.

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. 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. 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. Although the ocular surface can stabilize after surgical intervention, the success rates of these procedures remain unsatisfactory. 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. Recently, a staging system for LSCD was reported based on a global consensus by the International Limbal Stem Cell Deficiency Working Group. 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 |
|-------------------------------------------------|-----------------------------------|
| B. Examination findings                         | 2. Photophobia                    |
|                                                 | 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 PAX6 mutations |
| E. Genetic analysis                              | PAX6 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.

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). 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 categorized 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, aphakia, or pseudophakia. Ocular
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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 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 ± 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.

Table 2. Patient Characteristics at the Final Visit

| Characteristics                      | Cases        |
|--------------------------------------|--------------|
| 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 ± SD               | 40.5 ± 18.9  |
| Male: Female, cases                 | 15: 12       |
| Familial: Sporadic: Unknown, cases  | 13: 11: 3    |
| Follow-up duration, years, mean ± SD| 5.2 ± 6.3    |
| CDVA (logMAR), mean ± SD            | 1.52 ± 1.09  |
| Limbal 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.
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, 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
healing response, defective corneal epithelial differentiation, and conjunctival changes caused by the downregulated expression of cytokeratin-12, gelatinase-B, and cell adhesion molecules. 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. 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. 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. Additionally, Mayer et al. reported that visual acuity continued to decrease even after successful cataract extraction in patients without glaucoma. 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%. 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.

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. 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%. Therefore, from the standpoint of surgical indications, each condition should be separated, 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.

### Acknowledgments

Supported by Research on Rare and Intractable Diseases, Health, Labour, and Welfare Sciences Research Grants (Grant Number: H29- Nanchitou (Nan)-Ippan -011, Principal Investigator: Kohji Nishida).

Disclosure: S. Komoto, None; Y. Oie, None; S. Kawasaki, None; R. Kawasaki, None; N. Nishida, None; T. Soma, None; S. Koh, None; K. Maruyama, None; S. Usui, None; K. Matsushita, None; M. Tsujikawa, None; N. Maeda, None; K. Nishida, None

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