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

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 progressed 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 approximately 90% of aniridia cases have a genetic origin in the PAX6 gene.3–5 Aniridia is associated with a mean difference of +0.021 logMAR (95% confidence interval [CI] 0.01–0.03; P < 0.001).
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 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.

Stagel: Conjunctival invasion not extending to central cornea with the diameter of 5 mm
Involvement of conjunctivalization in limbus
A: less than 180 degrees
B: from 180 to 360 degrees
C: 360 degrees

StageII: Conjunctival invasion extending to central cornea with the diameter of 5 mm
Involvement of conjunctivalization in limbus
A: less than 180 degrees
B: from 180 to 360 degrees
C: 360 degrees

StageIII: Complete conjunctival invasion

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.

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.

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

Glaucoma was defined as having both a funduscopic glaucomatous 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).\textsuperscript{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.\textsuperscript{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 $\pm$ 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 trephination 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 LCSD 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 $\pm$ 16.8 / 32, 12–53 in Ia, 30.1 $\pm$ 15.0 / 25, 14–53 in Ib, 49.0 $\pm$ 7.0 / 50, 37–54 in Ic, 50.5 $\pm$ 20.5 / 50.5, 36–65 in IIb, 51.8 $\pm$ 13.8 / 47, 32–79 in IIc, 45.0 $\pm$ 17.5 / 41, and 23–69 in III.

### TABLE 2. 
Patient Characteristics at the Final Visit

<table>
<thead>
<tr>
<th>Patients, cases, eyes</th>
<th>27, 52</th>
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<td>Definite: Probable, cases, eyes</td>
<td>19, 36: 8, 16</td>
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<td>FS: N / M: no mutations, cases</td>
<td>9: 7: 3 / 2</td>
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<td>Age, years, mean $\pm$ SD</td>
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<tr>
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<td>15: 12</td>
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<tr>
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<td>Follow-up duration, years, mean $\pm$ SD</td>
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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.

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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.

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.

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

<table>
<thead>
<tr>
<th>Factors</th>
<th>Unadjusted Simple Regression Model</th>
<th>Multivariate Regression Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Difference 95% CI P Value</td>
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</tr>
<tr>
<td>Follow-up duration, per +1 yr</td>
<td>0.025 0.016 0.034 &lt;0.001</td>
<td>0.025 0.014 0.033 &lt;0.001</td>
</tr>
<tr>
<td>Age at first visit, per +1 yr of age</td>
<td>0.007 −0.009 0.023 0.388</td>
<td>0.014 −0.002 0.030 0.094</td>
</tr>
<tr>
<td>Severity of LSCD, II or higher vs. I or lower</td>
<td>0.275 0.115 0.435 0.001</td>
<td>0.098 −0.059 0.256 0.222</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>(reference)</td>
<td>(reference)</td>
</tr>
<tr>
<td>Ocular hypertension</td>
<td>−0.073 −0.567 0.421 0.772</td>
<td>0.167 −0.251 0.583 0.434</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>0.227 −0.361 0.816 0.449</td>
<td>0.695 0.156 1.234 0.012</td>
</tr>
<tr>
<td>Lens status</td>
<td>(reference)</td>
<td>(reference)</td>
</tr>
<tr>
<td>No cataract</td>
<td>0.615 −0.658 1.888 0.344</td>
<td>−0.098 −1.248 1.051 0.867</td>
</tr>
<tr>
<td>Cataract</td>
<td>−0.177 −1.421 1.068 0.781</td>
<td>−1.000 −2.171 0.172 0.280</td>
</tr>
<tr>
<td>Pseudophakia/apakia</td>
<td>(reference)</td>
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95% CI = 95% confidence interval; LSCD = limbal stem cell deficiency in aniridia.

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 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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