Ophthalmologic Findings in Cornelia de Lange Syndrome

A Genotype-Phenotype Correlation Study

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Objective: To evaluate individuals with Cornelia de Lange syndrome previously screened for mutations in the NIPBL gene for genotype-phenotype correlations with regard to severity of ophthalmologic findings.

Methods: Fifty-four patients with Cornelia de Lange syndrome (26 mutation positive and 28 mutation negative) with varying extent and severity of ophthalmologic findings participated in the study. We conducted a retrospective analysis of ophthalmologic data obtained through survey responses and medical records. The severity of nasolacrimal duct obstruction, myopia, ptosis, and strabismus was classified. The severity of eye findings was compared relative to the presence vs the absence of mutations in the coding region of NIPBL and relative to mutations predicted to result in a truncated protein (nonsense and frameshift mutations) vs missense mutations. Fisher exact test was used to determine the significance of these correlations.

Results: A trend toward increased ptosis severity was found among individuals with truncating (nonsense and frameshift) mutations compared with individuals with missense mutations (P = .07).

Conclusion: NIPBL may be directly involved in ptosis pathogenesis.

Clinical Relevance: Elucidating the pathogenetic mechanisms of ophthalmologic morbidities in patients with de Lange syndrome may lead to more effective treatment.

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Children’s Hospital of Philadelphia. Parents or legal guardians reviewed board–approved protocol of informed consent at The All patients were enrolled in the study under an institutional review board–approved protocol of informed consent at The Children’s Hospital of Philadelphia, most of whom were examined by one of us (T.L.Y.). The prevalences of NLD, myopia, ptosis, strabismus, and nystagmus were calculated in our overall cohort for comparison with published values.

The severity of NLD, myopia, ptosis, and strabismus was classified as summarized in Table 1. A child with severe ptosis with chin-up position is shown in Figure 1. Because myopia in children younger than 12 years is considered abnormal, the child was categorized as having class 2 myopia. Any individual 12 years or older with reported myopia but with unknown refractive error (ie, the complete ophthalmologic chart was unavailable) was classified as having class 1 myopia.

For analysis of mutation-positive vs mutation-negative individuals and truncating mutations (nonsense and frameshift) vs missense mutations, Fisher exact test was used because of the small sample. Significance was set at P<.05. Although Gillis et al defined the truncating mutation group as individuals with nonsense, frameshift, and splice-site mutations, we excluded subjects with splice-site mutations from our truncating mutation group. It is difficult to predict the effect of splice-site mutations, which may merely cause exon skipping rather than protein truncation. Our data also demonstrate that the 3 individuals with splice-site mutations had milder phenotypes for NLD, myopia, ptosis, and strabismus than those with other mutations, suggesting that splice-site mutations may not cause profound defects in the NIPBL protein product (Table 2).

Although some patients had incomplete ophthalmologic data, any additional findings were noted. These included hyperopia, nystagmus, astigmatism, anisometropia, microphthalmia, cataracts, optic nerve abnormalities, and glaucoma.

Table 1. Ophthalmologic Phenotype Classifications

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Class 0</th>
<th>Class 1</th>
<th>Class 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLD</td>
<td>No evidence of NLD</td>
<td>Symptomatic NLD with medical management</td>
<td>Severe NLD requiring P&amp;I or dacryocystorhinostomy</td>
</tr>
<tr>
<td>Myopia</td>
<td>No myopia</td>
<td>Mild or moderate myopia (≤ −5.0 D in subjects &lt;12 years of age)</td>
<td>Severe myopia (≥ −5.0 D in children or myopia &gt; −5.0 D in subjects of any age)</td>
</tr>
<tr>
<td>Ptosis</td>
<td>No ptosis</td>
<td>Measurable ptosis without pupil obstruction (MRD ≥ 2 mm)</td>
<td>Severe ptosis with upper eyelid elevation deficit, marked chin-up position, or requiring surgery (MRD &lt; 2 mm)</td>
</tr>
<tr>
<td>Strabismus</td>
<td>No strabismus</td>
<td>Mild or moderate strabismus not requiring surgery</td>
<td>Severe strabismus requiring surgery</td>
</tr>
</tbody>
</table>

Abbreviations: MRD, marginal reflex distance; NLD, nasolacrimal duct obstruction; P&I, probe and irrigation.

All patients were enrolled in the study under an institutional review board–approved protocol of informed consent at The Children’s Hospital of Philadelphia. Parents or legal guardians of the cohort of 120 patients with CdLS previously genotyped5 received mailings requesting the completion of an eye history survey and copies of ophthalmologic records. Parents and guardians were unaware of the mutation status of their children at the time of this request. Data were obtained for many patients from medical records already on file at The Children’s Hospital of Philadelphia, most of whom were examined by one of us (T.L.Y.). The prevalences of NLD, myopia, ptosis, strabismus, and nystagmus were calculated in our overall cohort for comparison with published values.

The severity of NLD, myopia, ptosis, and strabismus was classified as summarized in Table 1. A child with severe pto-
positive and mutation-negative groups ($P = .26$), a slight trend for increased overall ptosis prevalence was found among mutation-positive individuals (57.7% [15/26]) compared with mutation-negative individuals (35.7% [10/28]) ($P = .14$). In addition, those with truncating mutations were more likely to have increased ptosis severity than those with missense mutations ($P = .07$). The only 2 individuals who required strabismus surgery were in the mutation-negative group. However, the mutation-positive group had a slightly higher prevalence of any degree of strabismus (34.6% [9/26]) than the mutation-negative group (21.4% [6/28]). Mutation-positive individuals were more likely to have class 1 (mild or moderate) strabismus, and mutation-negative individuals were more likely to have class 2 (severe) strabismus ($P = .09$), contrary to our hypothesis. Those with missense mutations and truncating mutations were equally likely to have strabismus.

Among the mutation-positive patients in our study, only 2 (patients 32 and 33 in Table 2) had the same mutation, a missense mutation in exon 40 (6893 G→A, R2298H). Both of these individuals had some degree of NLDO and myopia at a young age but no ptosis or strabismus.

Some patients had additional ophthalmologic findings (Table 2) previously reported in the literature but uncommonly found in CdLS.8-13 Patients 48 and 51 had congenital glaucoma, one with a 5-base pair (bp) deletion in exon 31 causing a frameshift and another with a 1-bp insertion in exon 45 causing a frameshift. The fundus photographs for patient 48 are shown in Figure 2. Patients 33, 36, and 51 had microphthalmia, all of whom had different mutations (a missense mutation in exon 40 [6893 G→A, R2298H], a nonsense mutation in exon 10 [2389 C→T, R797X], and a frameshift mutation in exon 45 [7825-7826 ins G], respectively). Patient 42 had a nonsense mutation, and patient 18 (mutation negative) had microcornea.

Fifteen percent (8/54) of our study cohort had hyperopia: 5 patients were mutation positive (with a missense mutation at exon 17, nonsense mutations at exons 10 and 27, a frameshift at exon 24, and a splice site mutation at exon 7), and 3 patients were mutation negative. Seventeen percent (9/54) had nystagmus: 7 patients were mutation positive (with missense mutations at exons 28 and 43, nonsense mutations at exons 26 and 27, and frameshift mutations at exons 9, 31, and 45), and 2 patients were mutation negative. Because refractive errors were unavailable for all patients, we cannot give prevalence rates for anisometropia or astigmatism. However,
3 mutation-negative individuals had known anisometropia (difference in refractive error of ≥2.00 diopter [D] between eyes). Thirteen individuals (6 mutation positive and 7 mutation negative) had astigmatism; those with reported refractive errors had astigmatism of at least 1.00 D. Nine individuals in our cohort had known optic nerve abnormalities (such as cupping, tilted optic discs, pallor, hypoplasia, staphyloma, and coloboma), 4 of whom were mutation positive and 5 of whom were mutation negative. Although only 1 mutation-positive subject (patient 42) had a cataract, 3 mutation-negative individuals had cataracts. Of these 3 mutation-negative individuals, 1 had a posterior subcapsular cataract associated with high myopia, another had dot opacities of the lens, and a third had a morgagnian cataract with pigmentary changes in the retina and numerous areas of vitreoretinal traction, adhesion, and fibrosis over the macula and posterior pole.

**COMMENT**

Individuals with truncating mutations displayed a nonsignificant trend (P=.07) toward increased ptosis severity compared with individuals with missense mutations. However, no trend in severity was found for NLDO or myopia between mutation-positive and mutation-negative groups or between truncating mutation and missense mutation groups. In addition, contrary to our hypothesis, mutation-negative individuals were more likely to have more severe strabismus than mutation-positive individuals (P = .09). This suggests that NIPBL may have greater implications for ptosis pathogenesis than for pathogenesis of NLDO, myopia, and strabismus in patients with CdLS.

The severity ratings for NLDO and myopia have been problematic and may help explain the absence of trends in our study. Our survey questioned parents and guardians about NLDO symptoms and any required surgery and provided space for inclusion of the details. Some family members may not have recalled or specified multiple probe and irrigation procedures or Silastic tube placement, leading to erroneous low NLDO classifications. Similarly, some parents and guardians were unable to specify the degree of refractive error, and any individual 12 years or older with unknown myopia severity was placed in the class 1 myopia group by default. If indi-
Individuals with unknown myopia severity (patients 7, 37, 44, 49, and 53 in Table 2) are excluded from the analysis, the trend values were \( P = .60 \) for the mutation-positive vs mutation-negative groups and \( P = .22 \) for the truncating mutation vs missense mutation groups. The small study sample may explain the nonsignificant \( P \) values. In addition, because myopia is a common complex disorder, it is a problematic classification variable. Our rating system allowed for a wide spectrum of spherical error in the severe myopia group, ranging from very mild myopia in a child younger than 12 years to very severe myopia (highest refractive error, \(-25.00\) D OU) causing retinal detachments in 2 individuals (patients 11 and 34).

Patients 32 and 33, who had the same mutation, had similar presentations with regard to the presence or absence of NLDO, myopia, ptosis, and strabismus. This offers insight into the specific regional function of exon 40, at which this missense mutation occurs. However, patient 33 had microphthalmia by parental report, whereas patient 32 did not. In addition, patient 33 had astigmatism, but whether patient 32 had astigmatism is unknown.

There has been 1 reported case of CdLS with glaucoma in a newborn with aniridia who demonstrated profound buphthalmos at initial examination.\(^{14}\) The 2 individuals in our cohort with congenital glaucoma have novel presentations, to our knowledge, because they had normal irides. Both of these individuals have truncating mutations in \(NIPBL\).

Many individuals in the mutation-negative group had NLDO, myopia, ptosis, and strabismus, among other eye findings. Only 2 individuals who had severe enough strabismus to warrant surgery were mutation negative, and 1 individual with high myopia complicated by retinal detachments was mutation negative. These findings support the suggestions by Gillis et al\(^7\) that (1) a large number of mutations in \(NIPBL\) may have been missed as a result of the large size of the gene and the use of conformation-sensitive gel electrophoresis (a minimally sensitive method of gene screening) for mutational analysis or (2) CdLS may be genetically heterogeneous (ie, another gene is involved).

It is unlikely that our study has selection bias with regard to who responded to the requests to return a com-

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**Table 3. Prevalence Rates of Ophthalmologic Findings**

<table>
<thead>
<tr>
<th>Cohort</th>
<th>NLDO (n=54)</th>
<th>Myopia (n=54)</th>
<th>Ptoosis (n=54)</th>
<th>Strabismus (n=54)</th>
<th>Nystagmus (n=54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>66.7 (36)</td>
<td>57.4 (31)</td>
<td>46.3 (25)</td>
<td>25.9 (14)</td>
<td>16.7 (9)</td>
</tr>
<tr>
<td>Levin et al,(^{13}) 1990</td>
<td>59.1 (13)</td>
<td>60.0 (24)</td>
<td>45.5 (10)</td>
<td>13.6 (3)</td>
<td>36.4 (8)</td>
</tr>
</tbody>
</table>

Abbreviation: NLDO, nasolacrimal duct obstruction.

*Data are given as percentage (number/total number) of patients with each finding unless otherwise indicated.
†Data are given as percentage (number/total number) of eyes.

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**Table 4. Distribution of Severity of Ophthalmologic Findings and Results of Genotype-Phenotype Correlation Analysis**

<table>
<thead>
<tr>
<th>Phenotype and Classification</th>
<th>Mutation Positive (n=26)</th>
<th>Truncating</th>
<th>Nonsense (n=8)</th>
<th>Frameshift (n=9)</th>
<th>Splice Site (n=3)</th>
<th>Missense (n=8)</th>
<th>Mutation Negative (n=28)</th>
<th>Truncating</th>
<th>Nonsense (n=8)</th>
<th>Frameshift (n=9)</th>
<th>Splice Site (n=3)</th>
<th>Missense (n=8)</th>
</tr>
</thead>
</table>

Abbreviation: NLDO, nasolacrimal duct obstruction.

*Data are given as number (percentage) of patients.
†\( P = .76 \) and \( P = .74 \) for mutation-positive vs mutation-negative groups and for truncating mutation vs missense groups, respectively.
‡\( P = .43 \) and \( P = .75 \) for mutation-positive vs mutation-negative groups and for truncating mutation vs missense groups, respectively.
§\( P = .26 \) and \( P = .07 \) for mutation-positive vs mutation-negative groups and for truncating mutation vs missense groups, respectively.
||\( P = .90 \) and \( P = .25 \) for mutation-positive vs mutation-negative groups and for truncating mutation vs missense groups, respectively.
completed survey or to send in ophthalmologic records, as parents and guardians were unaware of their children’s mutation status at the time of the request. In addition, there was a wide range of eye findings (from negligible observations to severe structural defects) in the mutation-positive and mutation-negative groups. The percentage of the cohort that was mutation positive was 48.1% (26/54), which is comparable to the 47% found by Gillis et al. It is possible that some of the survey responses were inaccurate, as they are partly dependent on the recall accuracy of parents and guardians. Data for 48.1% (26/54) were solely obtained from survey responses, with this number evenly distributed between the mutation-positive (46.2% [12/26]) and mutation-negative (50.0% [14/28]) groups. Also, some of the data from the ophthalmologic records were subjective, such as the assessment of ptosis. Various physicians (F.K., L.G.J., I.D.K., and T.L.Y.) performed the examinations, and some ophthalmologic records may be incomplete because families may have moved and original records were not always available.

To minimize the effect of possible inherent inaccuracies, we are continually increasing the size of our study cohort of patients with CdLS. A future research direction is direct sequencing of the NIPBL coding region in mutation-negative individuals, which may detect mutations overlooked by conformation-sensitive gel electrophoresis. In addition, untranslated, enhancer, and promoter regions of NIPBL will be screened for mutations. As more is learned about NIPBL and its protein product, we will be better able to postulate its role in the pathogenesis of the extensive and variable array of ophthalmologic abnormalities in CdLS.

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