CRBI Gene Mutations Are Associated with Keratoconus in Patients with Leber Congenital Amaurosis

Timothy T. McMahon, Linda S. Kim, Gerald A. Fishman, Edwin M. Stone, Xinping C. Zhao, Richard W. Yee, and Jarema Malicki

PURPOSE. To present an association of mutations in the CRBI gene with keratoconus in patients with Leber congenital amaurosis (LCA).

METHODS. Sixteen patients with genotyped LCA (having the CRBI, CRX, RflGC, RPE65, and AIPL1 mutations) were recruited from one ophthalmology practice and examined for the presence of keratoconus. Corneal topography, visual acuity, and slit lamp biomicroscopic examination were performed in all cases.

RESULTS. The mean age of the patients was 34.5 years (range, 13–74). Visual acuities ranged from 20/40 to light perception. Corneal topography was successfully collected in 15 of the cases. Five of the 16 cases had slit lamp and/or topographic features consistent with keratoconus. One patient had a clinical picture that was keratoglobus-like. Of these six cases, four had a CRBI mutation and two had a CRX mutation. Of the three subjects with the CRX mutation, one had keratoconus, one had the keratoglobus-like presentation, and one was normal. Our cohort represents 14 separate, unrelated families. Only one family comprised multiple members with LCA. These were three affected brothers, one with keratoconus, all with CRBI mutations.

CONCLUSIONS. Although the results cannot exclude other gene mutations, they suggest that LCA patients with a CRBI mutation may have a particular susceptibility to keratoconus. (Invest Ophthalmol Vis Sci. 2009;50:3185–3187) DOI:10.1167/iovs.08-2886

Lever congenital amaurosis (LCA) is an uncommon disease of hereditary origin. Several different mutations identified in this disorder have been described. Keratoconus has been associated with LCA in several studies. The relationship of a concordance of LCA and keratoconus is most likely explained by four mechanisms: (1) a complication of LCA, as yet unexplained; (2) a direct genetic mutation(s) that affects both the retina and the cornea; (3) multifactorial or polygenetic causes where different genes cause each disorder but there is some form of linkage present; and (4) a casual but not necessarily a causal link between the two disorders. Keratoconus has a prevalence of between 50 and 230 per 100,000.7 LCA has a prevalence of 1/30,000 to 1/81,000.8 With these frequencies it is conceivable that an association is a matter of chance. Searches for genes responsible for keratoconus, to date, have not disclosed definitive or consistent findings.10–20 We sought to determine, in a clinical setting, whether there is an association between various genotypes of LCA and the presence of keratoconus.

METHODS

Sixteen genotyped patients with LCA were recruited by one of the investigators (GAF). These cases had been previously genotyped by a co-investigator (EMS). Genotypes included in our cohort included CRBI, CRX, RflGC, RPE65, and AIPL1. All patients were examined at the Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, by three of the investigators (TTM, LSK, GAF). The study adhered to the tenets of the Declaration of Helsinki and was approved by a University of Illinois at Chicago institutional review board. Informed consent was obtained from each patient after the nature of the procedures had been explained. Examinations were conducted in accordance with the Health Insurance Portability and Accountability Act regulations.

Diagnostic criteria for LCA composed of an early onset of visual impairment, nystagmus, amaurotic pupils, and marked reduction or absence of electroretinogram (ERG) signal.21 Examination of the posterior pole typically revealed pigmentary retinopathy, though the phenotype can vary significantly. Genotyping was conducted previously by using the methods described by Lotery et al.22 The patients in this report were studied over several years. As with all things, as technology changes, the way in which patients are screened for genetic variations also changes. A more complete chronology of molecular genetics for LCA is referenced in the paper by Stone.23 Keratoconus is defined as a noninflammatory corneal thinning disorder that is bilateral in 96% of cases.24 Stromal thinning affects the central and para- central cornea. A pigmented ring of hemosiderin located in the basal epithelium and found at the edge of thinning (Fleischer ring) is a hallmark and a common clinical finding. Fine lines, generally vertical in orientation in Descemet’s membrane near the center of the thinning (Vogt striae) are also a hallmark of keratoconus. Last, anterior stromal scarring may occur in more severe stages of the disease. Keratoconus is a slowly progressive disorder presenting as early as 8 to 10 years of age, but most often is not diagnosed until adulthood.6–7,15

Our cohort represents 13 unrelated families. Each patient was examined for slit lamp findings of keratoconus. In addition, corneal topography was attempted on all subjects, as were visual acuities. Corneal topography was obtained with a corneal topographer (Kera- tron Corneal Analyzer; 2000; Optikon, Rome, Italy; with Scout software, ver. 3.6.4). Slit scanning topography was attempted (Orbscan II, Investigative Ophthalmology & Visual Science, July 2009, Vol. 50, No. 7
Copyright © Association for Research in Vision and Ophthalmology
The association between keratoconus and LCA has been well described.7 Of interest, how an observer examines the association determines how commonly these two disorders occur together. Studies of LCA cases not infrequently report a concordance with keratoconus.1,24 Studies of keratoconus populations on the other hand, rarely find LCA cases.25 It is presumed that the visual disability resulting from LCA is generally quite profound rendering keratoconus, when present, irrelevant to the patient, and thus patients are more likely to be seen by retinal specialists rather than corneal or contact lens specialists. Keratoconus has variously been described as both an acquired disorder26 and a hereditary disease,13,27 or some combination of both. Recent explorations for a genetic origin have resulted in a confusing ensemble of candidate genes or linked regions of the human genome.12 Some have been discredited by additional studies.26 The candidates identified thus far are listed in Table 3. Further evidence that genetics play a role in the development of keratoconus are a 13.5% occurrence in first-degree relatives of patients with keratoconus,25 which is a 15 to 64 times greater likelihood than in the general population.13 Twin studies commonly find concordance for disease in patients with keratoconus, although not uniformly.29–31

Dharmaraj et al.32 reported that 26% of 19 LCA patients with an AIPL1 mutation had keratoconus and cataracts. Hameed et al.33 also report a novel locus for LCA and keratoconus to the same 17p13 location. In our series, no patients with AIPL1 mutations presented with keratoconus. CRB1 has been shown to be expressed in the retina and iris of the fly, mouse, and human.34–36 It is unknown whether CRB1 is expressed in the cornea. CRB1 expression in the retina is responsible for the apicobasal polarity of the neuro-epithelium and later, the photoreceptor cell.45,46 Two of our cases with keratoconus had a CRX mutation. CRX is a photoreceptor-specific transcription factor expressed during development and plays a role in photoreceptor differentiation and maintenance. Similar to CRB1, CRX has not been shown to be expressed in the cornea.

To our knowledge, there are no reports linking CRB1 or CRX and keratoconus, nonetheless, although we cannot exclude other gene mutations, our study suggests that patients with LCA with a CRB1 mutation may (and possibly a CRX mutation) have a particular susceptibility for the development of keratoconus.

Acknowledgments

The authors thank Jill Beyer for providing comments on this work.

References


---

### Table 1. Genotypes, Visual Acuities, and Presence or Absence of Keratoconus

<table>
<thead>
<tr>
<th>No.</th>
<th>Genotype</th>
<th>Age</th>
<th>VA OD</th>
<th>VA OS</th>
<th>KCN</th>
<th>KSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CRB1</td>
<td>20/40-2</td>
<td>6/350</td>
<td>Yes</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CRB1</td>
<td>74</td>
<td>HM</td>
<td>HM</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>AIPL1</td>
<td>18</td>
<td>LP</td>
<td>LP</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>CRX</td>
<td>13</td>
<td>10/350</td>
<td>10/400</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>RetGC</td>
<td>50</td>
<td>20/400</td>
<td>20/400</td>
<td>No</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>CRX</td>
<td>32</td>
<td>LP</td>
<td>LP</td>
<td>Yes</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>CRB1*</td>
<td>40</td>
<td>LP</td>
<td>LP</td>
<td>Yes</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>CRB1*</td>
<td>46</td>
<td>LP</td>
<td>LP</td>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>RetGC</td>
<td>31</td>
<td>LP</td>
<td>LP</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>CRB1</td>
<td>31</td>
<td>LP</td>
<td>LP</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>CRX</td>
<td>39</td>
<td>LP</td>
<td>LP</td>
<td>Yes</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>RPE65</td>
<td>47</td>
<td>HM</td>
<td>HM</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>RPE65</td>
<td>58</td>
<td>LP</td>
<td>LP</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>CRB1*</td>
<td>47</td>
<td>CF</td>
<td>CF</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>RPE65</td>
<td>39</td>
<td>LP</td>
<td>LP</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>AIPL1</td>
<td>19</td>
<td>HM</td>
<td>HM</td>
<td>No</td>
<td>0</td>
</tr>
</tbody>
</table>

* Members of a single family. KSS: 0, normal; 1, atypical but not keratoconus; 2, keratoconus suspect; 3, mild keratoconus; 4, moderate keratoconus; 5, severe keratoconus. VA, visual acuity; KCN, keratoconus.

### Table 2. Distribution of KCN by Genotype for Individuals and Families

<table>
<thead>
<tr>
<th>Genotype (Location)</th>
<th>KCN</th>
<th>No KCN</th>
</tr>
</thead>
</table>
| CRB1 (1q13-1q21.1) | 4 (4) | 2 (*)
| CRX (1q13.3)       | 2 (2) | 1 (1)  |
| RetGC (17p13.1)     | 0 (0) | 2 (2)  |
| RPE65 (1p31)        | 0 (0) | 3 (3)  |
| AIPL1 (1p13.1)      | 0 (0) | 2 (2)  |

* Of the four families with individuals having keratoconus, one affected brother had two siblings positive for LCA but without keratoconus.

### Table 3. Candidate Genes/Regions for Keratoconus Identified by Linkage Studies

<table>
<thead>
<tr>
<th>Genotype/Location</th>
<th>Location</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIPL1</td>
<td>17p13</td>
<td>Finland</td>
</tr>
<tr>
<td>RPE65</td>
<td>17p13</td>
<td>Tasmania</td>
</tr>
<tr>
<td>CRB1</td>
<td>21q12</td>
<td>Utah</td>
</tr>
<tr>
<td>CRX</td>
<td>20p11q12</td>
<td>Canada</td>
</tr>
<tr>
<td>RetGC</td>
<td>3p14q13</td>
<td>Italy</td>
</tr>
<tr>
<td>Aquaporin 5</td>
<td>6q25</td>
<td>United States</td>
</tr>
</tbody>
</table>

---

These results are consistent with previous studies and demonstrate the importance of genetic factors in the development of keratoconus. Further investigation into the genetic basis of keratoconus is necessary to better understand the underlying mechanisms and to develop targeted therapeutic interventions.


