Autosomal Dominant Retinal Degeneration and Bone Loss in Patients with a 12-bp Deletion in the CRX Gene

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PURPOSE. To define the phenotypic expression of a deletion in the gene encoding the transcription factor CRX in a large, seven-generation, white family.

METHODS. Fourteen affected individuals, all heterozygous for the Leu146del12 mutation in the cone–rod homeobox gene (CRX), and four nonaffected relatives from the same family were examined with visual function tests, and 10 underwent bone mineral density (BMD) measurement.

RESULTS. The ability of the mutated CRX protein to transactivate rhodopsin promoter was decreased by approximately 25%, and its ability to react synergistically with neural retinal leucine zipper (NRL) was reduced by more than 30%. The affected members of the family had an autosomal dominant ocular condition most closely resembling Leber congenital amaurosis (LCA) with severe visual impairment at an early age. Depending on age, affected members showed varying degrees of significant visual acuity loss, elevated dark-adaptation thresholds, significantly reduced cone and rod electroretinogram (ERG) amplitudes, and progressive constriction of the visual fields, in most cases leading to complete blindness. Six affected members had reduced levels of BMD in the spine and the hip (osteofr); Four affected female members were receiving long-term hormonal replacement therapy (HRT) demonstrating normal values of BMD.

CONCLUSIONS. This large deletion of the CRX gene is associated with a severe form of autosomal dominant retinal degeneration. Affected members not receiving HRT showed reduced BMD (osteopenia). This phenotype may reflect the abnormal ability of the mutated CRX protein to transactivate rhodopsin promoter. This large deletion of the CRX gene is associated with a severe form of autosomal dominant retinal degeneration. Affected members not receiving HRT showed reduced BMD (osteopenia). This phenotype may reflect the abnormal ability of the mutated CRX protein to transactivate rhodopsin promoter. This large deletion of the CRX gene is associated with a severe form of autosomal dominant retinal degeneration. Affected members not receiving HRT showed reduced BMD (osteopenia). This phenotype may reflect the abnormal ability of the mutated CRX protein to transactivate rhodopsin promoter. This large deletion of the CRX gene is associated with a severe form of autosomal dominant retinal degeneration. Affected members not receiving HRT showed reduced BMD (osteopenia). This phenotype may reflect the abnormal ability of the mutated CRX protein to transactivate rhodopsin promoter. This large deletion of the CRX gene is associated with a severe form of autosomal dominant retinal degeneration. Affected members not receiving HRT showed reduced BMD (osteopenia). This phenotype may reflect the abnormal ability of the mutated CRX protein to transactivate rhodopsin promoter. This large deletion of the CRX gene is associated with a severe form of autosomal dominant retinal degeneration. Affected members not receiving HRT showed reduced BMD (osteopenia). This phenotype may reflect the abnormal ability of the mutated CRX protein to transactivate rhodopsin promoter.

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Materials and Methods

Subjects
All subjects were evaluated by medical, ocular, and family history and clinical ophthalmic examination. Five of them (individuals III:3, IV:2, IV:3, V:2, and V:4 in branch RFS900_B) were tested at Jules Stein Eye Institute (Los Angeles, CA) in the late 1980s.7 10 Those individuals have been re-evaluated during the past 3 years at the Retina Foundation of the Southwest (Dallas, TX). The proband (V:4) was initially seen at age 11 at the University of California Los Angeles and was subsequently seen at age 27 at the Retina Foundation. In addition to the original five
patients, nine more affected family members also were examined at the
Retina Foundation. Proceedings followed the tenets of the Declaration
of Helsinki and were approved by the appropriate institutional review
boards.

**Mutation Analysis**

The details of the mutation analysis have been published elsewhere. 26
Briefly, DNA was isolated from peripheral blood by a DNA extraction
kit (Puregene; Gentra, Minneapolis, MN). Single-strand conformational
polymorphism (SSCP) analysis was performed, and a second amplifi-
cation was performed from a stock DNA sample, as a template for
sequencing. The fragment was treated with shrimp alkaline phospha-
tase and exonuclease (US Biochemical, Cleveland, OH) and was se-
quenced with a kit (AmpliCycle; Perkin Elmer, Norwalk, CT). To
confirm deletion size and location, the amplimer was also subcloned
using the a site-directed mutagenesis kit (QuickChange; Stratagene)
according to the manufacturer's instructions. The primers used for
mutagenesis were 5'-CAGGTTTGGTTCAAGAACTGGAGGGCTAAATG-
CAGGC-3' (forward primer) and 5'-GCCTGCATTTAGCCCTCCAGT-
TCTTGAACCAAACCTG-3' (reverse). The mutation was confirmed by
sequence analysis.

Calcium phosphate–mediated transfections and luciferase and β-ga-
lactosidase assays were performed as previously described, 19 except
that transfections with glycerol shock were performed with 50% con-
fluent, 10-cm plates of 293 cells grown in Dulbecco's modified Eagle’s
medium (DMEM) with 10% fetal bovine serum, and 1% penicillin-
streptomycin (Gibco, Grand Island, NY). Cells were harvested 48 hours
after transfection. Each transfection experiment was performed in
triplicate. Aliquots of pCDNA-CRX expression construct varying from
0.1 to 1.0 μg were cotransfected with bovine rhodopsin promoter-
luciferase reporter (pBR130-luc; 5.0 μg), with and without the Nrl
expression plasmid pED-bNrl (1.0 μg). 27 The plasmid pCMV-LacZ was
included to normalize for transfection efficiency.

**Visual Function Testing**

Ophthalmic examination included best corrected visual acuity, direct
and indirect ophthalmoscopy and fundus photography. Visual acuity
was tested on the Early-Treatment Diabetic Retinopathy Study (ETDRS)
chart whenever possible. If necessary, the Distance Test Chart for the

**FIGURE 1.** (A) Pedigree of RFS900 family. Circular drawing of the whole family. Roman numerals indicate the number of generations. (B, C, and
D) Abbreviated presentations of the three branches of the RFS900 family with affected living members. Solid line crossing the symbol diagonally
indicates a deceased person. Vertical black band crossing the symbol indicates that individual is affected by hearsay. Numbering is independent
for each branch and starts at generation II of the main pedigree.
Partially Sighted (Designs for Vision, Ronkonkoma, NY), or forced-choice preferential looking tests were used. Dark-adapted thresholds with an 11° test were obtained on a Goldman–Weekers adaptometer (Haag-Streit, Berne, Switzerland) after 45 minutes of dark adaptation. Because patients were unable to see a fixation light, they were allowed to use any strategy to detect the test target.

Standard full-field electroretinograms (ERGs) were elicited by methods previously described. Whenever detectable, rod and cone a-waves were recorded according to an established protocol. Briefly, a set of white flashes (2–4-log scotopic trolands [scot td]) were first presented in the dark. The cone a-waves, elicited by the same stimuli presented against a rod-saturating background, were then subtracted from the dark-adapted responses to produce rod-only a-waves. Rod-only responses and cone-only responses were fit with a computational model.

Bone Mineral Densitometry
We measured BMD by dual-energy x-ray absorptiometry (DEXA) at various skeletal sites (lumbar spine, total hip, femoral neck, and forearm; QDR-2000; Hologic, Waltham, MA). One subject with previous spinal fusion had only hip and femoral neck BMD tested. One subject had body weight in excess to the maximum recommendations for the QDR-2000 and was tested only for BMD on the forearm. BMD was recorded in grams per square centimeter. For all locations, z-scores were calculated (as SD of BMD compared with age- and sex-matched control data from the Hologic database). BMD was expressed as a z-score and compared with the reference population of the Hologic database. Additional correction for the femoral neck analysis (provided by Hologic in 1997) was used. Four postmenopausal women had received hormonal replacement therapy (HRT) for at least 5 years. Because HRT can influence bone density, these patients were analyzed separately. Differences between mean z-scores at each site in HRT-treated and untreated patients were assessed by Student’s t-test.

RESULTS
Pedigree and History
We were able to trace the origins of the family up to seven consecutive generations with more than 250 individuals (Fig. 1). Labels were assigned to branches of the family in order of the age of siblings in generation II. This report focuses on individuals from three branches of the family. From those, 25 individuals were contacted, and 20 agreed to participate in the study.

The mode of disease inheritance is clearly autosomal dominant. The pedigree has been traced back to a couple living during the second half of the 19th century in Oklahoma. According to census data, the husband was from mixed white and Native American ancestry. The wife (of white ancestry), who moved to Oklahoma from Missouri, had low vision during her adult life. According to family members, three of the nine children of this couple (generation I on Fig. 1) had low vision beginning in childhood. These individuals are represented by black-striped symbols in generation I of the subpedigrees on Figure 1. There are no known cases of consanguinity in the pedigree. No systemic abnormalities apart from reduced BMD were reported in the family. There were no cases of mental retardation.

Genetic Testing
The initial results from genetic testing of this family have been reported. Recently, DNA samples were obtained from an additional eight affected and six unaffected family members. Segregation of the 12-bp deletion with retinal degeneration in these samples was confirmed by PCR amplification of CRX amplimer 3b, followed by separation on 5% 3:1 agarose gels, as demonstrated in Figure 2.

Transactivation Activity
To explore the mechanism by which the CRX Leu146del12 mutation leads to retinal degeneration, we tested the activity of...
mutant CRX in a transient transfection-based transactivation assay. Compared with wild-type CRX, the CRX Leu146del12 mutant demonstrated an ~25% decrease in its ability to transactivate a bovine rhodopsin promoter-reporter construct (Fig. 3A). It retained the ability to act synergistically with the bZIP transcription factor Nrl but its ability to do so was reduced 30% to 40% compared with wild-type CRX (Fig. 3B).

Fundus Appearance

Typical fundus photographs from three affected members of the family are presented in Figure 4. The youngest family member (RFS900_I, VI:1; Fig. 4A) showed irregular macular reflex and minimal changes in the RPE. Fundus photography of the left eye of her mother at age 27 (RFS900_I, V:2; Fig 4B) demonstrated widespread atrophy of the RPE, irregular macular reflex, and mild vessel attenuation. Another family member at age 45 (RFS900_I, IV:3; Fig. 4C) showed pallor of the optic disc, moderate arteriolar attenuation, diffuse RPE atrophy and a bull’s-eye lesion in the macula.

Psychophysical and Electrophysiological Testing

Clinical results from affected family members are shown in Table 1. Severely reduced visual acuity was documented at age 2. Visual acuity loss was gradual over the years, but in most cases ultimately led to light perception or total blindness. The only case of cone-mediated central visual acuity (better than 20/100) was observed in the left eye of RFS900_I V:4.

Determination of the dark-adapted threshold was possible in half the affected members (7/14). With one exception (RFS900_C IV:3), the threshold was elevated by at least 4 log units, even at 8 years of age. No visual field was measurable in eight patients. The remaining six had an overall constriction of the visual field even at a young age (~30° field with the I-4e isopter at ages 7–11 years). In some patients (RFS900_I, V:2), the size of the visual field was even more reduced.

Affected members of the family had greatly reduced ERG rod responses, severely attenuated cone responses (>95% amplitude loss), and delayed 30-Hz flicker responses. Patients aged more than 15 years had nondetectable or greatly reduced single cone responses. Typical ERG responses are presented in Figure 5.

In one of the branches, there was an exception to the general trend (IV:3, RFS900_C). The psychophysical and electrophysiological measurements in this patient revealed relatively preserved rod and cone function even at age 42, when most of the other members barely retained the ability to detect light. Although rod and cone responses were decreased in amplitude and delayed, they were an order of magnitude higher than these in any other family member, regardless of age. Furthermore, this was the only patient in the pedigree in whom a-waves were detectable with high-intensity stimulus. An analysis of the leading edge of the a-wave revealed that log S (an indication of phototransduction efficiency) was within 0.3 log units of normal, whereas log RmP3 (the maximum photoreceptor amplitude) was decreased by 0.6 log units. Cone a-waves to high-intensity stimuli were not detectable (Fig. 6).

Bone Mineral Density

The results from the DEXA BMD and z-scores are summarized in Table 2. Affected family members are separated into two groups: affected members without HRT and affected members with HRT. At three of four locations tested, patients without hormonal treatment had results that were more than 1 z-score below mean normal. According to the established diagnostic criteria of the World Health Organization, a low bone mass condition (osteopenia) is present when BMD is more than 1 SD
<table>
<thead>
<tr>
<th>Family Member</th>
<th>Age at Initial Visit (y)</th>
<th>Follow-up (y)</th>
<th>Refraction (D)</th>
<th>Visual Acuity (Initial–Last Visit)</th>
<th>Nystagmus Since Birth</th>
<th>Fundus Appearance</th>
<th>Visual Field† at Age, y</th>
<th>DA Threshold‡ at Last Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV:3</td>
<td>42</td>
<td>12</td>
<td>OD: -5.00 + 2.0 x 70 OS: -4.00 + 2.0 x 70</td>
<td>OD 20/300–20/320 OS 20/225–20/320</td>
<td>+ Localized macular and peripapillary atrophy, bone spicule formation</td>
<td>OD: 15 deg (42) OS: 45 x 50 deg (42)</td>
<td>2.5 log masb</td>
<td></td>
</tr>
<tr>
<td>V:1</td>
<td>32</td>
<td>—</td>
<td>OD: -7.75 + 1.25 x 57 OS: -9.25 + 2.75 x 95</td>
<td>OD 20/800 OS 20/800</td>
<td>+ Vessel attenuation, mild to moderate granular changes of RPE, early atrophy in the macula</td>
<td>ND</td>
<td>6.6 log masb</td>
<td></td>
</tr>
<tr>
<td>IV:6</td>
<td>40</td>
<td>—</td>
<td>OD: +1.5 + 0.75 x 86 OS: +1.25 + 1.25 x 105</td>
<td>OD 20/320 OS 20/320</td>
<td>+ Localized macular and perimacular RPE atrophy, bone spicule formation in those areas</td>
<td>ND</td>
<td>5.7 log masb</td>
<td></td>
</tr>
<tr>
<td>III:15</td>
<td>71</td>
<td>—</td>
<td>Mild myopia (&lt; -3.0)</td>
<td>PD LP OS LP</td>
<td>Diffuse RPE atrophy, bone spicule formation, moderate vessel attenuation, macular atrophy</td>
<td>ND</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>IV:8</td>
<td>50</td>
<td>—</td>
<td>OD: +1.25 + 2.75 x 98 OS: +0.25 + 2.25 x 87</td>
<td>OD 5/350 OS 5/350</td>
<td>Diffuse RPE atrophy, bone spicule formation, moderate vessel attenuation, macular atrophy</td>
<td>ND</td>
<td>6.6 log masb</td>
<td></td>
</tr>
<tr>
<td>III:11</td>
<td>65</td>
<td>—</td>
<td>Mild hyperopic</td>
<td>OD NLP OS NLP</td>
<td>Moderate vessel attenuation, macular atrophy, isolated areas of bone spicule formation</td>
<td>ND</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>IV:16</td>
<td>25</td>
<td>16</td>
<td>N/A</td>
<td>OD HM-NLP OS CF1-NLP</td>
<td>+ N/A (subcapsular cataract in both eyes)</td>
<td>ND</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>III:3</td>
<td>52</td>
<td>—</td>
<td>OD: -3.0 OS: -3.5</td>
<td>OD LP-NLP OS LP-NLP</td>
<td>Diffuse RPE atrophy, areas of bone spicule formation, moderate vessel attenuation</td>
<td>ND</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>IV:2</td>
<td>35</td>
<td>16</td>
<td>OD: -1.75 + 1.0 x 135 OS: -1.25</td>
<td>OD LP-NLP OS LP-LP</td>
<td>Generalized depigmentation of the RPE with granularity, bull's-eye lesion in the macula</td>
<td>ND</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>IV:3</td>
<td>30</td>
<td>16</td>
<td>OD: -4.75 + 1.75 x 85 OS: -2.5 + 1.0 x 75</td>
<td>OD 20/100–20/100 OS 20/200–20/100</td>
<td>+ Generalized depigmentation of the RPE with granularity, bull’s-eye lesion in the macula</td>
<td>OD: 8 deg (30) OS: 12 deg (30)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>V:2</td>
<td>11</td>
<td>16</td>
<td>OD: +3.5 + 2.0 x 105 OS: +4.75 + 2.0 x 75</td>
<td>OD CF3-NLP OS CF3-NLP</td>
<td>+ Vessel attenuation, mild to moderate granular changes of RPE, early atrophy in the macula</td>
<td>Temporal islands OU (11)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>V:4</td>
<td>11</td>
<td>16</td>
<td>OD: -2.0 OS: N/A</td>
<td>OD 20/20–20/320 OS 20/60–20/80</td>
<td>+ Attenuated vessels, irregular macular reflex, pigment epitheliopathy outside the posterior pole</td>
<td>OD: 25 x 15 deg (11) OS: 20 deg (11)</td>
<td>5.6 log masb</td>
<td></td>
</tr>
<tr>
<td>VI:1</td>
<td>5</td>
<td>2</td>
<td>OD: +3.75 + 1.25 x 104 OS: +4.00 + 1.25 x 90</td>
<td>OD 3/300–3/300 OS 3/225–3/300</td>
<td>+ Paramacular RPE atrophy</td>
<td>OD: 25 x 30 (7) OS: 25 x 30 (7)</td>
<td>5.7 log masb</td>
<td></td>
</tr>
<tr>
<td>VI:2</td>
<td>2</td>
<td>6</td>
<td>OD: +3.50 + 1.0 x 90 OS: +2.50 + 1.0 x 96</td>
<td>OD 20/200–3/180 OS 20/200–3/140</td>
<td>+ Initial signs of bull’s-eye maculopathy</td>
<td>OD: 30 x 40 (8) OS: 30 x 40 (8)</td>
<td>5.2 log masb</td>
<td></td>
</tr>
</tbody>
</table>

CF1, count fingers at 1 foot; CF3, count fingers at 3 feet; HM, hand movement; LP, light perception; NLP, no light perception; ND, nondetectable; N/A, not applicable.

† Determined by preferential looking testing.
‡ Isopter IV-4.
§ Upper limit of normal is 2.0 log microapostilbes (masb).
but less than 2.5 SD below the young adult mean. For scientific purposes, the use of the age-corrected $z$-scores is preferable.

Overall, BMD in the hip and the spine was lower in the affected members than in age-matched normal subjects or members receiving HRT, whereas BMD was not lower in the forearm (Table 2, Fig. 7). The normal bone mineral content and its relationship with age differed in both genders and at different measurement sites. Changes in BMD versus age for the two most illustrative measurement locations (total spine and total hip) are demonstrated separately in Figure 7 for each gender group.

From the data in Figure 7, it is clear that there is a tendency for a faster than normal decrease in BMD with the age of the affected individuals. This is most evident for the BMD changes in males at the hip, where BMD loss with age of the affected individuals increased more than twice as fast with age, compared with the normal group. The BMD loss was $-0.08 \text{ g/cm}^2$ per decade for the affected males, compared with $-0.03 \text{ g/cm}^2$ average loss per decade for the normal male population ($P < 0.0001$).

**DISCUSSION**

The history of decreased vision since birth, presence of nystagmus, normal shape of the cornea, progressive visual field loss, abnormal dark adaptation, diffuse RPE changes in advanced stages and nonrecordable or greatly reduced ERG responses found in this family are consistent with the diagnosis of LCA. Diagnostically, with all the clinical findings considered, the nonrecordable or greatly reduced ERG responses in all cases differentiates them as affected by LCA rather than early-

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**FIGURE 5.** Full-field rod and cone responses from one nonaffected (RFS900_C IV:4) and two affected members of the RFS900 family. Pedigree localization, gender, and age at the time of the testing are indicated above each column of recordings. *Horizontal rows* show computer-averaged responses. Scotopic blue: a rod response to blue test; 30-Hz flicker: cone responses to a rapidly repeated stimulus (30-Hz white flicker). Superimposed spikes indicate stimulus timing.

**FIGURE 6.** Standard full-field ERGs (A) and high-intensity a-wave series (B) from the affected family member RFS900_C IV:3. See Figure 5 for details.
onset classic retinitis pigmentosa or cone–rod degeneration. The only feature that is not consistent with the diagnosis of LCA is the mode of inheritance. However, mutations in \textit{CRX} have been associated with autosomal dominant inheritance in cases with cone–rod dystrophy.\textsuperscript{17,26,35} Furthermore, two cases of de novo mutations in the \textit{CRX} gene were associated with LCA,\textsuperscript{13,14} suggesting new autosomal dominant mutations, and four other LCA pedigrees have been described with an autosomal dominant mode of inheritance.\textsuperscript{23,24,36}

Variable phenotypic expressivity was demonstrated in the described pedigree. By electrophysiological and psychophysical criteria, one case of incomplete penetrance (IV:3, RFS900_C) was present. In two other cases (V:4, RFS900_I and IV:6, RFS900_C), visual acuity was relatively preserved beyond puberty. Those findings are consistent with other examples of variation in the \textit{CRX-LCA} phenotype.\textsuperscript{16}

The actual molecular mechanism by which the \textit{CRX} \textit{Leu146del12} deletion leads to retinal degeneration remains unclear. The four amino acids that are deleted by the \textit{Leu146del12} mutation are conserved among the murine, bovine, and human \textit{CRX} proteins. Although the region affected by the mutation is outside the DNA-binding homeodomain region and the \textit{OTX} tail, its proximity to the WSP motif suggested that it may significantly affect the protein’s biologic activity. Consistent with this possibility, we found that the deletion led to a 25% to 40% decrease in transactivation activity. Presumably, this transient transfection-defined abnormality translates in vivo into an alteration of photoreceptor gene expression that directly or indirectly causes retinal degeneration. In relation to this it should be noted that mutations that lead to increases in transactivating activity, as well as those that lead to decreases, could lead to photoreceptor degeneration.\textsuperscript{37} Interesting questions remain about how different mutations in \textit{CRX} and other transcription factors can lead to different clinical phenotypes, and how, as shown in this study, even a single mutation can be associated with substantial clinical heterogeneity.

An unexpected result in our study was the finding of reduced axial BMD in affected members of the RFS900 family, without other systemic abnormalities. Such an observation has not been described previously. However, osteopenia does not manifest obvious clinical symptoms and requires specialized testing to be detected. Therefore, we cannot exclude the possibility that families with other reported \textit{CRX} mutations demonstrate the same abnormality.

The tendency for osteopenia in this family is suggestive, but we were unable to demonstrate a direct link with the \textit{CRX} mutation. A major confounding factor was HRT for at least 5

\begin{table}
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
 & Spine BMD & Total Hip BMD & Femoral Neck BMD & Total Forearm BMD \\
 & Spine \textit{z}-Score & Total Hip \textit{z}-Score & Femoral Neck \textit{z}-Score & Total Forearm \textit{z}-Score \\
\hline
\textit{CRX}, no HRT & 0.932 ± 0.095 & 0.786 ± 0.152 & 0.689 ± 0.122 & 0.650 ± 0.071 \\
 & −1.32 ± 0.75* & −1.40 ± 0.78* & −1.24 ± 0.70* & −0.37 ± 0.99 \\
\textit{CRX}, HRT & 1.112 ± 0.153 & 0.902 ± 0.060 & 0.848 ± 0.090 & 0.552 ± 0.068 \\
 & 1.35 ± 1.08 & 0.35 ± 0.36 & 0.93 ± 0.86 & 0.42 ± 0.97 \\
\hline
\end{tabular}
\caption{BMD and \textit{z}-Scores from Adult Patients Carrying the Leu146del12 \textit{CRX} Mutation}
\end{table}
years in three of the five affected female members of the family who were tested for BMD. Several studies have shown that HRT has a substantial beneficial effect in preventing bone loss in postmenopausal women. Such an effect is comparable to the difference that we observe between HRT-treated and non-treated affected family members.

Although it is possible that a reduced level of moderate exercise can influence bone mass acquisition, this relationship is still uncertain. It cannot be ruled out that, in patients with LCA, the reduced exercise level due to very low visual acuity may affect the buildup of bone mass. There is an association between low visual acuity and increased risk of hip fracture, but it can be due to other factors, such as poor visuomotor control, besides reduced levels of exercise. The effect of reduced exercise in patients with LCA and other blinding disorders on BMD has not been studied systematically and at present is unknown. Alternatively, lower bone density could involve altered pinacle function. It has been shown that CRX is expressed in the pinacle gland and can regulate pinacle gene expression in vivo.

Melatonin (MT) is the major secretory product of the pinacle gland. Totally and partially blind (light perception only) persons demonstrate increased daytime level of MT secretion and phase-advanced or phase-delayed rhythm. We did not have the opportunity to study MT levels in our patients. However, mice with anull mutation in the CRX gene demonstrated reduced expression of several pinacle genes and altered photoentrainment. Therefore, it is conceivable that there was a substantial alteration in the MT secretion in the affected family members due to abnormal pinacle function in addition to any alteration due to blindness alone. Studies have shown that MT affects calcium blood levels and homeostasis, which are important factors for maintaining bone mineral content. There is also recent direct evidence that MT stimulates proliferation and synthesis of type I collagen in human bone cells in vitro and that oral administration of MT increases bone mass in young growing mice. It also has been demonstrated that MT directly promotes osteoblast differentiation and bone formation. Even before the publication of these findings, a relationship between osteoporosis and altered pinacle function had been proposed based on indirect evidence. Our findings are consistent with the possibility that altered function of the pinacle gland resulting from the CRX mutation may be the cause of faster than normal age-related bone loss (especially in the hip) in the affected family members.

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