Clinical Manifestation of a Novel PAX6 Mutation Arg128Pro

We have restudied a Norwegian family previously examined owing to nystagmus. A panocular malformation with peripheral corneal opacities, correctopia, iris hypoplasia, early cataract formation, highly variable axial lengths, and foveal hypoplasia was found, causing a secondary nystagmus. A novel missense mutation in the PAX6 gene predicting an arginine-to-proline substitution (p.Arg128Pro) in the C-terminal part of the paired domain was identified. Isolated foveal hypoplasia with normal anterior segments has previously been seen in members of 2 unrelated families where the same amino acid is substituted with cysteine. This illustrates how different missense mutations affecting the same codon in the paired domain of the PAX6 gene may result in distinctly different phenotypes.

The study was approved by the Regional Medical Research Ethics Committee West.

Report of Cases. After obtaining informed consent, 9 affected and 3 unaffected adults were examined and blood samples were obtained. In addition, the 2 affected children, VI:1 and VI:2, underwent a less comprehensive clinical examination.

The family pedigree is shown in Figure 1. Individual IV:17 had amblyopia due to inadequately treated strabismus. Otherwise, the unaffected family members had normal eye examination results. All of the affected individuals had nystagmus shortly after birth and variable strabismus (Table). Mild to moderate ptosis was also present. However, only 1 patient (IV:1) was in need of surgery. No systemic abnormalities or malformations were recorded. The 2 children had nystagmus, iris hypoplasia, correctopia, and foveal hypoplasia similar to the adult family members (data not shown) but no corneal or lens abnormalities.

Slitlamp examination revealed 11 corneas with peripheral opacities concentric with the limbus resembling but less sharply defined than a typical embryotoxon posterior (III:20, IV:1, IV:7, IV:10, V:1, and V:4). Individual III:20 had bilateral primary open-angle glaucoma. Gonioscopy performed in 3 other patients (IV:10, V:1, and V:7) revealed normal angles. Slight correctopia and iris hypoplasia were observed in all of the eyes (Figure 2). Eleven eyes (III:20, IV:1, IV:6, IV:7, IV:9, and IV:10) underwent cataract surgery. In addition, bilateral cataracts were observed in individuals V:1, V:4, and V:7. One eye in individual IV:1 was amaurotic due to retinal detachment. Individual V:1 had a nonsymptomatic retinal detachment in the left eye. Both eyes were myopic, at −7 and −10. Neither eye had undergone pre-

Figure 1. Family pedigree updated from Odland. Double lines above the symbols indicate the affected family members examined in this study, all of whom were heterozygous for the Arg128Pro mutation. The unaffected individuals V:2, IV:16, and IV:17 also participated, whereas the children VI:1 and VI:2 only underwent a brief clinical examination. Individuals III:20, IV:1, IV:7, IV:10, V:1, and V:4 had peripheral linear opacities resembling embryotoxon posterior.
vious surgery. Retinal detachment after cataract surgery was reported previously in 2 other affected family members (III:2 and III:16) (Figure 1). Examination of the fundus showed foveal hypoplasia in all of the eyes (Figure 3).

The corrected visual acuity varied from no light perception to 6/18. The mean corneal diameter in the 14 measured eyes was 10.9 mm (range, 10.0-11.5 mm). The mean corneal thickness of 13 affected eyes measured by pachymetry was 542 µm (range, 477-587 µm). The average axial length was 23.60 mm (range, 15.34-27.94 mm) in the 15 eyes examined. The refractive errors varied from −10.0 to +3.0 in the eyes where refractive values before surgery were available (Table).

Optical coherence tomography was performed in 5 patients (III:20, IV:1, IV:7, IV:9, and V:4) and showed absence of the fovea (Figure 3). When examined by full-field electroretinography, rod and cone responses were found to be of normal configuration but with reduced amplitude in the older patients (data not shown). Magnetic resonance imaging results of the brain and orbit of 2 patients (IV:10 and V:1) with special attention to the anterior commissure, corpus callosum, and pineal gland were normal. The visual evoked potentials of 2 patients (IV:10 and V:4) showed no chiasmal asymmetry.

The anterior and posterior segment anomalies suggested that the nystagmus was secondary to a particular disorder with PAX6 as a candidate causative gene. Microsatellite markers surrounding the PAX6 locus revealed full cosegregation between the trait and markers in the family. Subsequent sequencing of the PAX6 gene revealed a missense mutation c.383G>C predicting an arginine-to-proline substitution, p.Arg128Pro, in the C-terminal part of the paired domain (reference sequence, GenBank NM_000280.2; nomenclature, http://www.hgvs.org/mutnomen/). The mutation was present in all of the affected individuals, whereas none of the healthy family members examined carried the mutation. At the same codon, an arginine-to-cysteine substitution (originally named Arg125Cys) was previously found to cause isolated foveal hypoplasia with normal-

### Table. Clinical Characteristics of the Affected Family Members Examined, All Carrying the Arg128Pro Mutation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Strabismus</th>
<th>Cornea</th>
<th>Cornal Diameter, OD/OS, mm</th>
<th>Cataract</th>
<th>Age at Surgery, y</th>
<th>Refraction Before Surgery, OD/OS</th>
<th>Best VA, OD/OS</th>
<th>Axial Length, OD/OS, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>III:20</td>
<td>74</td>
<td>Exotropia</td>
<td>Peripheral opacity</td>
<td>10.0/10.0</td>
<td>Surgery</td>
<td>46</td>
<td>−1.0/−0.5</td>
<td>0.3/0.3</td>
<td>21.47/20.94</td>
</tr>
<tr>
<td>IV:1</td>
<td>68</td>
<td>NA</td>
<td>Peripheral opacity</td>
<td>ND</td>
<td>Surgery</td>
<td>57</td>
<td>−7.0/−7.0</td>
<td>NLP/0.1</td>
<td>ND/19.27</td>
</tr>
<tr>
<td>IV:6</td>
<td>65</td>
<td>Exotropia</td>
<td>Slight neovascularization</td>
<td>11.0/11.0</td>
<td>Surgery</td>
<td>40 and 48</td>
<td>−2.0/−1.0</td>
<td>0.3/0.3</td>
<td>22.07/22.19</td>
</tr>
<tr>
<td>IV:7</td>
<td>61</td>
<td>Exotropia</td>
<td>Peripheral opacity</td>
<td>11.0/11.0</td>
<td>Surgery</td>
<td>50</td>
<td>−2.0/−2.0</td>
<td>0.2/0.3</td>
<td>24.06/24.65</td>
</tr>
<tr>
<td>IV:9</td>
<td>55</td>
<td>Exotropia</td>
<td>Clear</td>
<td>10.5/11.0</td>
<td>Surgery</td>
<td>48</td>
<td>ND</td>
<td>CF/CF</td>
<td>27.94/27.83</td>
</tr>
<tr>
<td>IV:10</td>
<td>59</td>
<td>Exotropia</td>
<td>Peripheral opacity</td>
<td>11.5/11.5</td>
<td>Surgery</td>
<td>51</td>
<td>−4.0/−4.0</td>
<td>0.1/0.3</td>
<td>15.34/25.06</td>
</tr>
<tr>
<td>V:1</td>
<td>40</td>
<td>Esotropia</td>
<td>Peripheral opacity</td>
<td>11.0/11.0</td>
<td>Yes</td>
<td>NA</td>
<td>−10.0/−10.0</td>
<td>0.1/0.1</td>
<td>27.78/26.75</td>
</tr>
<tr>
<td>V:4</td>
<td>46</td>
<td>Esotropia</td>
<td>Peripheral opacity</td>
<td>10.5/11.0</td>
<td>Yes</td>
<td>NA</td>
<td>+3.0/−2.0</td>
<td>0.1/0.1</td>
<td>24.06/24.65</td>
</tr>
<tr>
<td>V:7</td>
<td>27</td>
<td>Esotropia</td>
<td>Slight keratopathy</td>
<td>ND</td>
<td>Yes</td>
<td>NA</td>
<td>+1.0/+1.0</td>
<td>0.3/0.1</td>
<td>ND</td>
</tr>
</tbody>
</table>

Abbreviations: CF, counting fingers; NA, not applicable; ND, no data; NLP, no light perception; VA, visual acuity.
appearing anterior segments in 2 unrelated pedigrees. 2,3 Azuma et al2 examined 4 affected family members in 2 generations with normal cornea, normal iris, and no occurrence of lens opacities. The visual acuity varied between 0.4 and 0.7 in these patients. The family briefly mentioned by van Heyningen and Williamson2 consisted of 3 affected family members. The family acuity was normal, iris, and no occurrence of lens opacities. The visual acuity varied between 0.4 and 0.7 in these patients. The family briefly mentioned by van Heyningen and Williamson2 consisted of 3 affected family members. The family briefly mentioned by van Heyningen and Williamson2 consisted of 3 affected family members. The family briefly mentioned by van Heyningen and Williamson2 consisted of 3 affected family members. The family briefly mentioned by van Heyningen and Williamson2 consisted of 3 affected family members.

The arginine-to-cysteine mutation in this position have more severe malformations than humans, with corneal, lens, and iris anomalies. 4

Comment. The PAX6 gene exerts important genetic control of eye development. Mutations in PAX6 have been associated primarily with aniridia but also with a number of other ocular anomalies. Variant phenotypes are mostly related to missense mutations in the highly conserved paired domain. 3,5 The paired domain and the homeodomain make extensive DNA contacts. Arg128 is important for DNA binding because it contacts both the methyl group of thymidine 19 of the recognition sequence and a phosphate in the DNA backbone. 6

The arginine-to-proline substitution of amino acid 128 in the C-terminal part of the paired domain reported here has a phenotypic outcome clearly different from that seen when the same residue is substituted with cysteine. The observation of both intrafamilial and interfamilial phenotypic variations corroborates a difference in phenotype, although the influence of other genetic factors cannot be excluded. Excluding patients with several PAX6 mutations, the Human PAX6 Allelic Variant Database (http://pax6.hgu.mrc.ac.uk) lists 6 codons where more than 1 variant missense base-pair mutation leading to production of different amino acids is found (viz, codons 1, 18, 44, 87, 255, and 353). In 3 codons, the phenotype reported is the same or highly similar, independent of the amino acid substitution (codons 18, 44, and 255). In the remaining codons, the phenotypic outcome is not described in sufficient detail for 1 of the mutations (codons 1 and 353) or is described in only a single person (codon 87), making further genotype-phenotype comparison difficult.

Our study illustrates the importance of codon 128 with respect to PAX6 function. Further, it supports the hypothesis that missense mutations in the paired domain are likely to form a complex allelic series in which the precise consequence of the mutation might be highly variable and associated with specific subphenotypes. 7

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