A Novel Mutation in the GJA1 Gene in a Family With Oculodentodigital Dysplasia

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Objectives: To describe a Brazilian family with oculodentodigital dysplasia (ODDD) and to screen for mutations in the gap junction protein alpha 1 (GJA1) gene in this family.

Methods: Twelve members of a 3-generation family with ODDD underwent screening for mutations of the GJA1 gene and a comprehensive ophthalmic examination. We defined ODDD on the basis of clinical characteristics described in this syndrome (microdontia, caries, enamel hypoplasia, thin nose, and syndactyly) and eye abnormalities such as microphthalmos, iris atrophy, and glaucoma. Direct sequencing of the GJA1 gene was performed using DNA collected from peripheral blood. A control group of 60 healthy individuals underwent evaluation by means of enzyme digestion.

Results: Among the 8 members of this family who were characterized as having ODDD, 2 showed chronic angle-closure glaucoma, and 1 had open-angle glaucoma. A new mutation in the GJA1 gene was identified, consisting of a change from proline to histidine at codon 59. This mutation segregated through members with the ODDD phenotype. Analysis of the control group by means of restriction fragment length polymorphism (MvaI enzyme) did not disclose this mutation.

Conclusion: Our results demonstrate a new mutation (P59H) in the GJA1 gene, identified in a family with ODDD syndrome.

Clinical Relevance: The presence of different forms of glaucoma in families with ODDD may indicate a new mutation in the GJA1 gene.

of the hyaloid artery and an increased number of retinal vessels at the optic disc.\(^8\) Other less frequent ocular features of ODDD are nystagmus, palpebral fissure hypoplasia, epicantal folds, and convergent strabismus.\(^8\)

Glaucoma is one of the causes of visual loss in ODDD. Open-angle and angle-closure glaucomas have been reported in eyes with ODDD, as well as goniodysgenesis.\(^5\)\(^-\)\(^13\)

In 1997, Gladwin et al\(^14\) evaluated ODDD in 6 families (autosomal dominant pattern) using linkage analysis. The authors identified the locus associated with ODDD at chromosome 6q22-q24 in a 28-centimorgan (cM) region, delimited by markers D6S474 proximally and D6S292 distally. Boyadjiev et al\(^12\) performed 2-point linkage analysis in 7 families with ODDD and significantly reduced the size of the critical region (11 cM in male and 20 cM in female subjects). Subsequently, Paznekas et al\(^15\) identified structural changes in the gene responsible for synthesis of connexin 43 (Cx43), the gap junction protein alpha 1 (GJA1) gene, in all 17 families with ODDD who underwent screening for mutations in this gene.

The goals of this study were to describe a Brazilian family with ODDD and to screen for mutations in the GJA1 gene in this family.

### METHODS

Twelve members of a family with ODDD from Cascavel, Brazil, underwent screening for mutations in the GJA1 gene and a comprehensive ophthalmic examination. We defined ODDD on the basis of clinical characteristics described in the literature. Informed consent was obtained from the members of the family. Ophthalmic examination included intraocular pressure measurement by means of applanation tonometry; slitlamp biomicroscopy and gonioscopy; evaluation of the optic disc with a 78-diopter lens; automated perimetry (Humphrey System 24.2; Zeiss-Humphrey-Zeiss Systems, Dublin, Calif); in individuals older than 15 years; measurements of the axial length and corneal diameters and keratometry; and ocular history obtained by interview, including the age at diagnosis of glaucoma and clinical and surgical treatment.

Glaucoma was defined as the presence of at least 2 of the following characteristics: (1) intraocular pressure above 24 mm Hg; (2) optic disc changes, including thinning of the neuroretinal rim, hemorrhage, notch, cup-disc ratio greater than 0.7, or asymmetry of cup-disc ratio greater than 0.2; and (3) glaucomatous visual field defect, defined as a corrected pattern standard deviation outside the 95% normal limits or a glaucoma hemifield test result outside the 99% limits.

We extracted DNA from peripheral blood and performed direct sequencing of the GJA1 gene using fluorescent deoxyribonucleotides on an automated sequencer (ABI 310; Applied Biosystems, Foster City, Calif), according to Paznekas et al.\(^15\) Connexin amino-acid alignment was performed with the ClustalW program (http://www.ebi.ac.uk/clustalw/). Sequences were obtained from the Entrez-Protein Web site of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/entrez/). A control group of 60 healthy individuals underwent evaluation by means of digestion of the GJA1 gene PCR product with the MvaI enzyme.

### RESULTS

This family included 13 subjects, with 9 affected individuals distributed among 3 generations, suggesting an autosomal dominant inheritance pattern (Figure 1). The affected individuals had the classical characteristics described in ODDD, including microdontia, caries, enamel hypoplasia, syndactyly involving the second and third toes, and mild clinodactyly (Figure 2). These individuals also had a thin nose due to a hypoplastic alae nasi.

The only member of the family who was not examined was individual I:1, described by relatives as having clinical characteristics typical of ODDD, who died at 63 years of age, blind due to glaucoma. The Table gives the ocular findings of all family members. Among the 8 affected individuals who were examined, all had microcornea (corneal diameters, 8-9 mm) and peripupillary iris atrophy (Figure 3). Three (40%) of the affected individuals, all of them belonging to the second generation, had glaucoma. One of these was found to have open-angle glaucoma and had undergone trabeculectomy in both eyes. In this individual, results of gonioscopy disclosed a wide open angle, allowing the visualization of the ciliary band. The other 2 family members had angle-closure glaucoma. Both had shallow anterior chambers, and gonioscopy allowed the visualization of the Schwalbe line. Results of indentation gonioscopy did not disclose peripheral anterior synechiae.

The screening for mutations in the GJA1 gene identified a new mutation at codon 59, changing a proline (CCT) for a histidine (CAT) in heterozygosis (P59H) (Figure 4). This alteration segregated in members with the ODDD phenotype (Figure 1). Results of analysis by means of restriction fragment length polymorphism (MvaI enzyme) in the control group did not disclose this structural alteration.

### COMMENT

This study described a Brazilian family with ODDD that carries a new mutation in the GJA1 gene. Among the ocular findings, open-angle and angle-closure glaucoma were observed in members of the second generation.

In a review by Loddenkemper et al\(^11\) that included 243 individuals with ODDD syndrome, 9 had the diagnosis of glaucoma, 15 showed reduced visual acuity, and another 20 were described with other ocular manifesta-
tions. However, that article emphasized the neurological aspects of ODDD. In another review by Judisch et al,9 the authors identified 6 patients with glaucoma, among whom 2 had open-angle glaucoma and the remaining patients had goniodysgenesis. Novotny and Sterbova10 described 102 members of a 5-generation family, in which 42 were found to have ODDD. The condition was complicated by glaucoma in the older individuals and strabismus in the younger ones. In 1996, Braun et al11 described a boy with ODDD and juvenile open-angle glaucoma who showed reduced corneal diameter (OD, 9.0/7.0 mm; OS, 8.5/6.5 mm) with axial lengths close to the normal range (OD, 25.3 mm; OS, 23.6 mm). Traboulsi and Parks8 described 2 individuals with ODDD associated with developmental glaucoma that started at 4 months and 5 years of age, respectively. Finally, 2 patients with angle-closure glaucoma were described by Sugar et al12,13 in 1966 and 1978.

In our study, among the 3 family members with glaucoma, 1 had open-angle glaucoma and 2 had angle-closure glaucoma. When we consider the individuals in the third generation, all 5 family members with ODDD were young (age range, 8-20 years), and glaucoma had not developed (Table). It will be interesting to follow up the association between glaucoma and ODDD among this generation of the family. It is highly possible that some of them will develop glaucoma later in life.

In 2003, Paznekas et al15 described 17 families with ODDD and 17 different structural alterations in the GJA1 gene. The mutations segregated with the disease and were absent in a control group of 100 individuals. The GJA1 gene is responsible for the synthesis of the Cx43 protein. Connexins are

Table. Ophthalmic Characteristics of the Family With ODDD

<table>
<thead>
<tr>
<th>Individuals</th>
<th>Visual Acuity*</th>
<th>Refraction, SE*</th>
<th>IOP, mm Hg*</th>
<th>Results of Biomicroscopy</th>
<th>Gonioscopy, Angle*</th>
<th>C/D Vertical Ratio*</th>
<th>Cornea, mm*</th>
<th>Axial Length, mm*</th>
<th>KT, D*</th>
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</thead>
<tbody>
<tr>
<td>I:2</td>
<td>25/25</td>
<td>0.00/0.00</td>
<td>14/16</td>
<td>Normal</td>
<td>Open/Open</td>
<td>0.4/0.4</td>
<td>12.0/12.0</td>
<td>22.15/22.16</td>
<td>43.25/43.50</td>
</tr>
<tr>
<td>I:3†‡</td>
<td>30/&lt;400</td>
<td>-0.75/NA</td>
<td>30/30</td>
<td>Peripapillary iris atrophy</td>
<td>Open/Open</td>
<td>0.4/0.5</td>
<td>8.5/8.2</td>
<td>21.53/23.63</td>
<td>47.50/48.12</td>
</tr>
<tr>
<td>I:4</td>
<td>20/20</td>
<td>-1.25/-0.75</td>
<td>12/12</td>
<td>Normal</td>
<td>Open/Open</td>
<td>0.4/0.4</td>
<td>12.2/12.3</td>
<td>23.02/22.94</td>
<td>44.25/43.75</td>
</tr>
<tr>
<td>I:5</td>
<td>20/20</td>
<td>0.00/0.00</td>
<td>16/16</td>
<td>Normal</td>
<td>Open/Open</td>
<td>0.3/0.3</td>
<td>12.0/12.0</td>
<td>22.95/23.20</td>
<td>46.00/45.50</td>
</tr>
<tr>
<td>I:6†‡</td>
<td>40/20</td>
<td>+1.75/+0.75</td>
<td>25/14</td>
<td>Peripapillary iris atrophy</td>
<td>Open/Open</td>
<td>0.7/0.6</td>
<td>8.0/8.0</td>
<td>20.87/20.79</td>
<td>45.37/45.25</td>
</tr>
<tr>
<td>III:1†</td>
<td>NA</td>
<td>NA</td>
<td>20/18</td>
<td>Peripapillary iris atrophy</td>
<td>Narrow/Narrow</td>
<td>0.3/0.4</td>
<td>8.8/8.8</td>
<td>21.78/21.86</td>
<td>41.75/41.25</td>
</tr>
<tr>
<td>III:2†</td>
<td>20/25</td>
<td>0.00/0.00</td>
<td>17/20</td>
<td>Peripapillary iris atrophy</td>
<td>Narrow/Narrow</td>
<td>0.3/0.3</td>
<td>8.4/8.2</td>
<td>21.22/21.14</td>
<td>44.62/44.75</td>
</tr>
<tr>
<td>III:3†</td>
<td>20/20</td>
<td>+1.75/+1.50</td>
<td>19/19</td>
<td>Peripapillary iris atrophy</td>
<td>Narrow/Narrow</td>
<td>0.6/0.7</td>
<td>9.0/9.0</td>
<td>20.62/20.78</td>
<td>47.50/47.37</td>
</tr>
<tr>
<td>III:4†</td>
<td>20/20</td>
<td>0.00/-0.25</td>
<td>14/15</td>
<td>Peripapillary iris atrophy</td>
<td>Open/Open</td>
<td>0.5/0.6</td>
<td>8.5/8.5</td>
<td>20.80/20.73</td>
<td>48.12/48.62</td>
</tr>
<tr>
<td>III:5†</td>
<td>30/30</td>
<td>+1.00/+0.25</td>
<td>16/16</td>
<td>Peripapillary iris atrophy</td>
<td>Open/Open</td>
<td>0.3/0.3</td>
<td>8.0/8.0</td>
<td>20.87/20.85</td>
<td>45.37/45.37</td>
</tr>
</tbody>
</table>

Abbreviations: C/D, cup-disc; D, diopter; IOP, intraocular pressure; KT, keratometry; NA, not available; ODDD, oculodentodigital dysplasia; SE, spherical equivalent; Ø, diameter.
*Data presented as result in the right eye/result in the left eye.
†Indicates family members with ODDD syndrome.
‡Indicates family members with glaucoma.

Figure 2. Phenotype of oculodentodigital dysplasia in the studied family showing syndactyly involving the second and third toes (A) and mild clinodactyly (B) in family members II:2, III:1, and III:2 (depicted in Figure 1).
molecules that are structurally important for the formation of gap junctions between adjacent cells, through which direct intercellular communication via diffusion of ions and metabolites can occur.\textsuperscript{16} Several human diseases are associated with mutations in connexin genes. These include disturbances of several biological processes such as cardiac conduction, auditory function, aging and senescence, neuronal function, pathfinding and glial signaling, immune system activation, bone and tooth development, neural tube defects, hematopoiesis, and myogenesis.\textsuperscript{17} For example, con-
genital cataract has been described as a result of Cx46 and Cx50 mutations. Both connexins are expressed during lens development, and their function seems to be dependent on the repertoire, rather than the number, of these 2 connexin subunits available.

In their study, Paznekas et al described 3 families with ODDD and glaucoma showing mutations located in the cytoplasm (Y17S) and in the first transmembrane domain (G22E) and second transmembrane domain (L190V) of the GJA1 gene. The Y17S mutation was associated with early cataract development, and the G22E mutation was identified in 2 children with developmental glaucoma.

In the family described herein, we identified the P59H mutation, which belongs to the first extracellular loop of the connexin protein and has not been reported in any of the previously described 17 families. The extracellular loops of the connexin proteins are important for the docking of gap junctional hemichannels (connexons) and are responsible for selective compatibility between different types of connexins that lead to the formation of heterotypic functional channels. The proline residue at codon 59 is close to the absolutely conserved cystein residues that are crucial for intramolecular stabilization. Furthermore, this proline has been shown to be a highly conserved residue among the connexins of other species and among different human connexins.

The coexistence in this family of open-angle and angle-closure glaucoma suggests that different mechanisms may explain the association between ODDD and glaucoma. These mechanisms could be related to the participation of connexins in embryonic development. Neural crest cells contribute to the angle development and appear to express Cx43 and functional gap junctions. It has been shown that the rate of migration of neural crest cells in vitro is correlated with the level of Cx43 expression. Thus, structural alterations of the GJA1 gene could interfere with the embryonic development of the angle and lead to an ocular anatomic predisposition to developmental glaucoma. On the other hand, an abnormality at the meshwork level could explain the occurrence of open-angle glaucoma, possibly associated with a dysfunction of gap junctions between the trabecular cells that could reduce conventional outflow. Finally, the frequent ocular abnormalities found in ODDD, including microphthalmos and microcornea, could predispose these eyes to chronic angle-closure glaucoma.

In the literature, all of these forms of glaucoma have been described in the ODDD syndrome. The presence of different forms of glaucoma in the same family may indicate the interference of other genes (including different connexins) or an environmental role in its phenotypic modulation. Further studies are needed to evaluate the expression of Cx43 and other connexins in the trabecular meshwork during angle development and in the trabecular meshwork of patients with glaucoma.

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