Clinical Description and Exclusion of Candidate Genes in a Novel Autosomal Recessively Inherited Vitreoretinal Dystrophy

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Objectives: To describe the clinical phenotype of a novel autosomal recessively inherited vitreoretinal dystrophy in one generation of a family originating from eastern Switzerland.

Methods: A clinical study including electroretinographic investigations followed by laboratory-based genetic and molecular analysis. Four affected and 3 unaffected members of the family were examined. Ten candidate regions were tested by linkage analysis with highly polymorphic molecular markers or with intragenic restriction fragment length polymorphisms.

Results: Of 8 siblings, 4 were affected, showing high myopia with pronounced vitreous liquefaction, retinitis pigmentosa–like retinal degeneration, diffuse retinal pigment epithelium atrophy, macular staphylomata, and premature cataract formation. Strikingly abnormal results on electroretinograms, affecting both the rod and the cone systems, revealed an extensive defect of retinal function, unlike those usually found in pathologic myopia. No extraocular manifestations were observed. Three types of nonsyndromic high myopia, Stickler syndrome I, II, and III, Wagner syndrome, Knobloch syndrome, Goldmann-Favre dystrophy, and multiple vitreoretinopathies were excluded by linkage analysis.

Conclusions: The reported phenotype as well as the results of molecular linkage analysis in the siblings described here suggest an autosomal recessively inherited vitreoretinal dystrophy, which, to our knowledge, has not been described until now.

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HEREDITARY vitreoretinal dystrophies are potentially blinding disorders characterized by an abnormal vitreous gel, associated retinal degeneration, and a heterogeneous origin. The phenotype is extremely variable, and both syndromic and nonsyndromic forms exist. Several of these vitreoretinal dystrophies are associated with axial myopia of different extents and retinal detachment. Disease-causing mutations have been identified in various genes that encode components of the extracellular matrix.

See also page 1184

Here, we describe a type of vitreoretinal dystrophy characterized by early-onset, high axial myopia with pronounced vitreous liquefaction, retinitis pigmentosa–like retinal degeneration, diffuse retinal pigment epithelium (RPE) atrophy, macular staphylomata, premature cataract formation, strikingly abnormal electroretinogram (ERG) results, and severe visual field constriction.

METHODS

SUBJECTS

The family described here originated from eastern Switzerland. Autosomal recessive inheritance was inferred, with affected individuals occurring in only one generation. Genealogic investigation (Manuel Aicher, Genealogische Forschung, Dietikon, Switzerland) indicated that most of the ancestors originated from a relatively isolated population. Informed consent and medical history were obtained from 3 healthy and 4 affected siblings aged 68 to 77 years at the time of examination. The eighth brother as well as both parents were not affected and died prior to this investigation. The 8 siblings have a total of 36 children and 50 grandchildren.

CLINICAL AND FUNCTIONAL INVESTIGATIONS

Phenotype characterization was based on medical and ophthalmic history and results of slit-lamp biomicroscopy and detailed fundus ex-
Table 1. Investigation of Candidate Genes/Loci

<table>
<thead>
<tr>
<th>Gene/Disease</th>
<th>Locus</th>
<th>Marker</th>
<th>Affected Persons</th>
<th>Unaffected Persons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myopia</td>
<td>MYP2</td>
<td>18p11</td>
<td>12 (pool)</td>
<td>12 (pool)</td>
</tr>
<tr>
<td></td>
<td>MYP3</td>
<td>12q21-23</td>
<td>12 (pool)</td>
<td>12 (pool)</td>
</tr>
<tr>
<td>Familial high myopia</td>
<td>Sticker syndrome</td>
<td>7q36</td>
<td>D7S550</td>
<td>12 (pool)</td>
</tr>
<tr>
<td>COL2A1</td>
<td></td>
<td>12q13</td>
<td>PCRI7.2</td>
<td>22/12/22</td>
</tr>
<tr>
<td>COL11A1</td>
<td></td>
<td>12q11</td>
<td>D12S81</td>
<td>12 (pool)</td>
</tr>
<tr>
<td>COL11A2</td>
<td></td>
<td>6p21.3</td>
<td>D6S291</td>
<td>13/13/13</td>
</tr>
<tr>
<td>Wagner syndrome</td>
<td></td>
<td>5q13-14</td>
<td>D5S644</td>
<td>13/24/35/24</td>
</tr>
<tr>
<td>Knobloch syndrome</td>
<td></td>
<td>21q22.3</td>
<td>D21S1589</td>
<td>23/23/23/13</td>
</tr>
<tr>
<td>Goldmann-Favre</td>
<td></td>
<td>15q23</td>
<td>D15S991</td>
<td>12/11/12/11</td>
</tr>
<tr>
<td>症候群</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

The Genome database (http://gdbwww.gdb.org) was consulted for primer sequences of polymorphic sites in candidate genes and for allele frequencies of microsatellite markers. Online Mendelian Inheritance in Man (OMIM) (http://www.ncbi.nlm.nih.gov/Omim) was used for Knobloch syndrome (OMIM *120328), Stickler syndrome type I (OMIM #108300), Stickler syndrome type II (OMIM #608481), Stickler syndrome type III (OMIM #184840), Wagner disease (OMIM *143200), Goldmann-Favre dystrophy (OMIM #268100), MYP2 (myopia 2) (OMIM *160700), MYP3 (OMIM *603221), Marfan syndrome (OMIM #154700), and Doyne honeycomb retinal dystrophy (OMIM *126600).

**RESULTS**

The 4 affected siblings reported that the onset of their visual disturbance occurred in early childhood and was followed by progressive night blindness and visual field loss during the third to fifth decade of life. In addition, all of the affected siblings had premature cataract formation, which required cataract surgery around the age of 50 years. Retinal detachment surgery was needed in 2 pseudophakic eyes (both eyes of patient 1) and in one phakic eye (left eye of patient 2). Prophylactic laser treatment of retinal tears was performed in 2 eyes (right eye of patient 2 and right eye of patient 3). None of the 86 offspring of the 8 siblings were known to have any relevant visual problems except for minor refractive errors: 72.5% were emmetropic and only 2 individuals exceeded –3.0 diopters (D) (−4.0 D OU and –4.5 D OU).

**OPHTHALMIC HISTORY**

The pertinent ocular findings of all of the affected patients are presented in Table 2.

**Patient 1**

The right vitrectomized and silicone oil–filled eye (Figure 1A) disclosed scattered cryotherapy and laser therapy scars throughout the outer periphery and retinal pigment, clumping predominantly on the temporal side of the equatorial zone. The posterior pole showed diffuse RPE and retinal atrophy. A shallow posterior staphyloma was present, and the macula showed fine pigment irregularities. The retinal arterioles were attenuated, and the optic disc appeared normal. The left eye was phthisical; the posterior segment was barely visible.

**Patient 2**

The intact but cataractous lens was floating in the vitreous of the right eye. There were no signs of inflammation in the vitreous, and a posterior vitreous detachment was found. In the vitreous gel, band-shaped strands were described, which paralleled the equator on the nasal aspect of the midperipheral retina. The anterior retina

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showed multiple and scattered bone spicule–like pigmented clumps. At the 2–o’clock meridian, a pigmented retinal hole with a free-floating operculum was detected, with the retina attached. The posterior pole disclosed a diffusely atrophic RPE and a circumscribed scar temporoinferiorly adjacent to the border of a moderately deep posterior staphyloma. On the left side, posterior segment visualization was impossible owing to the small pupil size and a retropupillary mass.

**Patient 3**

Both eyes showed symmetric changes. The vitreous was liquefied and contained fibrillar strands and scattered pigmented clumps in the far periphery of the retinas. In addition, midperipheral lattice degeneration on the temporal aspect, diffuse RPE atrophy, and deep posterior staphylomata including the macula were on both sides (left eye, Figure 1B). The optic nerve heads showed tilted discs with a myopic configuration and shallow cupping.

**Patient 4**

Both eyes contained liquefied vitreous gel with band-shaped condensations. The peripheral retina showed scattered bone spicule–like pigmentary clumps. The midperipheral and posterior RPE were diffusely atrophic. The posterior poles demonstrated deep, central staphylomata with associated circumscribed chorioretinal atrophy exposing areas of bare sclera, mainly on the right side (Figure 1C). There was some remaining macular tissue in the left eye (Figure 1D). The retinal arterioles were attenuated, and both optic discs showed glaucomatous excavation.

**KINETIC PERIMETRY AND DA**

After 7 minutes, the DA curve for patient 1 showed a monophasic pattern above the normal cone threshold without detectable adaptation of the rod system. Kinetic perimetry revealed severe and irregular constriction of the isopters with a remaining central island and a deep supranasal scotoma (Figure 2A). Owing to technical problems, no DA was available for patient 2. The visual field of the right eye showed a concentric constriction for all tested isopters (V4e, II4e, and I4e) but no ring scotoma. In patient 3, DA revealed a monophasic pattern above the normal cone threshold and no rod adaptation. Kinetic perimetry showed a highly and irregularly constricted visual field with a remaining central island of residual sensitivity (Figure 2B). In patient 4, DA disclosed a monophasic pattern for the first 18 minutes, then a slight increase in the sensitivity, with a final rod threshold of 2.5 log units above normal. Kinetic perimetry showed a marked and irregular constriction, far more advanced on the right side (Figure 2C and D). In the 3 unaffected siblings, findings on perimetry and DA were normal.
ELECTROPHYSIOLOGIC FINDINGS

The ERG recordings are shown in Figure 3, and the corresponding b-wave amplitudes and latencies are presented in Table 3. In patient 1, a recordable signal (small residual b-wave of 30 µV) was only detected on maximal stimulation (1 dB) in the dark-adapted right eye. Selective cone signals were not detectable (data not shown). Stimulation of the rod system in the right eye of patient 2 (Figure 3 row 4) demonstrated small remaining b-waves. Intense stimulation with white light on DA elicited b-waves with maximum amplitudes of 80 µV. The cone system showed small residual b-waves with markedly prolonged latencies. Ganzfeld ERGs (Figure 3 row 5) on DA showed no recordable responses on rod-matched red and blue stimulation in patient 3. A 40-µV b-wave amplitude was detectable on intense white stimulation. The light-adapted cone ERG disclosed only residual responses. A 30-Hz flicker ERG was not recordable. In patient 4, all ERG recordings (Figure 3 row 6) revealed reduced b-wave amplitudes both in the rod and cone systems. The cone responses were markedly reduced, with prolonged implicit time, and no 30-Hz flicker responses were recordable.

Overall, the electrophysiologic investigations in the affected patients showed severe retinal damage affecting both the rod and cone systems. This loss of function exceeded that caused by pathologic myopia...
alone, which suggests a superimposed widespread retinal dystrophy. The ERG responses in the other 3 siblings were within normal limits (Figure 3 rows 2,3, and 7).

**LINKAGE ANALYSIS**

All markers tested for high myopia (MYP2, MYP3, and for the newly described locus on 7q36), Goldmann-Favre disease, Wagner syndrome, Knobloch syndrome, familial exudative vitreoretinopathy, and the Stickler syndrome genes \( \text{COL2A1} \), \( \text{COL11A1} \), and \( \text{COL11A2} \) were informative. At least 2 alleles were found in affected individuals, thereby excluding homozygosity at these loci (Table 1). In addition, no common haplotype was found for any of the markers in the affected or the unaffected group. For marker D5S644, 5 different alleles were reproducibly found in this family, providing evidence for a microsatellite mutation event.

**COMMENT**

We described a nonsyndromic vitreoretinal dystrophy that has not been described previously and is characterized by excessive early-onset axial myopia with deep posterior staphylomata, pronounced vitreous liquefaction, retinal degeneration with diffuse RPE atrophy, and premature cataract formation. The clinical picture and the striking electrophysiologic and visual field defects suggest the presence of a primary vitreoretinal dystrophy rather than merely late sequelae of pathologic myopia. However, there are secondary degenerations due to the advanced age of the affected patients and the surgical interventions performed. This makes the retrospective understanding of the pathogenic process in these 4 patients more difficult. Nevertheless, the phenotype is very similar but not equivalent to a number of inherited syndromic and nonsyndromic ocular diseases.
Figure 3. Electroretinogram (ERG) traces of 3 affected (row 4, 5, and 6) and 3 unaffected (row 2, 3, and 7) siblings. The traces of row 1 represent an age-matched control. All ERG responses of the affected members revealed reduced b-wave amplitudes of both the rod and cone systems. The cone responses were markedly reduced in amplitude with prolonged implicit time. No responses to 30-Hz flicker stimulation were recordable.
The Knobloch syndrome (OMIM *120328) is an autosomal recessively inherited disorder characterized by axial myopia, vitreous degeneration, retinal detachment, and additional nonocular findings, such as posterior encephalocele or scalp defects, and has been mapped to chromosome 21q22.3. A homozygous mutation in the COL18A1 gene encoding the short type XVIII collagen was recently identified in a large consanguineous family with Knobloch syndrome. The rather heterogeneous Stickler syndrome is caused by mutations in at least 3 different collagen genes, COL2A1 (“beaded” vitreous phenotype; Stickler syndrome type I, OMIM #108300), COL11A1 (“membranous” vitreous phenotype; Stickler syndrome type II, OMIM #604841), and COL11A2 (no ocular phenotype; Stickler syndrome type III, OMIM #184840). Prominent ocular findings include progressive axial myopia with vitreoretinal degeneration and a high incidence of retinal detachment. Orofacial and skeletal abnormalities are frequent but none of them were present in our family. Apart from the phenotypical differences, both Stickler syndrome and Knobloch syndrome were excluded in our family by molecular investigations.

Typical features of Wagner disease (OMIM *143200) are an empty vitreous cavity with avascular strands and veil, mid peripheral retinal dystrophy, mild myopia, and cataract. Rhegmatogenous retinal detachments are observed infrequently, whereas peripheral traction retinal detachment is present in most of the affected elderly individuals. No extraocular findings have been reported. Two loci for autosomal dominant myopia, MYP2 (OMIM *160700) and MYP3 (OMIM *603221) were identified by linkage, one to a 7.6-centimorgan region on chromosome 18p11.31 and the other to a 3-centimorgan region on chromosome 12q24.13-15 Recently, a third locus was mapped to an 11.7-centimorgan region on chromosome 7q36.15 All of these loci were excluded by linkage analysis and the additional clinical findings in our family.

In conclusion, in the siblings described here, the ophthalmologic findings are not compatible with high myopia or any previously described vitreoretinal dystrophy. Furthermore, the results of molecular linkage analysis gave convincing evidence for an autosomal recessive entity form of retinoschisis or edema and pigmentary degeneration of the retina with hemeralopia, and an extinguished ERG. Goldmann-Favre dystrophy is allelic with the enhanced S cone syndrome, and both are caused by mutations in a nuclear receptor gene, NR2E3, on chromosome 15q23.8 In both diseases, posterior staphyloma have never been observed, and therefore, from a clinical view, both are unlikely. In addition, linkage analysis did not provide evidence for homozygosity at this locus in the 4 affected individuals.

Nonsyndromic myopia is a common condition affecting approximately one third of the world population. The term high myopia, or pathologic myopia, usually refers to a condition in which individuals have myopia greater than −6 D or an axial length exceeding 26 to 27 mm. It results primarily from axial overgrowth of the globe, and is often associated with rhegmatogenous retinal detachment, macular degeneration, glaucoma, and a posterior staphyloma. Most cases of myopia probably have a multifactorial origin, and the susceptibility genes contributing to the development of this condition are unknown. Families with X-chromosomal, autosomal dominant, and autosomal recessive modes of inheritance of myopia have been described, especially in high myopia or complex eye anomalies. Two loci for autosomal dominant myopia, MYP2 (OMIM *160700) and MYP3 (OMIM *603221) were identified by linkage, one to a 7.6-centimorgan region on chromosome 18p11.31 and the other to a 3-centimorgan region on chromosome 12q24.13-15 Recently, a third locus was mapped to an 11.7-centimorgan region on chromosome 7q36.15 All of these loci were excluded by linkage analysis and the additional clinical findings in our family.

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within the extremely heterogeneous group of inherited vitreoretinopathies, which has not been described previously. Elucidation of the genetic and molecular mechanism in our family may provide further insight into the pathogenesis and classification, not only of vitreoretinal degeneration, but also of other forms of inherited myopia. We have also described a genomewide homozygosity mapping approach and the molecular analysis of a candidate gene located on 22q13 (FBLN1 [fibulin 1]).

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