Phenotypic variations in a family with retinal dystrophy as result of different mutations in the ABCR gene

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Abstract

Aims—To describe two phenotypic variations of autosomal recessive retinal dystrophy occurring in a consanguineous family in a pseudodominant pattern, resulting from mutations in the ATP binding cassette transporter (ABCR) gene.

Methods—Patients of this family underwent an extensive ophthalmic evaluation, including fundus photography, fluorescein angiography, and electroretinography (ERG). Genetic analysis comprised sequence analysis of the retina specific ABCR gene.

Results—Five patients presented with decreased visual acuity in the second decade, central choriotinal atrophy associated with a central scotoma, and severely decreased photopic and scotopic ERG responses. This clinical picture, which in our opinion resembles a cone-rod dystrophy (CRD), was associated with compound heterozygosity for IVS30+1g→t and IVS40+5g→a mutations in the ABCR gene. The four remaining patients presented with night blindness in the first decade because of a retinitis pigmentosa-like dystrophy (RP-like). In addition to a pale “waxy” optic disc, attenuated retinal vessels and bone spicule deposits, a widespread choriotinal atrophy was observed. The scotopic ERG was extinguished and the photopic ERG was severely diminished. Genetic analysis revealed a homozygous 5’ splice mutation IVS30+1g→t in the ABCR gene. Conclusion—Mutations in the ABCR gene can cause clinical pictures resembling autosomal recessive RP and autosomal recessive CRD.

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Stargardt’s disease (STGD) presents in the first or second decade of life with a bilateral gradual diminution of vision due to progressive atrophy of the macular retinal pigment epithelium and the choriocapillaris in combination with degeneration of the photoreceptors of the posterior pole.1 STGD is caused by mutations in the retina specific ATP binding cassette transporter (ABCR) gene, which also has been shown to be involved in age related macular degeneration (AMD), although the latter finding has been disputed.2–4 We describe a single consanguineous family with two different phenotypes: cone-rod-like dystrophy (CRD-like) and a retinitis pigmentosa-like dystrophy (RP-like). These phenotypes co-segregated with DNA markers flanking the ABCR gene and subsequent sequence analysis revealed compound heterozygosity (CRD-like) and homozygosity (RP-like) for ABCR mutations.
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GENOTYPING
DNA was extracted from leucocytes as described previously. D1S406 (UT2069) and D1S236 (AFM205ta11) marker analysis was carried out as described elsewhere. Sequence analysis of the ABCR exons was performed as described by Allikmets et al and Gerber et al.  

Results
The pedigree of the RP-like/CRD-like family is depicted in Figure 1. Nine members of the family were affected, two sibs in generation IV and seven sibs in generation V. No abnormalities were found in individuals IV-1, V-1, V-2, V-4, V-6, and V-7. After the ophthalmic history was taken and a full ophthalmic examination was performed (as summarised in Table 1), two different phenotypes could be distinguished.

Five individuals (IV-3, IV-7, V-5, V-10, V-11) presented with initial complaints of decreased central vision or a grey spot in the central field of vision in the second decade. At the time of our examination the visual acuity of these five patients was reduced to counting fingers. Funduscopy disclosed a circumscribed area of central chorioretinal atrophy. Waxy pallor of the optic disc, attenuated retinal vessels, and pigmentary retinopathy with bone spicule deposits in the (mid) periphery. Furthermore, large areas of poorly demarcated chorioretinal atrophy were noted in these patients, also in the posterior pole (Fig 2D, E, F). Goldmann perimetry of patient V-3 showed a central scotoma and peripheral restriction at the age of 22. Patient V-8 demonstrated restricted visual fields at age 13, the visual field of patient V-9 revealed a constriction nasally at age 21 and patient V-12 showed a central scotoma and concentric impairment of sensitivity at the age of 11. ERG recordings performed in patients V-8, V-9, and V-12 showed severely decreased photopic ERG responses and an extinguished scotopic ERG (Table 1).

Linkage analysis using highly polymorphic DNA markers from 1p21 showed that all affected sibs in generation V inherited the same chromosomal haplotype from their father.
HTZ = compound heterozygosity for IVS30+1g→a mutation; HMZ = homozygosity for IVS30+1g→a mutation.

Table 1 Minimal values for ERG recordings: 100 µV for the photopic ERG, 150 µV for the scotopic ERG with a white stimulus after 12 minutes of dark adaptation, 170 µV for the scotopic ERG with a blue stimulus after 15 minutes of dark adaptation.

<table>
<thead>
<tr>
<th>Number</th>
<th>Genotype</th>
<th>Age (years)</th>
<th>Onset of symptoms</th>
<th>Visual acuity (1995)</th>
<th>Funduscopy</th>
<th>Photopic ERG (µV) right eye</th>
<th>Photopic ERG (µV) left eye</th>
<th>Scotopic ERG (µV) white right eye</th>
<th>Scotopic ERG (µV) white left eye</th>
<th>Scotopic ERG (µV) blue right eye</th>
<th>Scotopic ERG (µV) blue left eye</th>
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</thead>
<tbody>
<tr>
<td>IV-3</td>
<td>HTZ</td>
<td>84</td>
<td>12</td>
<td>Decrease in visual acuity</td>
<td>CF</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IV-7</td>
<td>HTZ</td>
<td>74</td>
<td>20</td>
<td>Decrease in visual acuity</td>
<td>CF</td>
<td>20</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>75</td>
<td>30</td>
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<tr>
<td>V-3</td>
<td>HMZ</td>
<td>59</td>
<td>6</td>
<td>Night blindness</td>
<td>LP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>V-5</td>
<td>HTZ</td>
<td>56</td>
<td>15</td>
<td>Decrease in visual acuity</td>
<td>CF</td>
<td>30</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>V-8</td>
<td>HMZ</td>
<td>51</td>
<td>7</td>
<td>Night blindness</td>
<td>LP</td>
<td>20</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>V-9</td>
<td>HMZ</td>
<td>50</td>
<td>7</td>
<td>Night blindness</td>
<td>LP</td>
<td>0</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>V-10</td>
<td>HTZ</td>
<td>48</td>
<td>12</td>
<td>Decreased visual acuity</td>
<td>CF</td>
<td>30</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>V-11</td>
<td>HTZ</td>
<td>46</td>
<td>12</td>
<td>Grey spot in the central field of vision</td>
<td>CF</td>
<td>80</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>75</td>
<td>30</td>
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<tr>
<td>V-12</td>
<td>HMZ</td>
<td>43</td>
<td>6</td>
<td>Night blindness</td>
<td>HM</td>
<td>20</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

HTZ = compound heterozygosity for IVS30+1g→t and IVS40+5g→a mutation; HMZ = homozygosity for IVS30+1g→t mutation; CF = counting fingers; HM = hand movements; LP = light perception; NP = not performed.

Discussion

In this study we describe a consanguineous family in which the affected members display one of two distinctly different phenotypes, either RP-like or CRD-like. These different disease phenotypes completely match two different genotypes. Apparently, different combinations of gene defects in the ABCR gene result in different phenotypic effects. The four patients who are homozygous for the IVS30+1g→t mutation present with a clinical picture resembling RP, with an age of onset of approximately 6 years. Although this retinal dystrophy harbours several aspects of a classic RP (night blindness, peripheral restricted visual fields, the ERG findings, and attenuation of the retinal vessels, bone spicule deposits, and a waxy optic nerve head on funduscopy), there are some aspects of this phenotype which are not typically associated with RP. The extensive chorioretinal atrophy and pigmentary changes in the posterior pole, the central scotomas in patients V-3 and V-12, and the substantively more reduction in visual acuity than is normally noted in patients with RP lead us to refer to this entity as RP-like.

The patients displaying compound heterozygosity for the IVS30+1g→t and IVS40+5g→a mutations develop a retinal dystrophy which occurs later in life, with an age of onset varying from 12 to 20 years. In these patients decrease of visual acuity and/or a cataractous area of chorioretinal atrophy, especially located in the posterior pole. No ophthalmoscopic abnormalities were observed that could be attributed to RP. Strikingly, the central localisation of the retinal dystrophy in
patients with compound heterozygosity is not reflected in their ERG recordings, which in general demonstrate a severely affected cone system but an even more affected rod system. This cannot simply be attributed to the fact that most patients have reached an end stage, since early ERG recordings of V-5, at 27 years of age, show the same phenomenon—that is, the scotopic ERG is more affected than the photopic ERG. These ERG responses are very similar to the ERG findings in sibs with homozygous mutations and the resulting RP-like phenotype. It must be mentioned, however, that the measurement of the scotopic responses was unconventional, for reasons stated earlier, and the 12 minute period of dark adaptation when a white stimulus was used, may not have disclosed the entire potential for the development of a rod response. Although the chorioretinal atrophy is far more widespread in this group compared with the central atrophy observed in the compound heterozygous patients, the results of functional tests such as the ERG are virtually identical. Thus, the retinal disorder in the compound heterozygous patients is more difficult to classify. Since perifoveal yellow spots were never observed and the choroidal fluorescence was never obscured in fluorescein angiography, it is our belief that the original diagnosis of STGD disease is not appropriate. Nevertheless, all these patients initially presented with blurred central vision rather than nyctalopia and the fundus of patient V-5 at 28 years of age shows a well demarcated oval shaped depigmentation of the retinal pigment epithelium in the posterior pole. The visual fields of patients IV-3 and V-5 show a central scotoma or decreased central sensitivity in combination with a mild peripheral restriction. The ERG findings are atypical...
and suggest severely affected cone and rod systems with a slight emphasis on damage to the latter. In view of these findings the most likely diagnosis in our opinion is CRD, although the ERG recordings make it difficult to fit this phenotype into previous subtypes of CRD.\textsuperscript{11, 12} We therefore have referred to this type of dystrophy as CRD-like. When classified as CRD this phenotype would fit best in the group described by Szlyk \textit{et al} as type 2a CRD.\textsuperscript{11} It is also possible that these patients have a form of RP which is mainly located centra- lly, so called central RP or inverse RP,\textsuperscript{11, 14} although this entity is not very well defined in literature. The fact that this retinal dystrophy cannot be accurately classified underscores that these diseases need to be defined in genetic terms rather than the current subjective and variable phenotypic terminology.

Allikmets \textit{et al} recently identified mutations in a photoreceptor cell specific ATP binding cassette transporter gene (\textit{ABCR}) in STGD.\textsuperscript{2} Another report from the same group describes alterations in one allele of the \textit{ABCR} gene in some AMD patients, although these findings were not confirmed in a correspondence by Stone \textit{et al}.\textsuperscript{4} Our findings suggest that mutations in \textit{ABCR} not only result in STGD but can also cause autosomal recessive CRD-like phenotypes and autosomal recessive RP-like phenotypes. Compound heterozygosity for IVS30\,+\,1g\,\,\rightarrow\,\,t and IVS40\,+\,5g\,\,\rightarrow\,\,a mutations results in a clinical picture resembling CRD, whereas homozygosity for the IVS30\,+\,1g\,\,\rightarrow\,\,t mutation causes a RP-like phenotype. Based on the greater severity of the RP-like phenotype compared with CRD-like phenotype and STGD the IVS30\,+\,1g\,\,\rightarrow\,\,t mutation can be regarded as a true null allele. A mutation at the +1 position of a 5' splice site invariably inactivates the corresponding splice site. The possible effects of this mutation are discussed in more detail elsewhere.\textsuperscript{22} In accordance with our hypothesis we and others thus far have not found two \textit{ABCR} null mutations in STGD patients\textsuperscript{2} (J. Kaplan, personal communication; own observation). In addition, Martinez-Mir \textit{et al} recently identified a homozygous 1-bp deletion in the \textit{ABCR} gene of six siblings from a consanguineous RP family.\textsuperscript{113} This deletion results in a frameshift early in the coding region and thus represents a true null allele. The IVS40\,+\,5g\,\,\rightarrow\,\,a mutation lowers, but prob- ably does not abolish, the splice potential of the corresponding splice site, which may explain the less severe phenotype observed in the compound heterozygous CRD-like patients.

The fact that both CRD-like and RP-like phenotypes can be caused by mutations in the same gene is not without precedent. Previously, mutations in the RDS/peripherin gene were associated with a myriad of different phenotypes, including autosomal dominant forms of RP,\textsuperscript{17} CRD,\textsuperscript{18} macular dystrophy,\textsuperscript{19} pattern dystrophies of the retinal pigment epithelium,\textsuperscript{20, 21} and central aracnel choroidal dystrophy.\textsuperscript{22, 23}

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