A Population-Based Epidemiological and Genetic Study of X-Linked Retinitis Pigmentosa

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PURPOSE. To perform a nation-wide elucidation of the prevalence and the mutation spectrum in X-linked retinitis pigmentosa (XLRP), and to make genotype–phenotype comparisons.

METHODS. The study comprised 96 affected males and 149 female carriers from 42 families representing all identified XLRP individuals in the Danish population (5.4 million inhabitants). RPGR and RP2 were screened for mutations in 34 families, the medical files of the patients were scrutinized, and phenotype data were extracted.

RESULTS. The prevalence of affected males was estimated to be 1.26 per 200,000 and 1.18 per 1,000,000 of female carriers. A rough estimate, however, indicates that the real prevalence of affected males was approximately 1:15,000. The cumulated life risk of developing of XLRP in carriers was strongly age dependent and included one third of the carriers older than 60 years. Molecular analysis of RP2 and RPGR uncovered 28 different mutations in 33 of 34 index cases analyzed. Twelve patients carried a mutation in RP2, 12 in exons 1 to 14, and 9 in open reading frame (ORF) 15 of RPGR. Males with RP2 mutations tended to have higher degrees of myopia, lower visual acuities, and more preserved visual fields than did males with RPGR mutations at the same age. No significant differences in phenotype were found in age of onset and type of mutation in either RP2 or RPGR.

CONCLUSIONS. A very high mutation detection rate in familial cases makes genetic testing a valuable clinical tool for genetic counseling and prenatal testing. The proportion of RP2-mediated XLRP in the Danish population is higher and the proportion of RPGR-ORF15 is lower than reported in other studies. Thus, strategies for diagnostic procedures should take into account the population-specific mutation spectrum. (Invest Ophthalmol Vis Sci. 2007;48:4012–4018) DOI:10.1167/iovs.07-00071

Retinitis pigmentosa, the most common cause of inherited blindness, has an overall prevalence of 1:3000 in the Danish population,1 comparable with prevalences in other study populations.2–5 The proportion of X-linked cases, however, varies significantly between different studies.4–10 Haim11 calculated 17% of the Danish RP cases to be X-linked, as opposed to 6% of the families, and also pointed out that the discrepancies between different studies may to some extent be ascribed to methodological differences. The pathogenesis of the disease involves a progressive degeneration of photoreceptors. To date, more than 35 genes involved in RP have been described (according to RetNet, Retinal Information Network, http://www.sph.uth.tmc.edu/Retnet/provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX). The X-linked pattern of inheritance (XLRP) is observed in approximately 10% to 15% of RP cases,6,12 and is generally associated with the most severe course of the disease. In the first or second decade of life, affected males experience night blindness and restriction of visual fields, and the disease often leads to complete blindness in the fourth or fifth decade. Female carriers are usually not affected, but can sometimes be identified by the detection of a so-called tapetal reflex or peripheral pigmentary changes in the retina. Most X-linked diseases show either recessive or dominant inheritance. In XLRP, however, some families are characterized by incomplete or partially penetrant dominant inheritance, female carriers being almost as severely affected as males, even though the age of onset seems to be later.13–15 Although five loci have been proposed in XLRP, more than 90% are caused by mutations in the RPGR and RP2 genes.16,17 Previous studies performed in European and North American XLRP cases18–20 detected mutation frequencies of 85% to 90% in the RPGR gene and 8% to 15% in the RP2 gene.

Numerous splice variants of RPGR transcripts have been described in different tissues. One of these transcripts, expressed in the retina, includes exon 1 to 14 and the 3′-terminal ORF15.21 All mutations identified so far affect this transcript. As 50% to 80% of RPGR mutations have been found in open reading frame (ORF) 15 (size, ∼1.7 kb) versus 20% to 35% of mutations in the exons 1 to 14 (size ∼1.8 kb), ORF 15 is said to be a mutational hotspot.18–21

Despite a broad overlap in phenotype between males with RP2 and RPGR mutations, statistically significant differences have been established. Sharon et al.20 showed that patients with RP2, at the same age, had lower visual acuities on average than did RPGR patients. Among patients with RPGR mutations, those with ORF15 mutations had a larger visual field area than those with mutations in exons 1 to 14. Furthermore, the length of ORF15 wild-type sequence in patients with truncating or frame-shift mutations was proportionate to the remaining dark adaptation and ERG amplitude, and the length of the abnormal ORF amino acid sequence created by frame-shift mutations had the opposite effect.

In 1988, a national epidemiologic investigation evaluated the prevalence of RP in Denmark. Among 974 families, 24 XLRP families were assessed.12 Before the present study, the mutation was identified in seven Danish families with XLRP,22,23 and the phenotype in part of family RP200322 was described.24 Since 1988, 18 additional XLRP families have been collected, leading to a final number of 42 families. Herein we provide the molecular characterization of so far uncharacter-
ized XLRP families and give an overview of all known cases in Denmark.

METHODS

Families
The study used medical files from patients and relatives recorded at the National Eye Clinic for the Visually Impaired (NEC). A national RP register kept at NEC since 1990 is based on voluntary reports from patients and/or ophthalmologists in private practice or departments of ophthalmology. In addition to the electronic registration a medical file is established on every patient examined at the NEC (primary cases) or reported (secondary cases), and existing ophthalmic reports are collected from other ophthalmologists. Family information was retrieved from a national register on hereditary eye diseases at the NEC. Part of the material was included in a comprehensive epidemiologic analysis of retinitis pigmentosa in the Danish population. Patients were selected on the basis of pedigree information including 38 families, which were classified into six groups based on a modification of the criteria of Haim and Bader et al., no father-to-son transmission, no transmission through unaffected males, and at least one of the following events: (1) at least two affected males in two generations connected by at least two females (n = 2), (2) at least two affected males in two generations connected by a single female (n = 2), (3) at least two affected maternal male cousins (n = 2), (4) two affected brothers only (n = 2), (5) affected maternal half-brothers with unaffected and unrelated fathers (n = 3), (6) An affected male and carrier signs in mother, daughter, and/or sister. In addition, four families that did not fulfill any of these criteria were included based on clinical findings such as an affected female with late-onset RP and a son with early onset (n = 3) and an affected female with late onset and carrier signs in a daughter (n = 1).

Some of the Danish families have been subjected to studies before the identification of the RP2 and RPGR genes. Family RP200310 was described in 1968 and fluorometric studies were performed on the carriers. Linkage studies were performed on family RP200309 and random X chromosome inactivation was discussed based on a carrier study in family RP200316. Population counts according to January 1, 2006, and data on live births during the period from 1900 to 2000 were provided from Statistics Denmark. The vital status of all included persons was updated through The Danish Civil Registration System kept by the Ministry of Interior Affairs and Health.

The following data were extracted from the files of live affected males: family, age of onset, age, visual acuity (VA), refractive value in diopters (D) (spherical [sph], astigmatism [cyl]), spherical equivalent value (eq), and visual field group at last examination. Visual fields (mainly based on Goldmann perimetry with a large object, IV-4e or V-4e) were grouped in a semiquantitative way as follows: 0, normal fields; 1, slightly peripheral constriction or central scotomas; 2, ring scotoma occupying one hemiﬁeld or less; 3, ring scotoma affecting both hemiﬁelds; 4, tunnel vision of 10° or less with peripheral islands; and 5, tunnel vision of less without peripheral islands.

If available, data on age at examination, VA, refractive values, ERG, and visual fields from all examined female carriers were recorded. In addition, all carrier females were characterized according to family and status with regard to being obligate/nonobligate and affected or not, and finally we adopted the grading system of morphologic retinal findings presented by Grover et al.: 0, no changes; 1, tapetal reflex only; 2, regional, peripheral pigmentary changes affecting less than 180°; and 3, diffuse peripheral pigmentary changes with or without macular involvement. Arbitrarily, a female was considered affected if two or more of the following criteria were fulfilled: fundus grade 2 or 3, best corrected VA reduced below 0.5 due to RP, dark-adapted threshold elevated by 2 log units or more, visual fields defects, and/or dark adapted rod response reduced by 50% or more.

The phenotypic data were extracted from the files without prior knowledge of genotype and were analyzed with respect to RP2 and RPGR mutation groups. The project adhered to the tenets of the Declaration of Helsinki, and all participants gave informed, written consent to participate.

Mutation Screening of the RP2 and RPGR Genes
DNA was extracted from peripheral leukocytes by using standard protocols. For PCR amplification oligonucleotide primers were designed for the entire coding region of the RP2 and RPGR genes. Primer sequence and conditions for PCR are available on request.

PCR products were purified with DNA purification kits (Macherey and Nagel, Düren, Germany). Sequencing of the sense and antisense strands was performed with the same primers as for PCR amplification (BigDye Terminator, ver. 3; and a model 3100 Genetic Analyzer; Applied Biosystems [ABI], Naerum, Denmark). ORF15 screening was performed as described previously. For molecular characterization of the 15.2kb deletion in the RP2 gene, longerage PCR was performed with a kit by Stratagene (Aarhus, Denmark).

Functional Analysis of the Potential Splice Variants
RNA was isolated from blood samples using an RNA collection tube (PAXgene) and an RNA isolation kit (both from Qiagen, Albertslund, Denmark). Full-length first-strand cdNA was prepared with random hexamer primers. RPGR transcripts were amplified using primers located in exons 12 and 17 of the RPGR gene followed by nested PCR with primers located in exons 13 and 16. RP2 transcripts were amplified by primer located in exons 1 and 5. Primer sequence and conditions for PCR are available on request.

RESULTS

Epidemiology
We identified 42 families with familial XLRP including 96 live affected males 5 to 99 years of age and 149 female carriers aged 0 to 99 years. The corresponding age- and sex-specific prevalence rates according to January 1, 2006, of diagnosed and reported males with XLRP and female carriers were 1:26,200 and 1:18,000 respectively (Table 1). The prevalence of XLRP in relation to the whole population (5.4 million) was 1:44,000 (21 affected females included).

Table 2 shows the cumulative life risk for development of XLRP in 10-year birth cohorts during the last decennium. The risk of development of RP in carriers is obviously strongly age dependent, as no female born after 1970 was affected. In women born before 1971 (45 years and older) the risk increased by a factor of three to about one third among the eldest.

Molecular Genetics
Eight of the recognized 42 families did not participate in the genetic study, and in a further seven families, the mutation had been identified before this investigation (Table 3). Index patients from the remaining 27 families were included in the present study. Mutations were found in 33 of the 34 molecularly investigated families. One in-frame deletion of a serine at position 6 of the RP2 gene (p.S6del) was found in four apparently independent families. This finding prompted a supplemental genealogic study that revealed a common ancestry in all four families descending from a couple born in 1791 and 1801. A further two identical mutations were ascertained in two RP2 families (RP200307 and RP200308) and in two RP3 families (RP300304 and RP300318), reducing the total number of families with separate mutations from 33 to 28. Eight (29%) of 28 families carried a mutation in the RP2 gene and 20 (71%) of the 28 families carried a mutation in the RPGR (Table 3, Fig. 1). In nine cases, the mutation resided in ORF 15, and the
remaining mutations were located in exons 1 to 14 of the RPGR gene. These results differ from data in previous studies performed in Germany and North America, where a higher percentage of ORF15 mutations and a lower percentage of RP2 mutations have been described. 18–20

Among 28 different mutations detected in the Danish population, 25 were identified in a single family only and 17 (61%) of the 28 were novel, indicating that most mutations are very rare. The majority (82%) were loss-of-function mutations, which is consistent with previous findings. 20,32 The p.S66del leads to loss of membrane localization of the RP2 protein in HeLa cells. 33–35 In one patient, we detected a novel p.T255I missense mutation in RPGR, which is located distant from an exon was observed: a c.1573–3C>T transition (Fig. 2B). RT-PCR of the full-length transcript in blood of the patient revealed skipping of exon 4 of RP2, which was confirmed by sequencing. Skipping of exon 4 results in a frameshift with a stop codon introduced after five amino acids.

In another patient, exon 4 of the RP2 gene failed to be amplified. Multiple primers were designed for introns 3 and 4 in increasing distance from exon 4 until PCR amplification was successful in the patient’s DNA (Fig. 3). Long-range PCR with these primers revealed a 1.3-kb PCR product in the patient compared with the expected 16.5-kb PCR product in control subjects. By sequencing the breakpoints a 15,231-bp deletion was confirmed.

In a further patient, an insertion of an Alu element of the AluYb8 subfamily into the coding sequence of ORF 15 was detected. The 356-bp transposon contained a poly-A-tail of 28 adenines. The insertion created a nonsense mutation (data not shown).

### Phenotype–Genotype Correlation

Among 93 affected males, the age of onset varied between 3 and 45 years, with a median of 9 years. Within families the age of onset also varied, with a maximum difference of 30 years. No significant difference between RP2 and RPGR families was present. Six males belonging to the three families with missense mutations did not show a tendency toward late age of onset compared with those patients with nonsense or frameshift mutations. Some interfamilial variation, however, was exemplified by the family with the RP2 frame-shift mutation c.858delT, from which seven males had age of onset between 3 and 7 years (median, 4 years), as opposed to the RP2 p.s66del family from which nine males had age of onset between 4 and 44 years (median, 25 years).

Early-onset myopia was prevalent among the affected males. Because anisometropia was moderate (mean 0.68 D; range 0–3.25), only the right eye was used for comparison.

### Table 2. Cumulative Life Risk of Development of XLRP in 10-Year Birth Cohorts

<table>
<thead>
<tr>
<th>Birth Year Cohort</th>
<th>Population Live-Born Males*</th>
<th>Affected Males, n (n Alive)</th>
<th>Relative Risk 10,000 Males</th>
<th>Population Live-Born Females</th>
<th>Affected Carriers†</th>
<th>Unaffected Carriers‡</th>
<th>Percent Affected Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1901–1910</td>
<td>380,137</td>
<td>9 (0)</td>
<td>0.24</td>
<td>361,410</td>
<td>4</td>
<td>7</td>
<td>36</td>
</tr>
<tr>
<td>1911–1920</td>
<td>371,903</td>
<td>15 (0)</td>
<td>0.40</td>
<td>355,972</td>
<td>8</td>
<td>16</td>
<td>33</td>
</tr>
<tr>
<td>1921–1930</td>
<td>365,561</td>
<td>8 (2)</td>
<td>0.22</td>
<td>346,587</td>
<td>3</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>1931–1940</td>
<td>340,441</td>
<td>11 (5)</td>
<td>0.32</td>
<td>321,949</td>
<td>6</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>1941–1950</td>
<td>439,689</td>
<td>26 (21)</td>
<td>0.59</td>
<td>413,424</td>
<td>10</td>
<td>22</td>
<td>31</td>
</tr>
<tr>
<td>1951–1960</td>
<td>392,551</td>
<td>14 (14)</td>
<td>0.36</td>
<td>369,097</td>
<td>3</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>1961–1970</td>
<td>407,410</td>
<td>21 (20)</td>
<td>0.52</td>
<td>384,787</td>
<td>3</td>
<td>24</td>
<td>8</td>
</tr>
<tr>
<td>1971–1980</td>
<td>345,252</td>
<td>14 (14)</td>
<td>0.41</td>
<td>326,843</td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>1981–1990</td>
<td>286,150</td>
<td>10 (10)</td>
<td>0.35</td>
<td>271,129</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>1991–2000</td>
<td>345,889</td>
<td>10 (10)</td>
<td>0.29</td>
<td>327,765</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>2001–2000</td>
<td>3,692,983</td>
<td>138 (96)</td>
<td>0.37</td>
<td>3,567,852</td>
<td>37</td>
<td>150</td>
<td>19</td>
</tr>
</tbody>
</table>

* The numbers were not corrected for infant deaths.
† Among the affected and unaffected carriers, 11 and 34, respectively, had died.
One quarter of the 89 individuals with known mutations and known refraction had refraction of −6.00 D or more. Among 20 subjects with RP2 mutations, the median spherical equivalent was −4.75 D (range, +2.50 to −15.50) and 52 subjects carrying an RPGR mutation had a median of −2.88 D (range, +0.75 to −12.50). This finding is in contrast to the results obtained by Sharon et al.,20 who showed in a statistical analysis a tendency for less severe myopia in males with RP2 mutations, whereas only 8 (18%) of 45 in the RPGR families had values from 1 to 2 D. The study included 131 carriers from families with an identified mutation, 50 in RP2 families and 81 in RPGR families. Of interest, seven affected females with RP2 mutations were encountered. The fraction of RP2 families with affected females was 3 of 8, compared with 12 of 20 among RPGR families, and affected live carriers accounted for 8% of the carriers in RP2 families and 21% of the carriers in RPGR families.

Four of eight affected RP2 carriers belonged to a family with a missense mutation p.R118H that has been shown to abolish tubulin-GAP activity in the RP2 protein.36 The four females had RP2 mutations, as opposed to 11 (69%) of 16 with RP2 mutations in the milder visual field groups 2 and 3, irrespective of VA. Considerable intrafamilial variation in disease course was common. The RP2 del6S family represented the extremes: An affected male who at the age of 55 had a VA of 6/6 and normal visual fields for large objects had a refractive error of −2.50 to −2.88 D, whereas a 49-year-old who had a VA of hand movements and tunnel vision below 10° had an error of −14.00 D. The study included 131 carriers from families with an identified mutation, 50 in RP2 families and 81 in RPGR families. Of interest, seven affected females with RP2 mutations were encountered. The fraction of RP2 families with affected females was 3 of 8, compared with 12 of 20 among RPGR families, and affected live carriers accounted for 8% of the carriers in RP2 families and 21% of the carriers in RPGR families.

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years and by then had reduced VA of 6/36 and 6/60. The same family also counted two unaffected carriers examined at the age of 55 and 57 years. They were both low hypermetropic.

One of them had VA of 6/12 and 6/9 and tapetal fundus reflexes, whereas the other had VA of 6/60 in both eyes, fundus grade 0, normal ERG, and serious constriction of the visual fields, which was considered to be due to simulation. For comparison, two affected males from the \textit{RP2} p.R118H family examined, 17 and 27 years old, were moderately affected, with VA 6/60, refraction of \(-8.25\) and \(-2.00\) D, and visual fields of grade 3 and 2, respectively.

Two affected carriers with an \textit{RP2} p.Q26X mutation aged 40 and 50 years showed moderately constricted visual fields, peripheral RPE atrophy with numerous bone-spicule hyperpigmentations in four quadrants, and reduced ERGs. Two deceased women from the same family had also been affected according to history.

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The RP2 mutation p.L129FfsX10 was shared by a 46-year-old mother and her son. She had peripheral RPE atrophy and bone spicule hyperpigmentations in all quadrants and the ERG showed several reduced rod- and cone responses and prolonged cone implicit times.

Among the 12 families with affected carriers and RPGR mutations, seven frame-shifts or nonsense mutations were located in exons 1 to 14, whereas another five mutations were located in ORF 15. In the RP2 p.6del family, none among 16 carriers was affected. Two families with intronic mutations in RP2 and RPGR had no affected carriers.

Information of comorbidity in affected males was generally not included in the files. An audiologic investigation of an unselected RP cohort was performed in an earlier study. Haim also noted a male preponderance among elderly carriers, mainly affecting high frequencies, was noted in one patient from each of families RP300313, RP300316, and RP300320, with an RPGR deletion of exons 4 to 13, a frame-shift deletion in ORF 15, and a 6.4-kb deletion affecting ORF 15, respectively. In addition a dome-shaped audiogram with a 30- to 40-dB low-and high-frequency loss was found in a patient aged 34 years from family RP200315, who had a 15.2-kb RP2 deletion. Clefting of the lip and palate was present in a patient from family RP200310 with a frame-shift RP2 mutation.

**Discussion**

The present study was based on a nationwide registration of RP cases diagnosed over more than half a century. A few patients could not be identified in the Civil Registration System or had emigrated and were therefore excluded from the investigation. The appraisal of familial cases may be considered nearly complete, because of the presence of a single national diagnostic and rehabilitation clinic for the visually impaired and the compulsory registration of children with low vision. The same conditions favored the elucidation of a relatively high number of multigeneration families known for generations and allowed for the identification of X-linked families with apparent dominant transmission. Thus, the relative number of XLRP families in the Danish population has always been high. Still, the calculated prevalence of 1:26,200 males is a minimum value, because of the methodological limitations of unclassified affected males. Haim noted a male preponderance of 10 in 100,000 among sporadic RP cases aged 35 to 60 years and suggested that it partly was due to males with XLRP. The entire male population in this age interval was approximately 1 million. If the population remained in a steady state, it would mean a doubling of the prevalence of male cases. Breuer et al. found 29% of simplex RP males had RPGR or RP2 mutations. If the same statistic is applied to the Danish counts from 1981, approximately 60 prevalent cases should be added to obtain a more reasonable impression of the real number. Thus, a rough estimate of the true prevalence of XLRP is approximately 1:15,000. In addition, some small families were missed according to the inclusion criteria.

Haim also noted a female preponderance among elderly sporadic cases and hypothesized that it may represent heterozygotic late-onset cases. According to Table 2, approximately one third of the familial cases manifested RP after the age of 50 years, indicating a further increase in the estimated XLRP prevalence. The skew proportion between obligate and nonobligate carriers (111 vs. 38, Table 1) clearly demonstrates a deficit of ascertainment. Theoretically, the risk for carrier females to have heterozygotic female or hemizygotic male offspring is identical. Furthermore, mutation-carrying nonfamilial female cases without affected offspring are only identified in the presence of symptoms. Including male cases before RP onset, we estimate that at least 100 undiagnosed, asymptomatic, mutation-carrying individuals should be added to the observed cases. In conclusion, a final approximation point to a total load of at least 450 RP mutation-carrying X chromosomes (180 males and 250 females) among the 8,169,072 X chromosomes in the population is equivalent to a prevalence rate of 1:19,000. Accordingly, a more accurate account of the prevalence of XLRP mutations within a population should include RP2 and RPGR mutation screening strategies involving sporadic male cases as well as female cases of late-onset RP (after the age of 50 years).

Affected carriers occur in roughly half of both RP2 and RPGR families and in approximately one fifth of all carriers. The proportion of affected females is highly age-dependent as appears in Table 2 and may rise to approximately 50% among the elder generations. The small number of unaffected carriers examined prevents a firm conclusion on the proportion between carriers with and without tapetal reflexes. RP2 and RPGR carriers however seem to be equally affected as well.

Hearing impairment, mainly affecting high frequencies, was noted in one patient from each of families RP300313, RP300316, and RP300320, with an RPGR deletion of exons 4 to 13, a frame-shift deletion in ORF 15, and a 6.4-kb deletion affecting ORF 15, respectively. In addition a dome-shaped audiogram with a 30- to 40-dB low-and high-frequency loss was found in a patient aged 34 years from family RP200315, who had a 15.2-kb RP2 deletion. Clefting of the lip and palate was present in a patient from family RP200310 with a frame-shift RP2 mutation.

The retrospective nature of the clinical material imposes limitations on the investigation of phenotypes. First, the medical files were derived from a period of more than 50 years, representing the observations of several ophthalmologists and the use of many different examination techniques and instruments is making comparisons among cases difficult. Second, the number of examinations and observation periods varied from a single visit to many over several decades, and some patients had had their last examinations performed years ago. These conditions caused us to refrain from obtaining a measure of visual decline over time. Age of onset is an important clinical parameter in RP, but due to several reasons it was not easy to determine. In general, patients were unable to point out a certain age of onset. Night blindness, which most often was indicated as the initial symptom had, according to patients, been present “always” or “from birth on.” A subgroup noticed...
reduced VA as the first sign. The use of this information was complicated, however, by the early and frequent occurrence of myopia and astigmatism. A smaller group reported restricted visual fields. As the age of onset, we chose the age when visual symptoms were affecting daily life, despite optimal spectacle correction.

A cross-sectional analysis of refractive values, VA, and visual field category revealed some distinct differences related to the involved gene. Patients with RP2 mutations had higher degrees of myopia, a higher frequency of macular involvement as deduced from VA, and milder visual field defects compared with RPGR mutation carriers. These differences were not due to differences in age composition of the two groups, which included 26 RP2 patients with a mean age of 30.4 years (range, 14–55) and 55 RPGR patients with mean age 27.5 years (range, 5–53).

Acknowledgments

The authors thank the German National Genome Research Network (SMP-DNA: 01GR0411) for providing access to the genotyping platform.

References