Contribution of Mutation Load to the Intrafamilial Genetic Heterogeneity in a Large Cohort of Spanish Retinal Dystrophies Families

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Purpose. The aim of this study was to deepen our knowledge on the basis of intrafamilial genetic heterogeneity of inherited retinal dystrophies (RD) to further discern the contribution of individual alleles to the pathology.

Methods. Families with intrafamilial locus and/or allelic heterogeneity were selected from a cohort of 873 characterized of 2468 unrelated RD families. Clinical examination included visual field assessments, electrophysiology, fundus examination, and audiogram. Molecular characterization was performed using a combination of different methods: genotyping microarray, single strand conformational polymorphism (SSCP), denaturing high pressure liquid chromatography (dHPLC), high resolution melt (HRM), multiplex ligation-dependent probe amplification (MLPA), Sanger sequencing, whole-genome homozygosity mapping, and next-generation sequencing (NGS).

Results. Overall, intrafamilial genetic heterogeneity was encountered in a total of 8 pedigrees. There were 5 of 873 families (~0.6%) with causative mutations in more than one gene (locus heterogeneity), involving the genes: (1) USH2A, RDH112, and TULP1; (2) PDE6B and a new candidate gene; (3) CERK1 and CRB1; (4) BBS1 and C2orf71; and (5) ABCA4 and CRB1. Typically, in these cases, each mutated gene was associated with different phenotypes. In the 3 other families (~0.35%), different mutations in the same gene (allelic heterogeneity) were found, including the frequent RD genes ABCA4 and CRB1.

Conclusions. This systematic research estimates that the frequency of overall mutation load promoting RD intrafamilial heterogeneity in our cohort of Spanish families is almost 1%. The identification of the genetic mechanisms underlying RD locus and allelic heterogeneity is essential to discriminate the real contribution of the monoallelic mutations to the disease, especially in the NGS era. Moreover, it is decisive to provide an accurate genetic counseling and in disease treatment.

Keywords: mutation load, intrafamilial heterogeneity, retinal dystrophies

Inherited retinal dystrophies (RD) are a group of diseases characterized by a progressive degeneration of photoreceptors and retinal pigmented epithelium cells (RPE) leading to visual impairment. Of all types of inherited RD, retinitis pigmentosa (RP; MIM 268000) is the most prevalent subset, with a prevalence of 1:4000 people.1 It is characterized by primary degeneration of rod photoreceptors. Typically, night blindness is the first symptom of the disease, followed by a loss of peripheral vision and, in most of the cases, cone degeneration in the late stage. Leber congenital amaurosis (LCA; MIM 204000), with a prevalence of approximately 1:30000,2 is the earliest and most severe form of inherited RD and is responsible for congenital impaired vision or blindness. Congenital nystagmus and a nonrecordable electroretinogram (ERG), before 1 year of life, are frequent signs of the disease, which may be accompanied by sluggish or absent pupillary reaction and eye poking. Cone–rod dystrophy (CRD; MIM 120970), with a prevalence of approximately 1:35000,3 is characterized by primary cone dysfunction in the early stage and subsequent rod degeneration. Clinical manifestations include photoversion, reduced visual acuity, color vision defects, and paracentral scotomas. Nystagmus may be present in some cases. Within macular dystrophies (MD), Stargardt disease type I (STGD1; MIM 248200) is the most common
The tremendous heterogeneity of this group of diseases makes the genetics of RD really complicated. Multiple findings underscore this heterogeneity: Different mutations in the same gene may cause different phenotypes (the PRPH2 gene involved in adRP and adMD, ABCA4 in arCRD and STGD, USH2A in arRP or Usher type II, RPGR in xLDR and xLMR, RD due to mutations in the same gene may display different inheritance patterns (autosomal dominant or recessive due to mutations in the RP1, CRX, and NR2E3 genes), and the same mutation may exhibit intra- or interfamilial phenotypic variability (mutations in the BEST1 and PRPF31 genes). Moreover, to our knowledge genotype-phenotype correlations have not been fully established yet.

This scenario shows the real challenge linked to the molecular characterization of RD. The high frequency of RD variants random carriers has been widely described. The aim of this article is to know the basis of RD intrafamilial locus and allelic heterogeneity to further discriminate the contribution of the monoallelic mutations to the pathology.

### Patients and Methods

#### Patients

Patients clinically diagnosed with RD were recruited from the Fundacion Jimenez Diaz Hospital (Madrid, Spain). The study was reviewed and approved by the Ethics Committee of the hospital, and adhered to the tenets of the Declaration of Helsinki and further reviews. Informed consent was obtained from all subjects before their participation in this study.

We included 2468 nonsyndromic and syndromic unrelated Spanish families with RD who had been studied according to the molecular methods described below; 873 of them (55.4%) were fully characterized. They were distributed in 354 (40%) of MD, 308 (35%) of RP, 107 (12%) of syndromic RD, 52 (6%) of LCA, and 52 (6%) of CRD cases.

#### Clinical Evaluation

Diagnosis of RD was focused mainly on measurements of visual acuity (VA) and visual field (VF) tests, fundus examination, and ERG responses. Differential diagnosis of RP, LCA, CRD, MD, STGD, and syndromic RP forms (Usher type II and Bardet-Biedl syndromes) was determined conforming to their respective mode of inheritance (genetic classification was performed according to the study of Ayuso et al.17 and the clinical criteria described in multiple studies.2,18-22

#### Molecular Analysis

Molecular characterization of the RD families was performed by combining the following genotyping tools: APEX technology-based commercial genotyping chip (Asper Biotech, Tartu, Estonia)2,12,15,23,24; direct mutational screening by denaturing high pressure liquid chromatography (dHPLC), single-strand conformational polymorphism (SSCP), high resolution melt (HRM), multiplex ligation-dependent probe amplification (MLPA), or Sanger sequencing; indirect analysis by microsatellites, whole-genome homoyzosity mapping using high-resolution commercial single nucleotide polymorphism (SNP) arrays from Affymetrix (Santa Clara, CA, USA) or Illumina (San Diego, CA, USA); or next-generation sequencing (NGS) technologies using 2 targeted RD gene panels, including more than 70 genes, or by whole exome sequencing (WES).14,27,28

All identified variants were annotated according to the nomenclature recommendations of the Human Genome Variation Society. To predict the potential impact of the variants on protein function, missense mutations were analyzed by bioinformatics programs, including Sorting Intolerant from Tolerant (SIFT, available in the public domain at http://sift.jcvi.org) and Polymorphism Phenotyping v2 (Polyphen-2; available in the public domain at http://genetics.bwh.harvard.edu/pph2). The effect on splicing of the variants identified was analyzed by different softwares: Analyzer Splice Tool (AST; available in the public domain at http://ibis.tau.ac.il/ssat/SpliceSiteFrame.htm) and Berkeley Drosophila Genome Splice Site Prediction (BDGP; available in the public domain at http://www.fruitfly.org/seq_tools/splice.html). All changes were checked by Sanger sequencing, and segregation of the potentially pathogenic mutations was confirmed in all cases within the family and with the pathology.

#### Selection of Cases

Among the 873 fully characterized families, we searched for diverse mechanisms of intrafamilial genetic heterogeneity, including disease-causing mutations in more than one gene within the same family (locus heterogeneity) and/or different disease-causing mutations in the same gene within the same family (allelic heterogeneity).
RESULTS

Disease-Causing Mutations in More Than One RD Gene in the Same Family

We specifically described 5 of the 873 fully characterized families (0.6%) from our cohort, in which more than one RD gene were segregating within the same family. The initial clinical examination demonstrated distinct phenotypes (macular versus peripheral forms, early-onset versus congenital versus late-onset forms) that, in all cases except one, were clearly differentiated between patients within the family.

Family RP-0184 is a very large pedigree with 7 different branches and inbreeding events, in which 5 of the subfamilies were studied (Fig. 1). All members of this family were from a particular area of central Spain. The affected members of 2 of the subfamilies (VI:6, subfamily 1 and VI:10, subfamily 7) presented a clearly distinct phenotype with bilateral sensorineural hearing impairment along with typical symptoms of RP, characteristics of Usher II form (Table 2). Direct sequencing of the USH2A gene revealed that these individuals were compound heterozygotes for different mutations in the USH2A gene: p.Glu767Serfs*21/p.Arg303His (individual VI:6, subfamily 1) and p.Glu767Serfs*21/p.Cys3425Phefs*4 (individual VI:10, subfamily 7). These findings were consistent with the phenotype (Table 2). In all other branches, the affected individuals presented an early-onset RP phenotype. In the subfamily 3, the genotyping chip revealed the p.Tre49Met mutation in homozygosis in the RDH12 gene in the proband (VII:1). In the other branch (subfamily 6), still uncharacterized by conventional methods, we tested mutations in the proband, who had a very similar early-onset RP phenotype, with a NGS RD resequencing gene panel. Thus, we found a mutation in a third additional gene to be segregating in the family. The novel variant p.Arg149Trp was found in homozygosis in the TULP1 gene. This variant was predicted as highly pathogenic after in silico analysis, it is located in a very highly conserved region and it was discarded in 150 control alleles. The change was carried in the proband and in his affected sibling, and segregated with the pathology and exclusively within this subfamily (Table 2). It is not ruled out that other genes also would be involved in this family, as there still is one branch in study in which any contribution from these 3 genes was excluded, remaining yet molecularly uncharacterized.

Figure 1. Pedigrees of the RD families with disease-causing mutations in more than one RD gene within the family. The proband is marked by an arrow in each case. The genotype of each affected member is represented below the individual symbol, being “m, m1, m2, m3, and m4” the different mutated alleles and “?” individuals uncharacterized yet. All the variants were confirmed to be exclusive of each particular subfamily, being excluded in the rest. (A) Disease-causing mutations in three different genes within this family: USH2A, segregating with Usher II syndrome, and RDH12 and TULP1 with an EORP. NS, nonstudy. (B) Mutations in the PDE6B and in a new candidate gene were found in the 4 affected siblings with an EORP phenotype. (C) One mutation in the CERKL gene and a combination of distinct CRB1 alleles was found in this pedigree, causing different phenotypes within the affected members in the family. (D) Mutations in the C2orf71 and BBS1 genes were found within this family, segregating with their RP and Bardet-Biedl syndrome phenotypes, respectively. (E) Mutations in the CRB1 and ABCA4 genes segregating with LCA and STGD phenotypes, respectively, in the same family.
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<thead>
<tr>
<th>Family</th>
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<th>ID</th>
<th>Gene</th>
<th>Mutations</th>
<th>First Symptoms and Course</th>
<th>Age of Ophthalmic Evaluation, y</th>
<th>BCVA RE/LE</th>
<th>Visual Field RE/LE</th>
<th>ERG</th>
<th>Fundus Aspect</th>
<th>Additional Findings</th>
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<tbody>
<tr>
<td>RP-018i</td>
<td>Subfamily-1</td>
<td>VI:6</td>
<td>USH2A</td>
<td>p.E767S<em>21/p.C3425Ffs</em>4</td>
<td>NB, diminished VA (22 y), diminished VF (20 y)</td>
<td>32</td>
<td>ND</td>
<td>10'/10'</td>
<td>ND</td>
<td>ND</td>
<td>Cataract 55 y, progressive bilateral sensorineural hearing impairment</td>
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<tr>
<td>Subfamily-3</td>
<td>III:1</td>
<td>RDH12</td>
<td>p.T49M/p.T49M</td>
<td>32 NB (3 y), diminished VA (5 y), diminished VF (3 y)</td>
<td>15 0.7/0.2 Inferior nasal scotoma with reduced sensibility in the remaining field</td>
<td>Diminished Pale optic disc, retina vessels attenuation and bone spicule pigmentation Macular alteration</td>
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<tr>
<td>Subfamily-6</td>
<td>VI:16</td>
<td>TULP1</td>
<td>p.R419W/p.R419W</td>
<td>Novel NB (4 y), diminished VA (4 y), diminished VF (4 y)</td>
<td>36   IP/IP Macular unstructured and atrophy in left macula</td>
<td>Cataract (30 y), nystagmus</td>
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<tr>
<td>Subfamily-7</td>
<td>VI:10</td>
<td>USH2A</td>
<td>p.E767S*21/p.R305H</td>
<td>NB (25 y), diminished VA (25 y), diminished VF (36 y)</td>
<td>54   0.6/0.5 ND Normal hearing acuity (15 y)</td>
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<td>RP-1712</td>
<td>II:2</td>
<td>PDE6B</td>
<td>p.Q298*/p.Q298*</td>
<td>NB (7 y), diminished VA and VF</td>
<td>65 0.4/CF 1m Pale optic disc, retina vessels attenuation and bone spicule pigmentation covering the entire retina</td>
<td>Cataract, ocular hypertension</td>
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<tr>
<td>II:4</td>
<td>PDE6B</td>
<td>p.Q298*/p.Q298*</td>
<td>NB (7 y), diminished VA and VF</td>
<td>67 0.6/CF Pale optic disc</td>
<td>Cataract (21 y), glaucoma</td>
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<tr>
<td>II:5</td>
<td>New candidate gene</td>
<td>p.Q298*/p.Q298*</td>
<td>NB (8 y), diminished VA andVF</td>
<td>51 0.1/0.4 Severe scotoma</td>
<td>Pale optic disc</td>
<td>Gataract</td>
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<tr>
<td>II:6</td>
<td>PDE6B</td>
<td>p.Q298*/p.Q298*</td>
<td>NB (9 y), diminished VA and VF</td>
<td>54 0.5/0.25 10'/10' Pale optic disc, dispersed bone spicule pigmentation</td>
<td>Gataract (25 y)</td>
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<tr>
<td>MD-0092</td>
<td>Subfamily-1</td>
<td>IV:1</td>
<td>CERKL</td>
<td>p.R257*/p.R257*</td>
<td>NB (30 y), diminished VA (16 y) and VF (28 y)</td>
<td>36 0.2/0.2 Central scotoma Pathologic flash both eyes</td>
<td>Pale optic disc, slightly retina vessels attenuation and extensive RPE macular atrophy well demarcated</td>
<td>Photosensitivity (16 y)</td>
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<tr>
<td>Subfamily-2</td>
<td>III:4</td>
<td>CRB1</td>
<td>p.11674G169del/p.C948Y</td>
<td>NB (40 y), diminished VA (11 y) and VF (11 y)</td>
<td>70 CF 3 cm/CF 5 cm Absolutescotoma</td>
<td>General RPE and macular atrophy</td>
<td>Gataract, photophobia, hypermetropia, astigmatism</td>
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<tr>
<td>Subfamily-3</td>
<td>III:6</td>
<td>CRB1</td>
<td>p.C948Y/p.C948Y</td>
<td>NB (6 y), diminished VA (5 y) and VF (6 y)</td>
<td>59 LP/amaurotic ND</td>
<td>Gataract (LE), ocular hypertension RE, nystagmus (from born)</td>
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<tr>
<td>Family</td>
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<tr>
<td>RP-0622</td>
<td>III:1</td>
<td>RP-0622</td>
<td>C2ORF71</td>
<td>p.I210F/p.I210F</td>
<td>25</td>
<td>NB (18 y), diminished VA (25 y) and VF (26 y)</td>
<td>27</td>
<td>0.4/0.1</td>
<td>Absolute scotoma RE</td>
<td>Abolished</td>
<td>Pale optic disc, retina vessels attenuation and bone spicule pigmentation, macular unstructured and atrophy in left macula</td>
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<tr>
<td>II:7</td>
<td>BBS1</td>
<td>II:7</td>
<td>p.M390R/p.M390R</td>
<td>38</td>
<td>NB (3 y), diminished VA (3 y) and VF (3 y)</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Polydactyly, intellectual disability</td>
</tr>
<tr>
<td>RP-0280</td>
<td>II:1</td>
<td>RP-0280</td>
<td>ABCA4</td>
<td>p.N1805D/p.N1805D</td>
<td>39</td>
<td>No NB or restriction of VF, loss of VA</td>
<td>26</td>
<td>0.1/0.1</td>
<td>No restriction</td>
<td>Slightly reduced amplitude for rod, mixed cone-rod, cone single flash, and cone flicker</td>
<td>Maculopathy with RPE atrophy, hyperpigmentation, few central yellowish flecks, slight temporal papillary pallor, no constriction of retinal vessels</td>
</tr>
<tr>
<td>II:4</td>
<td>CRB1</td>
<td>II:4</td>
<td>p.C948Y/p.W822*</td>
<td>57,56</td>
<td>NB (14 y), diminished VF (2 y), and reduction central VA (14 y)</td>
<td>14</td>
<td>0.1/0.2</td>
<td>Not discernible from noise anymore</td>
<td>Concentrically constructed with small remaining central and nasal islands (≤10°)</td>
<td>Roundish pigments distributed across the entire retina, including peripheral retina, posterior pole, and macular region</td>
<td>Hyperopia, astigmatism, and nystagmus</td>
</tr>
</tbody>
</table>

ID, identification; BCVA, best corrected visual acuity; OD, right eye; OS, left eye; NB, night blindness; ND, no data; NR, nonrecordable; CF, counting fingers; LP, light perception.
The consanguineous RP-1712 family (Fig. 1) has 4 affected siblings with very similar phenotypes in terms of age of onset and progression, suggestive of an early-onset RP (Table 2). The genotyping chip revealed the previously described p.Gln298* mutation in homozygosis in the PDE6B gene, in only 3 of the siblings (II:2, II:4, and II:6). This change was confirmed by Sanger sequencing. The other affected sibling (II:5) was heterozygous for the mutation and no second pathogenic allele was found. Other pathological variants in associated genes in this patient were excluded by further NGS targeted RD gene resequencing. Thus, homozygosity mapping and WES was performed allowing the identification of a pathological variant in homozygosis in a new candidate gene (manuscript in preparation).

Pedigree MD-0092 has 3 different branches with suspected inbreeding in one of them. This family presented various affected individuals of different generations with different phenotypes and age of onset (Fig. 1, Table 2). This change was confirmed by Sanger sequencing. The other affected sibling (II:5) was heterozygous for the mutation and no second pathogenic allele was found. Other pathological variants in associated genes in this patient were excluded by further NGS targeted RD gene resequencing. Thus, homozygosity mapping and WES was performed allowing the identification of a pathological variant in homozygosis in a new candidate gene (manuscript in preparation).

We included 2 additional families reported previously. One of these, described by Nishimura et al., 25 was the RP-0622 family, with 2 affected individuals of different generations and a consanguinity event (Fig. 1). Initial clinical findings led us to suspect the existence of intrafamilial heterogeneity due to the different segregation of nonsyndromic RD and extraocular symptoms in the affected members. The proband presented typical clinical features of RP as summarized in Table 2, while his maternal aunt (II:7) showed additional intellectual disability and polydactyly, dealing with characteristic symptoms of BBS. Homozygosity mapping and sequencing revealed causative mutations in two ciliary genes: C2orf71 in the proband and BBS1 in his aunt, consistently segregating with their respective phenotypes.

The RP-0280 family, with two affected siblings, was described previously by Riveiro-Alvarez et al. 29 The proband 2 patients revealed a combination of 2 common mutations in the CRB1 gene (Table 2). Patient III:6 with an early-onset retinitis pigmentosa (EORP) phenotype, carried the p.Cys948-Tyr in homozygosis, while her sister (III:4), who exhibited late-onset and slower progression, was compound heterozygous for the mutations p.Ile167_Gly169del and p.Cys948Tyr.

In all these 3 families with different branches, all the variants were confirmed to be exclusive of each particular subfamily, being excluded in the rest.
TABLE 3. Clinical Findings in Patients Showing Intrafamilial Variability due to Different Mutations in the Same RD Gene

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<tbody>
<tr>
<td>RP-0714</td>
<td>II:3</td>
<td>ABCA4</td>
<td>c.4253+4C&gt;T/ c.4253+4C&gt;T</td>
<td>45</td>
<td>NB (30 y), diminished VA (10 y), diminished VF (30 y)</td>
<td>40</td>
<td>&lt;0.1/&lt;0.1</td>
<td>Central scotoma</td>
<td>ND</td>
<td>ND</td>
<td>Photophobia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III:3</td>
<td>ABCA4</td>
<td>c.4253+4C&gt;T/ p.R1129L</td>
<td>43, 44</td>
<td>Diminished VA (22 y)</td>
<td>22</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Photophobia</td>
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</tr>
<tr>
<td>RP-0069</td>
<td>Subfamily-1</td>
<td>IV:3</td>
<td>CRB1</td>
<td>p.C9-485Y/ p.C9-48Y</td>
<td>37</td>
<td>NB birth, diminished VA (30 y), diminished VF (20 y)</td>
<td>48</td>
<td>Amaurosis</td>
<td>Absolute scotoma</td>
<td>NR</td>
<td>Difficult to evaluate due to leukoma</td>
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<td></td>
<td>Nystagmus, dense cataracts, corneal leukoma secondary to keratoconus, microphthalmus</td>
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<tr>
<td></td>
<td>Subfamily-2</td>
<td>V:2</td>
<td>CRB1</td>
<td>p.C9-485Y/ p.I1100T</td>
<td>37, 42</td>
<td>ND</td>
<td>21</td>
<td>0.1/0.2</td>
<td>&lt;5°</td>
<td>NR</td>
<td>Bone spicule pigmentation, pale papilla, constricted arterioles</td>
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<td>Nystagmus (7 m)</td>
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<tr>
<td>LCA0038</td>
<td>Subfamily-1</td>
<td>V:1</td>
<td>CRB1</td>
<td>p.C9-485Y/ p.I1001N</td>
<td>45, 23</td>
<td>Diminished VA</td>
<td>1.5</td>
<td>ND</td>
<td>Partially preserved central vision with reduced sensitivity in inferior VF</td>
<td>NR</td>
<td>Slightly pale optic disc, attenuation of retinal vessels, granular and grayish aspect of RPE, dense yellowish area in all macular region</td>
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<td></td>
<td>Nystagmus, photophobia</td>
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<tr>
<td>Subfamily-2</td>
<td>III:4</td>
<td>CRB1</td>
<td>p.D564Y/ p.I1001N</td>
<td>25</td>
<td>NB (3 y), diminished VA (3 y), diminished VF (3 y)</td>
<td>51</td>
<td>LP/LP</td>
<td>Almost absolute scotoma</td>
<td>ND</td>
<td>ND</td>
<td>Cataracts (40 y)</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Photophobia, hearing loss (55%), 56 y, diplopia (27°) corrected by vitamins, hypermetropia, astigmatism, subcapsular cataract both eyes</td>
</tr>
<tr>
<td>Subfamily-3</td>
<td>III:12</td>
<td>CRB1</td>
<td>p.I1001N/ p.Y1161C</td>
<td>23, 46</td>
<td>NB (45 y), diminished VA (40 y), diminished VF (52 y)</td>
<td>55</td>
<td>0.5/0.4</td>
<td>Annular scotoma</td>
<td>NR</td>
<td>ND</td>
<td></td>
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RD Intrafamilial Genetic Heterogeneity

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RD Intrafamilial Genetic Heterogeneity

As reported here, there is great variability in intrafamilial genetic heterogeneity in RD. One common heterogeneity mechanism is to find two different RD genes segregating in the same family. This could be explained by the high rate of coincidental carriers in the general population, as we described. This mechanism frequently occurs in the \textit{ABCA4} and \textit{CRB1} genes, which are highly involved in either locus and allelic heterogeneity in the Spanish population, as reflected in the present research, as well as in other populations. Moreover, dealing with extended families with multiple branches, like RP-0184 and LCA-0038, and/or with consanguinity events, such as RP-0622, RP-1712, RP-0184, and RP-0714 families, increases the probability of finding intrafamilial genetic heterogeneity events, as previously described. As we observed in the cases displayed, intrafamilial genetic heterogeneity often is accompanied by phenotypic heterogeneity. However, as observed in the RP-1712 family, it is not a requisite condition. In cases in which there are not remarkable differences in the phenotypic expression, no cosegregation may be indicative of the presence of heterogeneity events.

In the MD-0092 pedigree with genetic and phenotypic heterogeneity, we found a remarkable finding in \textit{CRB1}, one of the genes involved mostly in genetic heterogeneity. In one of the individuals (III:4), we found the “a priori” uncertain significant clinical p.Ile167_Gly169del variant. This change was reported in other five families from our cohort, always in combination with other \textit{CRB1} pathogenic allele, which may suggest to be an hypomorphic allele. From a large cohort of 873 fully genetically characterized Spanish families, we identified a total of 8 pedigrees in which mutational load contributes to intrafamilial heterogeneity, which represents a frequency of almost 1%. Other complementary studies will be necessary, including NGS techniques, which help us to estimate the real rate of mutational load promoting RD intrafamilial heterogeneity.

To our knowledge, this is the first time that a systematic research of RD intrafamilial heterogeneity has been done. This study is an essential step toward identifying the genetic mechanisms underlying RD to discern the real contribution of the individual pathological variants in the disease, especially in the NGS era, when mutant alleles not underlying the pathology may be found. The collection of these sets of genetic mechanisms and their frequency is important in establishing a better genotype-phenotype correlation and to provide accurate genetic counseling, since events, such as pseudodominance (in pedigrees RP-0622 and MD-0092) or ambiguous inheritance patterns, could be observed.

Although this kind of research must be performed for each particular population, the present study evidences the estimated frequency of overall mutation load, which contributes to RD intrafamilial heterogeneity in a large cohort of Spanish population. Furthermore, this is essential in patient management and especially in disease treatment, as locus and allelic heterogeneity represent a barrier to the improvement of therapies focused on correcting the primary genetic defect.

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References


