Genetically isolated populations

Implications for genetic care

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Chapter 4

Long-term follow-up of patients with Retinitis Pigmentosa type 12 caused by CRB1 mutations; a severe phenotype with considerable interindividual variability

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ABSTRACT

Purpose
To examine the long-term clinical course and variability in a large pedigree segregating CRB1 type autosomal recessive retinitis pigmentosa.

Methods
An observational case study of 30 patients with CRB1 type autosomal recessive retinitis pigmentosa, homozygous for the $CRB1$ c.3122T>C; p.(Met1041Thr) mutation from a Dutch genetically isolated population in which the $CRB1$ gene was originally identified. The authors evaluated medical records, analyzed a questionnaire, and performed a comprehensive ophthalmic examination, including optical coherence tomography.

Results
Mean follow-up was 19 years (range 0-45 years, SD 15 years). With aging, patients showed progressive visual decline, deterioration of visual fields, increasing narrowing of the anterior chamber, increased prevalence of cataract, and an increase in the amount of intraretinal pigmentations. Fifty percent of patients had a visual acuity of ≤0.3 at Age 18 and of ≤0.1 at Age 35. Electroretinogram responses were severely reduced or absent already at a young age and optical coherence tomography showed increased retinal thickness with often cystoid maculopathy at young age, and thinning of the retina and disorientation of the photoreceptor layer in the late stages. The clinical course showed considerable interindividual variability, but intraindividual similarity between both eyes was the rule.

Conclusion
The wide and variable clinical spectrum in patients with the same $CRB1$ mutation supports the hypothesis that the CRB1 type autosomal recessive retinitis pigmentosa–phenotype is modulated by other factors. The clinical variability will make it harder to evaluate the effect of (upcoming) therapies for retinitis pigmentosa, although because of the intraindividual similarity between both eyes, the contralateral eye can be used as an excellent internal control.
INTRODUCTION

Retinitis pigmentosa (RP) (MIM #268000) is a clinically and genetically heterogeneous group of retinal dystrophies with a worldwide prevalence of about 1 in 4000. Onset, progression, and severity of the disease vary highly. Many patients initially notice night blindness, and subsequently become gradually aware of their restricted visual fields. Typical clinical findings are bone-corpuscle-like pigment deposits, attenuated vessels, and a pale optic disc. Visual fields generally show defects in the midperiphery (ringscotoma), and eventually tunnel vision will occur with loss of central vision. Amplitudes of the electroretinogram (ERG) are reduced or absent. Many patients become legally blind in early or middle life. Retinitis pigmentosa can segregate in an autosomal dominant, autosomal recessive, X-linked, or mitochondrial linked fashion. Today, more than 50 different genes for isolated (nonsyndromal) retinitis pigmentosa have been identified, and, in part, their structural or biochemical role in vision has been elucidated.

We previously identified a large pedigree, segregating autosomal recessive RP with preserved para-arteriolar retinal pigment epithelium (PPRPE) (RP12) (MIM *604210, #600105). Subsequently, we found genetic heterogeneity for RP in the pedigree, and we identified at least 1 causative disease gene—crumbs1 (CRB1). The mutation segregating in the pedigree is a missense mutation (CRB1 c.3122T>C; p.[Met1041Thr] with reference cDNA NM_201253.2). In other branches of the pedigree segregating different RP-like phenotypes, CRB1 mutations were excluded.

The CRB1 gene is a human homologue of the Drosophila gene coding for crumbs (crb) and is expressed in the human retina and brain. It is a key regulator of polarity of epithelial cells including photoreceptors. Mutations in the CRB1 gene mostly cause severe retinal dystrophies such as Leber congenital amaurosis and RP with preserved para-arteriolar retinal pigment epithelium. Other phenotypes reported are pigmented paravenous chorioretinal atrophy (as a dominant condition) and isolated macular atrophy. CRB1 mutations explain on average 3% (0-6.5%) of all autosomal recessive RP cases and 9 to 13% of patients with Leber congenital amaurosis. Patients with retinal dystrophies resulting from CRB1 mutations may have additional features including hypermetropia, acute glaucoma, nanophthalmos, giant optic nerve drusen, preserved para-arteriolar retinal pigment epithelium, and Coats-like exudative vasculopathy. More than 150 CRB1 mutations have been reported. No clear genotype-phenotype correlation has been established.

In the recent years, progress has been made to develop experimental gene therapy for RP12/Crumbs1 in our laboratories in mice. Targeted ablation of the mice homologues Crb1 and Crb2 in mice and in retinal progenitor cells mimicked the Leber congenital amaurosis/RP phenotype. Proof of therapeutic principle was recently established by rescue of the RP phenotype in mutant mice lacking CRB1 and having reduced levels of CRB2, using adeno-associated viral vectors.
Although several studies have been published concerning the clinical characteristics of patients with \textit{CRB1} mutations,\textsuperscript{15,23,28} these studies lack clinical follow-up information over time. Detailed description of the natural clinical course is essential for evaluation of upcoming therapeutical possibilities for RP12/CRB1 retinal dystrophy. Because all patients in our study carry the same homozygous \textit{CRB1} mutation, this is a unique population to study the phenotypic variability in patients with the same mutation.

In this follow-up study, we added recent clinical data to the extensive historical data collected from the Dutch pedigree of patients with RP, in which the \textit{CRB1} gene was originally identified. Our aim was to study the clinical course of RP in patients with the same homozygous \textit{CRB1} mutation, during a long period of follow-up.

**PATIENTS AND METHODS**

**Study population**

From the large pedigree in which the \textit{CRB1} gene was originally identified, we selected those patients whose medical records contained at least the diagnosis RP, visual acuity, and a funduscopic examination. Also, (almost) completely blind patients were included in the study to avoid ascertainment bias. Furthermore, we selected patients with the homozygous c.3122T>C; p.(Met1041Thr) mutation in the \textit{CRB1} gene, originating from the same genetically isolated population, from the Delleman register for genetic eye diseases at the Academic Medical Center in Amsterdam. The study was approved by the Medical Ethics Committee of the Academic Medical Center and adhered to the tenets of the Declaration of Helsinki. All participants provided signed, informed consent for participation in the study and retrieval of medical records.

**Molecular genetic analysis**

We obtained blood samples and extracted DNA from peripheral blood lymphocytes by standard procedures. We analyzed Exon 9 of the \textit{CRB1} gene by polymerase chain reaction and direct Sanger sequencing. Primer sequences and reaction conditions are available on request. In addition, we tested 370 non-selected adult controls from the genetic isolate for the \textit{CRB1} mutation to determine the mutation frequency in this population. More extensive molecular genetic investigations in patients of our previous published pedigree with an RP phenotype without the homozygous \textit{CRB1} mutation were performed.

**Collection of historical data and clinical examination**

After obtaining informed consent, we collected historical clinical data from our own
archives and from the patients’ ophthalmologists, including as many measurements
of visual function through time as possible. We invited the patients to the clinic for
a questionnaire (Table 1) and complete ophthalmic examination. Visual acuity was
determined with Early Treatment Diabetic Retinopathy Study charts at a distance of 4
m and expressed in Snellen ratios. We performed direct and indirect ophthalmoscopy
and fundus photography after pupillary dilatation. Further ophthalmological examina-
tion consisted of determination of refraction (autorefractometer), color vision (Ishihara
Test for Color Blindness and Lanthony’s Desaturated 15 Test and Saturated 28 Test),
intraocular pressure (applanation tonometry or noncontact tonometry), visual fields
(Goldmann and Humphrey 10-2 perimetry), ERG, and optical coherence tomography
(Topcon Europe Medical B.V., Capelle a/d IJssel, The Netherlands). Cumulative risks
of visual loss were studied with Kaplan-Meier product-limit survival analysis, with low
vision (visual acuity ≤0.3) or social blindness (visual acuity ≤0.1) as outcomes.

RESULTS

Molecular genetic analysis
All RP12 patients were homozygous for the c.3122T>C; p.(Met1041Thr) mutation
in the CRB1 gene. Genetic testing of 370 non-selected adult controls from the genetic
isolate revealed no homozygous individuals and 15 heterozygous carriers of the CRB1
mutation, resulting in a carrier frequency of 4% in this region.
More extensive molecular genetic investigation of our previously published pedigree\textsuperscript{6,7} revealed that in 2 branches, a homozygous \textit{LRAT} mutation c.12delC; p.(Met5CysfsX53) causing a distinct RP phenotype segregates. This finding is in line with our previous finding that genetic and clinical heterogeneity existed in the Autosomal Recessive Retinitis Pigmentosa pedigree. In the current study, we focused on the clinical follow-up in those patients with a homozygous \textit{CRB1} mutation without \textit{LRAT} mutation.

\textbf{Identification and clinical examination of RP12 patients}

We included 30 patients in our current study. Of them, 21 patients were also examined in the original pedigree.\textsuperscript{7} We detected 9 additional patients with a homozygous \textit{CRB1} c.3122T>C; p.(Met1041Thr) mutation from the Delleman Register for Genetic Eye Diseases at the Academic Medical Center in Amsterdam. They originated from the same geographical area and we were able to genealogically link 6 of them directly to the pedigree (Figure 1). The other 3 patients (Patients 28, 29, and 30), are most likely linked although we could not demonstrate this. Nineteen patients (12 for follow-up and 7 who did not participate in previous studies) were examined, all by the same ophthalmologist (M.J.S.). Eleven other patients agreed that we could use recent available data from their own ophthalmologist, but refused re-examination by the investigators. The mean follow-up period was 19 years (range 0-45 years, SD 15 years). The clinical findings of all patients are summarized in Table 2.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{pedigree.png}
\caption{The simplified pedigrees of all patients with RP with confirmed \textit{CRB1} mutations and their relatives. The dotted lines indicate the connections with the other pedigrees.}
\end{figure}
### Age of diagnosis

The mean age of diagnosis was 7.6 years (range 0-28 years; SD 7 years). Most patients (25/30; 83%) were diagnosed in the first decade, 1 in the second decade, and 4 in the third decade of life.

#### Table 2. Baseline characteristics of the patients.

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The pedigree number corresponds to Figure 1. + = thoroughly examined; - = permission to use data, no extra visit to the ophthalmologist; NA = not applicable, because no recent visit to an ophthalmologist took place; M = male; F = female; CF = counting fingers; HM = hand motion; LP = light perception; NLP = no light perception; OD = right eye; OS = left eye; VA = visual acuity.
In total, 224 measurements of visual acuity in 29 patients were available, with a mean of 7.7 measurements (range 1-25) per patient. One patient was too young to determine the visual acuity.

Visual acuity was decreased in all patients, ranging from slightly (20/25; 0.8) to no light perception. Visual loss usually started in the first decade. In Figure 2, we show the clinical course of the best corrected visual acuity for each patient. Temporary plateau phases of vision were a common feature. They differed both in age and in visual acuity level at which they occur, as well as in duration. Kaplan-Meier survival curves (Figure 3, A and B) show that there is a 50% risk of reaching visual acuity ≤0.3 at Age 18 years and of ≤0.1 at Age 35 years. The course of visual acuity loss was significantly different (p<0.05) between individuals when analyzed for the whole patient group. Also the clinical course of visual acuity varied considerably between siblings. Sisters 14 and 15 showed the same clinical course, whereas their sister 16 and grandniece 13 showed a more rapid decline resulting in a best-corrected visual acuity of 20/250 (0.08) at Age 28, and 20/200 (0.1) at Age 7 years, respectively. Also Sister 9 and Brother 10 showed a considerable difference in the course of visual acuity, with low vision from early on in life in the first and...

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**Figure 2.** Course of visual acuity (VA) per individual. Best-corrected visual acuity (BCVA) of the best eye is plotted against the age at the time of measurement. Points connected by a line are different measurements on the same patient.
Figure 3. Kaplan-Meier survival curves showing the cumulative incidence of (a) visual acuity ≤0.30 and (b) visual acuity ≤0.10 in the better eye as a function of age.

(a) Twenty-four out of 29 patients reached a visual acuity of 0.30 or less. Four patients of whom it was unclear when their visual acuity dropped under 0.30 were left out. There is a 50% risk of reaching low vision by the age of 18.2 years (SD 2.1; 95% confidence interval, 14-22 years). Patients have been censored if they still have a visual acuity >0.30 at the latest measurement of visual acuity.

(b) Sixteen out of 29 patients reached a visual acuity of 0.10 or less. There is a 50% risk of reaching a visual acuity of ≤0.10 by the age of 35.0 years (SD 3.1; 95% confidence interval, 28-41 years). Patients have been censored if they still have a visual acuity >0.10 at the latest measurement of visual acuity. VA, visual acuity.
a good visual acuity at onset and a gradual decline in visual acuity in the second. The visual acuity loss of both eyes in each individual patient was very symmetrical. Reading disability was present in 10 patients at a mean age of 30 years (range 21-42 years, SD 7). Eleven other patients were able to read with special aids, and 5 patients without special devices. Three patients were still too young to read. No information was available for 1 patient.

Three patients (10%) had no light perception in 1 of their eyes at examination at Age 49, 52, and 62. At previous examinations at the ages of 38, 51, and 47 years, respectively, only hand motion vision or light perception had been present.

Ten patients had a continuous nystagmus, three had intermittent nystagmus. All patients with continuous nystagmus had a severe impaired vision of 20/250 (0.08) or lower and in most patients, only light perception or no light perception was present. The patients with intermittent nystagmus had a vision between 20/160 (0.125) and 20/125 (0.16).

**Refractive error; hypermetropia in all**

Refraction (at the last examination) ranged from +0.5 diopters (D) to +8.75 D, with a mean of +4.5 D. In 27 of 30 cases (90%) a refractive error of more than +2 D was present and in 9 of 30 cases (30%), the refractive error was more than +6 D. In 22 of 30 (73%) cases, difference of refraction between both eyes was 0.50 D or less, and in 29 of 30 cases (97%), 1 D or less. We found no correlation between age and refractive error.

**Night vision and photoaversion**

All patients (27/27, 3 data were missing) experienced problems with night vision in the first decade, and this subjectively worsened over the years according to 14 patients. Twenty-four of 29 patients (1 missing) suffered from photoaversion of whom 4 were severely affected. Only five patients claimed not to be hindered by bright light. Three of them were young children, and two other patients only had light perception.

**Visual fields**

Twenty-seven of 28 patients reported subjective visual field impairment at the last examination or in the past (in patients with only light perception). Two patients were too young to answer the question. In 1 patient who reported no problems in daily life due to diminished visual fields, only a small area of central vision remained on visual field testing. Perimetry was performed repeatedly in 27 patients. Three patients were too young for reliable visual field examination. Relative or absolute scotomas were observed in all 27 patients. All visual fields revealed concentric narrowing, leaving only central vision <10° in 13/27
patients. Visual fields showed progressive narrowing in time. However, considerable interindividual difference was present. The visual fields between both eyes were very similar.

**Ophthalmic findings: anterior chamber, iris, lens, and intraocular pressure**
The anterior chamber was described as shallow at the last examination in 16/30 (53%) patients. It tends to become increasingly shallow with aging. Similarity between the depth of the anterior chamber of both eyes was high. In only 1 patient the anterior chamber of one eye was described as shallow and in the other eye as normal. In all other patients, the anterior chamber of both eyes was similar (shallow or normal). Four patients had had laser peripheral iridotomy because of bilateral acute glaucoma at the age of 30, respectively unilateral acute glaucoma at the age of 35, 46, and 50 years. Prophylactic iridotomy was performed in the other eye in patients with unilateral glaucoma, but not in patients without a history of acute glaucoma.

Median intraocular pressure was 10 mmHg (range 6-19) and pressures were not statistically different between left and right eye, nor between the group with normal anterior chambers and the group with shallow anterior chambers (Kruskall-Wallis, P<0.01). None of the patients was using antiglaucoma drugs.

At the last examination in 17/30 (57%) patients, cataract was present (n=15) or cataract surgery had been performed (n=2). The type of cataract was posterior subcapsular cataract, anterior subcapsular cataract, or both. We combined these data with the data from earlier eye examinations. In the age group of 0 to 10 years, none (0%) of the 10 patients had cataract; at Age 10 to 20 years cataract was present in 1/10 (10%), at 20 to 30 years in 2/3 (67%), at 30 to 40 years in 5/10 (50%), at 40 to 50 years in 11/13 (85%), and at 50 to 70 years all 6 patients (100%) had cataract. Although this clearly illustrates the increase in prevalence of cataract in RP12 patients with aging, the individual age of onset of the cataract varied widely; for example, the oldest patient with no cataract was 41 years old and the youngest patient with cataract was 19 years old. In most patients, both eyes were similar in the presence, absence, severity, and type of cataract. In 3 (7%) patients the eyes were incongruent, showing (beginning) cataract in 1 eye and a clear lens in the other eye at Age 19 and 34, and 35 years.

**Ophthalmic findings: optic nerve, macula and periphery**
The age of onset and severity of optic nerve atrophy varied widely between patients. Optic nerve pallor was present in the majority of patients (20/30 = 67%) at the last examination. We combined these data with the data from earlier eye examinations. In the age group of
0 to 30 years, 10/22 patients (45.5%) showed no optic nerve pallor, 10/22 (45.5%) mild, 2/22 (9%) moderate, and 0/22 (0%) severe optic nerve pallor. In the age group of 30 to 70 years, these numbers were, respectively, 3/21 patients (14%) no, 9/21 (43%) mild, 5/21 (24%) moderate, and 4/21 (19%) severe optic nerve pallor. These data show the increase in prevalence and severity of optic nerve pallor with aging. The presence/absence and the amount of optic nerve pallor were very similar between both eyes.

(Giant) optic nerve head drusen were seen in 10 (33%) patients; bilaterally in 7 and unilaterally in 3 patients.

Fundus alterations varied between family members (Figure 4). Within core families, the older subjects did not typically have a more severe phenotype compared with younger family members, although all patients showed an increase in macular changes and pigmentations with age.

Macular abnormalities were present in all 28 patients above the age of 3 years (2 younger patients missing). Macular or foveal reflections were absent in 27 patients. One 7-year-old patient had normal reflections. Twelve patients had macular sheens, seven an atrophic appearance of the retinal pigment epithelium in the macular region, and two patients bull's eye maculopathy. The appearance of the macula was very similar between both eyes.

Retinal pigmentations varied from granulated pigment epithelium changes to confluent bone-spicule pigmentations. Patches of preserved retinal pigment epithelium around the vasculature were regularly seen. Peripheral pigmentations were present in 29/30 (97%) patients. The only patient in whom no peripheral alternations were seen was a 1-year-old boy. The extent and pattern of the peripheral pigmentations in both eyes were very similar.

At the last examination in 26/29 (90%) patients (1 missing), narrow retinal vessels were present. Only three young patients of 1, 3, and 7 years showed a normal vessel caliber. Tortuosity of retinal arterioles was present in 10/23 (43%). In all 6 patients below 16 years, tortuosity was absent. The caliber and presence or absence of tortuosity was very similar in both eyes of each patient.

Coats'-like disease with exudates was (unilaterally) seen in two patients. In one 23-year-old patient, a vasoproliferative tumor was seen, surrounded by massive hard exudates in the inferior temporal retina.

Retrospectively, the tumor was seen at the age of 16 years, although it was smaller at that time and hard exudates were not seen. The other patient also had a vasoproliferative tumor, exudates and hemorrhage in the inferior nasal retina at the age of 36 years. This was not present at the age of 17 years.

In younger patients, small yellow dots (drusenlike) were seen in the posterior pole, but these faded away with age. See Figure 4, A and C.
Electroretinography and optical coherence tomography

Electroretinogram was performed in 28 patients (at ages 3-48). In the two remaining patients, aged 1 and 3 years, no ERG was performed. In 21/28 patients, (mean Age 24 years) no responses could be measured in the initial (available) ERG. Five additional
Figure 5. Optical coherence tomography (OCT) of different patients at different ages. 
(a) Normal OCT of unrelated patient. (b) OCT of patient 24, OS, at Age 6 years, and (c) OCT of the same patient and eye at Age 11 years, showing severe cystic maculopathy at first with resolution of the cysts after longstanding use of acetazolamide; vision in this eye has remained stable at 20/32 (0.63) although the photoreceptor layer is partly missing and disorganized just outside the fovea. (d) OCT of patient 13, OS, at Age 7 years. The OCT shows severe cystoid macular edema with confluent cysts. (e) OCT of patient 9, OD, at Age 35 years the macular area is still recognizable and the thickness of the retina is near normal, but the photoreceptor layer is largely absent and there are a few small cysts. (f) OCT of patient 16, OS at Age 52 years; atrophic retina with loss of architecture. OD, right eye; OS, left eye.
ERGs (mean Age 11 years) had minimal responses from both cones and rods and 2 more ERGs (mean Age 5 years) had no measurable rod responses and minimal cone responses. Electroretinogram was repeated at least once in 10 patients (at ages 3-53), with the same result on all occasions—absence of measurable photopic and scotopic responses. In each patient, no differences in rod and cone responses between both eyes were observed. Optical coherence tomography was performed in 13 patients (ages 5-57 years) (Figures 5 and 6). In three patients, optical coherence tomography was repeated several times in a period of 5 years. Retinal thickness at the fovea ranged from 120 to 480 µm (mean 276 µm; SD 85.8 µm). Retinal thickness at the outer-Superior area and outer-Inferior area was grouped because of similarity and ranged from 156 to 457 µm (mean 322 µm; SD 77.0 µm). Both are significantly higher than the average retinal thickness in normal eyes (fovea: mean 234 µm; outer-Superior area: 257 µm; outer-Inferior area: 247 µm, Topcon 3D OCT series normative database). Mainly at a young age, the retinal thickness is increased, with generalized thinning of the retina in the whole posterior pole in the late stages. In the younger patients, the retinal layers were well delineated and the outer limiting membrane preserved. In the older patients, there was coarse lamination and disorientation of the photoreceptor layer. Cystoid macula edema was seen in 5 patients (all between the age of 5 and 35 years).

**Nonocular findings**

Four patients had psychiatric disorders (psychosis in two, eating disorder in 1, and anxiety in 1). Two patients had migraine. Nonocular health problems reported in single patients were breast cancer, familial adenomatous polyposis, multiple sclerosis, epilepsy, disc herniation, thyroid dysfunction, hypercholesterolemia, hypertension, and surgery for ureteropelvic junction obstruction. None of the patients had congenital hearing problems. Three patients reported tinnitus above the age of 50.

**DISCUSSION**

In this study, we describe the long-term follow-up of 30 patients with RP Type 12 originating from the same genetically isolated community. This cohort of patients is unique and gives the opportunity to study clinical expression of one homozygous *CRB1* mutation. These data can be useful for counseling patients about the long-term clinical course of this type of RP. It also helps to select certain groups for future (gene) therapy trials and to determine the window of opportunity in which treatment can be performed.
Figure 6. Mean retinal thickness of (a) the fovea and (b) the outer-Superior area and outer-Inferior area against age. Dots represent the values of the individual patients, solid lines the linear regression lines of these dots, and dashed lines the normal values stratified by age (Topcon 3D OCT series normative database). Outer-S, outer-Superior; Outer-I, outer-Inferior.
Long-term follow up of RP type 12

Age of onset
The onset of RP12 is relatively early, and visual acuity declined to 20/200 (0.1) around the third decade. At the same time, both the age of diagnosis and the clinical course were remarkably heterogeneous. The age of diagnosis varied from 0 to 28 years with a mean of 7.2 years, although this difference can also be caused by difference in familiarity with the disorder in both patients and physicians.

Phenotype
All patients showed the characteristic progressive symptoms of RP: night blindness, visual field defects, and loss of visual acuity. The characteristic fundus abnormalities previously associated with CRB1 mutations were present in the majority of patients, such as preservation of the peripheral retinal pigment epithelium, optic atrophy (in 69%), (giant) optic nerve head drusen or hamartomas (in 33%), tortuosity of retinal arterioles (in 43%) and Coats-like vasculopathy (in 7%). Hypermetropia was present in all patients. Electroretinograms became nonresponsive already at a young age (early teens). Optical coherence tomography showed increased retinal thickness with often cystoid maculopathy at young age, and thinning of the retina and disorientation of the photoreceptor layer in the late stages. Because 4 of the patients developed acute glaucoma, monitoring patients with RP12 (anterior chamber depth estimation, gonioscopy, and lens status evaluation) and timely prophylactic iridotomy are very important.

In the earlier report about this pedigree, numerous yellow round deposits/dots in the posterior pole were detected in a subset of the patients. In some studies, a decrease in the number of dots have been demonstrated in these patients. This is in accordance with our observation of vanishing dots with age in Patients 23 and 12 (see Figure 4, A and C). We did not look for the dots systematically, but we want to mention them as a helpful sign of RP12 in younger patients.

Previously, several authors described that less bone spicule formation occurs in RP12 than in other forms of RP and that nummular pigment deposits are common. We observed that intraretinal pigmentations can take diverse shapes and forms and that pigmentation gradually increases over the years. The course of visual acuity loss was significantly different (P<0.05) between individuals. Also, other clinical parameters, such as refractive error, visual fields, and ophthalmoscopic findings show a remarkable difference between individuals.
**Interindividual variability**

All patients in the current study are homozygous for the same mutation and yet have a very different age of diagnosis and clinical course. However, there was little intraintividual variation between the two eyes.

In previous studies, mostly including small pedigrees or individual RP12/CRB1 RP patients, no clear genotype phenotype correlation was established.\(^{14,15,25,24}\) It has been proposed that other modifying factors (genetic and/or environmental) affect the phenotype in patients with \textit{CRB1} mutations.\(^{14,15,28,32,33}\) There is some evidence that the involvement of multiple mutated alleles in different genes may affect the phenotype.\(^{15}\) These genes might encode proteins, which interact with CRB1 during development, or in terms of structure or function (CRB2; PALS1/MPP5, MPP4, MPP3, and MUPP1).\(^{24,25,34-37}\)

As the interindividual difference between the individuals with the same homozygous mutation is very high, studying genotype-phenotype correlations are expected to be fruitless.

The interindividual difference and intraintividual similarity found in the current study supports the hypothesis that the phenotype of patients with \textit{CRB1} mutations is modulated by other factors. All patients in the current study come from the same genetically isolated region and are expected to share more than average other genetic and environmental factors. This population is a unique population to identify genetic factors modifying the phenotype. In this population, pathogenic mutations in the \textit{LRAT}-gene and \textit{USH2A}-gene are also present. Heterozygosity of a mutation in one of these genes could be a factor aggravating the phenotype.

We have described the variability in phenotype in patients with one specific homozygous mutation. We do not know whether other mutations might produce so much phenotypic variability. Further research is needed to determine the interindividual variation in other mutations.

**Therapy**

The main goal of therapies for RP is to delay or reverse the course of RP. The variation in onset, progression, and severity of the phenotype will make it harder to evaluate the effect of yet to be developed therapies, in particular, in therapies in which both eyes are treated at the same time (such as vitamin therapy). On the other hand, all patients in our study showed a very high degree of clinical symmetry between their eyes. This interocular symmetry makes it possible to treat 1 eye and use the contralateral eye as internal control. The main outcome measures of therapies of RP are visual acuity and visual fields. Because visual acuity and visual fields are very consistent between both eyes, for
these parameters the untreated eye can be used as an excellent internal control. Moreover, because of the progressive nature and the intraocular symmetry, we suggest that other parameters can also be used as outcome measures, such as intraretinal pigmentation, optic nerve pallor, narrowing and tortuosity of the retinal vessels, cataract, retinal structure on optical coherence tomography, and ERG (although already nonrecordable at a young age).

**Limitations of the study**

There are some potential limitations of the study. A potential weakness in this observational case study is that not all patients agreed to be (re-)examined by the ophthalmologist in our institute. Another limitation is that the time of observation of the patients varied both in age of the patient and in the length of the survey.

**Conclusion**

In conclusion, in this study we report on the longterm clinical follow-up of RP in a large pedigree with the same homozygous *CRB1* mutation. The patients showed a wide range in onset and clinical course of the disease, supporting the hypothesis that the phenotype of patients with *CRB1* mutations is modulated by other factors. The clinical variability, even in patients with the same homozygous mutation, will make it harder to evaluate the effect of (upcoming) therapies for RP. However, because of the similarity between both eyes, the contralateral eye can be used as an excellent internal control.

**CONFLICT OF INTEREST**

None of the authors have any conflicting interests to disclose.

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REFERENCES


