Generalized Choriocapillaris Dystrophy, a Distinct Phenotype in the Spectrum of *ABCA4*-Associated Retinopathies

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Purpose. We describe a particular form of autosomal recessive generalized choriocapillaris dystrophy phenotype associated with *ABCA4* mutations.

Methods. A cohort of 30 patients with identified *ABCA4* mutations and a distinct phenotype was studied. A retrospective review of history, fundus photographs, electroretinography, visual field testing, dark adaptometry, and optical coherence tomography was performed. Genetic analyses were performed by *ABCA4* microarray analysis, high resolution melting, and/or next generation sequencing of all protein-coding sequences of the *ABCA4* gene.

Results. The earliest recorded manifestation of *ABCA4*-associated disease was a central bull's eye type of macular dystrophy that progressed to chorioretinal atrophy of the macula with coarse rounded hyperpigmentations and expanding involvement of the periphery. The mean age at first presentation was 10.3 years, the longest follow-up was 61 years. All patients had two *ABCA4* mutations identified, confirming the molecular genetic diagnosis of an *ABCA4*-associated disease. Most patients harbored at least one mutation classified as "severe," the most common of which was the p.N965S variant that had been found previously at a high frequency among patients with *ABCA4*-associated retinal dystrophies in Denmark.

Conclusions. Generalized choriocapillaris dystrophy is a progressive *ABCA4*-associated phenotype characterized by early-onset macular dystrophy that disperses and expands to widespread end-stage chorioretinal atrophy with profound visual loss. All cases in this study were confirmed as harboring two *ABCA4* mutations. Most of the *ABCA4* mutations were classified as "severe" explaining the early onset, panretinal degeneration, and fast progression of the disease.

Keywords: chorioretinal dystrophy, ABCA4, phenotype–genotype

The retina-specific adenosine triphosphate (ATP)-binding cassette transporter (ABCA4) protein is a transporter of vitamin A derivatives in the visual cycle, a process that is vital for the maintenance of healthy photoreceptors. Mutations in the *ABCA4* gene are responsible for a wide range of retinal degeneration phenotypes, including, Stargardt disease,[1] cone-rod dystrophy (CRD), and retinitis pigmentosa (RP).[2,3]

A substantial fraction of patients in clinical practice present with a phenotype that differs from Stargardt disease and fundus flavimaculatus[4] by the lack of flecks in the early stages, and by a characteristic end-stage with generalized choriocapillaris dystrophy. The end-stage is characterized by severe retinal dystrophy in addition to the choriocapillaris dystrophy, but differs from the primary photoreceptor dystrophies, RP, and CRD by the significant involvement of the choriocapillaris layer. Other studies of the phenotypes associated with mutations in the *ABCA4* gene have reported similar cases with phenotypes resembling end-stage generalized choriocapillaris dystrophy, but these were classified as CRD,[5–8] RP-like,[9–11] and retinal dystrophy,[12] and did not describe the early stages. A study of several families of Arabic descent from a single village included a few cases resembling the early stages described in this study.[13]

Based on a follow-up study over a long time span of a relatively large selected group of patients with the distinct phenotype of generalized choriocapillaris dystrophy, we outlined the natural history of this subset within the spectrum of *ABCA4*-related retinopathies.

Methods

Patients

Patients were enrolled from the Danish Retinitis Pigmentosa Registry (DRPR) and the *ABCA4* mutation database, which are located at the National Eye Clinic for the Visually Impaired at

the Kennedy Center, Denmark. The mutation database holds information on all of the pathogenic mutations found in previous genetic studies of the *ABCA4* gene in Danish patients with ABCA4-related retinopathies, including Stargardt disease, CRD, and RP.[14,15] The DRPR comprises all patients in Denmark with generalized retinal and chorioretinal dystrophies.[16] The charts of patients registered with generalized choriocapillaris dystrophy and/or atypical RP were reviewed in the DRPR, and cases with two *ABCA4* mutations were included from the mutation database. The study adhered to the tenets of the Declaration of Helsinki II. According to Danish law, no ethical approval was required for this retrospective clinical study that used anonymized data.

Clinical Evaluation

All patients had undergone a complete ophthalmic examination by a medical retina specialist, including best-corrected visual acuity (BCVA) testing, slit-lamp examination, and direct and indirect ophthalmoscopy. The available records generally included color fundus photographs (from 1970, most photographs presented here were made using the TRC 50 DX camera [Topcon, Tokyo, Japan]), Goldmann perimetry plots, Goldmann-Weekers dark adaptometry curves, and electroretinograms (ERG). The Ganzfeld full field ERGs were performed according to the ISCEV standard guidelines with a Burian-Allen electrode. The recording equipment varied throughout the period. Patients seen more recently often also had undergone fundus autofluorescence photography and optical coherence tomography (OCT; Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) and wide-field scanning laser ophthalmoscopy (Optos 200Tx; Optos PLC, Dunfermline, UK). The initial review identified 42 patients with diagnoses related to chorioretinal dystrophy. A second review of the clinical records classified 12 patients as presenting with CRD, central areolar atrophy, pattern dystrophy, or serpiginous choroiditis, which left 30 patients with a mutual retinal appearance and course of disease who were strictly diagnosed with generalized choriocapillaris dystrophy. In a few patients with end-stages resembling generalized choriocapillaris dystrophy, no possibly disease-associated mutations were identified in the *ABCA4* gene. These patients were not included in the present study. The examinations were performed between 1952 and May 2013.

The mutations found in 10 of the 30 patients included in the present study (patients D137, D109, D147, D022, D112, D108, D135, D117, D186, and D173) were included in past reports on *ABCA4* disease[14,15] that described the cross-sectional genetic and clinical spectrum of *ABCA4* disease in Denmark, with a more detailed description of the clinical history and end-stage imaging of one patient (D137).[15] To our knowledge, this is the first large study to define the longitudinal characteristics of this particular form of generalized choriocapillaris dystrophy.

Genetic Analysis

DNA was isolated from EDTA-treated blood samples and the *ABCA4* gene was examined using *ABCA4* microarray analyses, high-resolution melting (HRM) following direct sequencing of heteroduplex positive bands or next-generation sequencing (NGS) methods. DNA samples from 23 patients were analyzed in 2002 to 2004 for known *ABCA4* mutations using a commercial APEX microarray (Asper Biotech AS, Tartu, Estonia),[17] which then included 386 pathogenic *ABCA4* variants.[15] DNA samples from 8 patients were analyzed using *ABCA4* microarray and HRM methods.[14] Finally, DNA samples from 6 patients were analyzed using NGS sequencing of the *ABCA4* gene, and 3 of these samples had already been analyzed with the *ABCA4* microarray analysis.[18]

All missense *ABCA4* variants, except for the known frequent polymorphisms, were analyzed with algorithms, such as Align GVGD,[19] Sorting Intolerant from Tolerant (SIFT; available in the public domain at http://sift.jcvi.org/), Polymorphism Phenotyping v2 (PolyPhen2; available in the public domain at http://genetics.bwh.harvard.edu/pph2/), and MutationTaster,[20] to predict the impact of variants on the ABCA4 function and, consequently, on disease susceptibility. Variants detected in the vicinity to exons/intron boundaries were analyzed with splice site prediction programs MaxEntScan,[21] GeneSplicer (available in the public domain at http://www.cbcb.umd.edu/software/GeneSplicer), NNSPLICE (available in the public domain at http://www.fruitfly.org/seq_tools/splice.html), and Human Splicing Finder.[22] All the prediction programs were accessed via Alamut 2.2 software (available in the public domain at http://www.interactive-biosoftware.com).

Results

The mean duration of follow-up in the 30 patients (14 males, 16 females) was 27.9 years (range, 9.4–60.7 years; Table 1). The mean age of clinical presentation was 10.3 years (range 4–18 years), and the year of presentation ranged from 1952 to 1996. Six patients were from the same family, GCCD0801, and the remaining patients were unrelated. All families were Caucasian, with four families of mixed descent (Danish and Swedish/Greek/Polish), while one family was from Portugal, and the rest of the families were from Denmark. None of the patients had high myopia.

Clinical Characteristics of Stage 1

The clinical course of generalized choriocapillaris dystrophy found in all patients presented in this study falls into three stages (Fig. 1). The initial stage (stage 1) is characterized by the development of bull's eye macular dystrophy without flecks characteristic for Stargardt disease or fundus flavimaculatus (Fig. 1, Stage 1), and in some cases by an additional speckled mid peripheral pattern with small, coalescent, round, or oblong atrophies resembling faded flavimaculatus flecks (Fig. 2A), and/or small, round atrophies with a punctate central pigmentation (Fig. 2B, white circle). At this stage the full-field dark-adapted ERG had rod responses within normal limits, while the 30 Hertz flicker cone responses had normal to subnormal amplitudes and moderately prolonged implicit times (0%–20%) depending on the duration of the disease. Half of the patients, however, did not have any available early ERG recordings. The symptoms in this stage were dominated by central visual loss, and in some cases dyschromatopsia. The mean BCVA in this stage was 0.31 (SD = 0.20), with early measurements available for 16 patients. Some patients also had difficulties adapting from light to dark surroundings. Most patients had normal dark adaptations and visual fields at stage 1 (Figs. 3-1A, 3-2A).

Clinical Characteristics of Stage 2

In the intermediary stage, the central atrophy started to disperse and spread centrifugally (Fig. 1, Stage 2). The scattered isles of chorioretinal atrophy increased in size and fused with time. Pigmentation clumps began to form in the center. The symptoms at this stage were more severe and were characterized by worsening of the central vision. In some cases, the peripheral vision and night vision began to

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Table 1. Demographic Characteristics of Patients With Generalized Choriocapillaris Dystrophy

Patient	Pedigree	Sex	Refraction	Age at Presentation	Date of Presentation	Most Recent Date of Examination	Period of Observation, y
D513	RP02270	M	Plano	10	January 19, 1952	February 25, 1983	31.1
D514	STG04071	M	Plano	8	March 16, 1984	April 27, 2011	27.1
D516	STG1	M	Plano	6	May 27, 1992	October 9, 2010	18.4
D517	STG2	M	−3.5/−4.7	12	October 6, 1970	December 8, 2010	40.2
D137	STG04078	M	Plano	18	January 16, 1968	September 3, 2002	34.7
D801	GCCD0801	M	Plano	7	April 29, 1987	September 21, 2001	14.4
D802	GCCD0801	M	Plano	7	May 30, 1990	February 23, 2000	9.8
D109	GCCD0801	M	Plano	18	April 14, 1969	September 16, 1997	28.5
D803	GCCD0801	F	−1.0/−1.0	6	May 9, 1969	April 20, 1994	24.9
D804	GCCD0801	M	Plano	14	February 7, 1969	October 26, 2012	43.7
D805	GCCD0801	F	−0.5/−0.5	15	February 1, 1971	January 14, 2005	33.9
D040	GCCD0804	F	−1.5/−2.0	4	August 23, 1985	March 7, 2013	27.6
D159	STG00215	M	N/A	7	May 25, 1984	March 22, 2004	19.8
D129	STG04042	F	−4.0/−4.0	13	March 12, 1964	August 31, 2000	36.4
D115	STG04073	M	Plano	10	April 16, 1953	November 19, 1996	43.6
D033	STG04012	F	−1.0/−1.0	11	September 11, 1975	May 16, 2012	36.7
D023	STG00205	F	Plano	9	January 21, 1985	May 5, 2013	28.3
D001	STG04003	F	Plano	8	November 19, 1996	August 28, 2012	15.7
D147	STG04069	F	−3.0/−3.0	11	August 14, 1981	January 8, 1991	9.4
D162	STG00216	F	−1.0/−1.5	18	September 4, 1992	November 11, 2003	11.0
D022	STG04092	M	Plano	9	September 15, 1983	June 6, 2003	19.7
D112	STG04077	F	−2.5/−1.2	9	November 27, 1984	February 28, 2011	26.2
D108	STG04088	F	Plano	11	August 15, 1977	February 5, 2008	30.5
D107	STG04087	F	−0.7/−0.5	13	September 7, 1990	December 3, 2003	13.1
D070	STG04023	F	Plano	16	March 10, 1975	August 17, 2011	36.4
D116	STG04081	M	Plano	10	February 11, 1977	April 29, 2013	36.2
D135	STG04074	M	+1.2/+3	7	September 27, 1950	March 16, 1995	44.5
D117	STG04082	F	Plano	7	August 22, 1984	January 22, 2003	18.4
D186	GCCD0810	F	Plano	12	June 2, 1975	April 4, 1991	15.8
D173	GCCD4067	F	+1.5/+1	13	May 29, 1952	January 28, 2013	60.7

deteriorate, with objective progression of the visual field scotomas and in some cases a subnormal dark adaptation (Figs. 3-1B, 3-2B). The mean BCVA for available 27 patients at this stage was 0.1 (SD = 0.05). The ERG recordings typically showed reduced cone and rod responses by approximately 50%. The patients mostly reached this stage in their 20s.

Clinical Characteristics of Stage 3

The final, end-stage (stage 3) was reached at the age of 30 to 50 years. This stage was the most characteristic for this phenotype with widespread diffuse chorioretinal atrophy. The choroidal space appeared clearly in the fundus with a gray-pink color. Distinct, coarse, rounded hyperpigmentations were present in the center (Fig. 1; D022 and D108, Stage 3), or in the entire retina (Fig. 1; D514, D033, and D116, Stage 3). The pigmentations were located superficially in the retina, in some cases enveloping the retinal vessels. The optic nerve was relatively well vascularized and the vessels were less attenuated compared to what is observed in the classical form of RP. In this stage, the symptoms were characterized by further worsening of the central vision, with BCVA of $\leq$2/60 in most cases. The mean BCVA was 0.05 (SD = 0.01), with measurements available for 29 patients. Dark adaptation was delayed and highly prolonged with a typically approximately linear middle phase. The visual fields showed further progression with visual field constriction and increasing central scotomas (Figs. 3-1C, 3-2C). The ERG recordings at this stage were either extinct or demonstrated severely reduced cone and rod responses.

In patients with advanced disease, the fundus was characterized by very severe or total areolar outer retinal atrophy with ophthalmoscopically visible sclera and confluent hyperpigmentation in the macula, whereas isolated or clustered lesions were seen in the periphery (Fig. 4). The 200° color images (Fig. 4A) show the coarse pigmentations in the macula and the presence of pigmented plaques in the outer periphery. Spectral domain OCT (Fig. 4D) revealed abnormal structural changes with widespread photoreceptor loss and complete absence of the photoreceptor layer in the center. Intraretinal hyperreflective deposits corresponding to larger pigmentation clumps observed in the fundus also were evident (Fig. 4D2, white arrow).

Mutations

Among the 52 mutations found in the 26 patients with generalized choriocapillaris dystrophy and unique genotypes (only including one affected family member from each of the two generations in family GCCD0801), 34 mutations (65%) were missense (Tables 2, 3), and 11 (42%) of the 26 subjects with unique genotypes patients had two missense mutations. The remaining mutations were 4 nonsense mutations (found in 15% of the patients), 6 deletions (found in 23% of the patients), 6 splice-site mutations (found in 23% of the patients), and 2 other intron mutations. Most of the variants were already known pathogenic mutations; however, the c.5169C>G; p.Y1723* variant found by NGS is a novel and likely deleterious mutation, since it generates a stop codon that terminates translation at codon 1723 of *ABCA4*. The most frequent mutations in the cohort were the p.N965S Danish founder

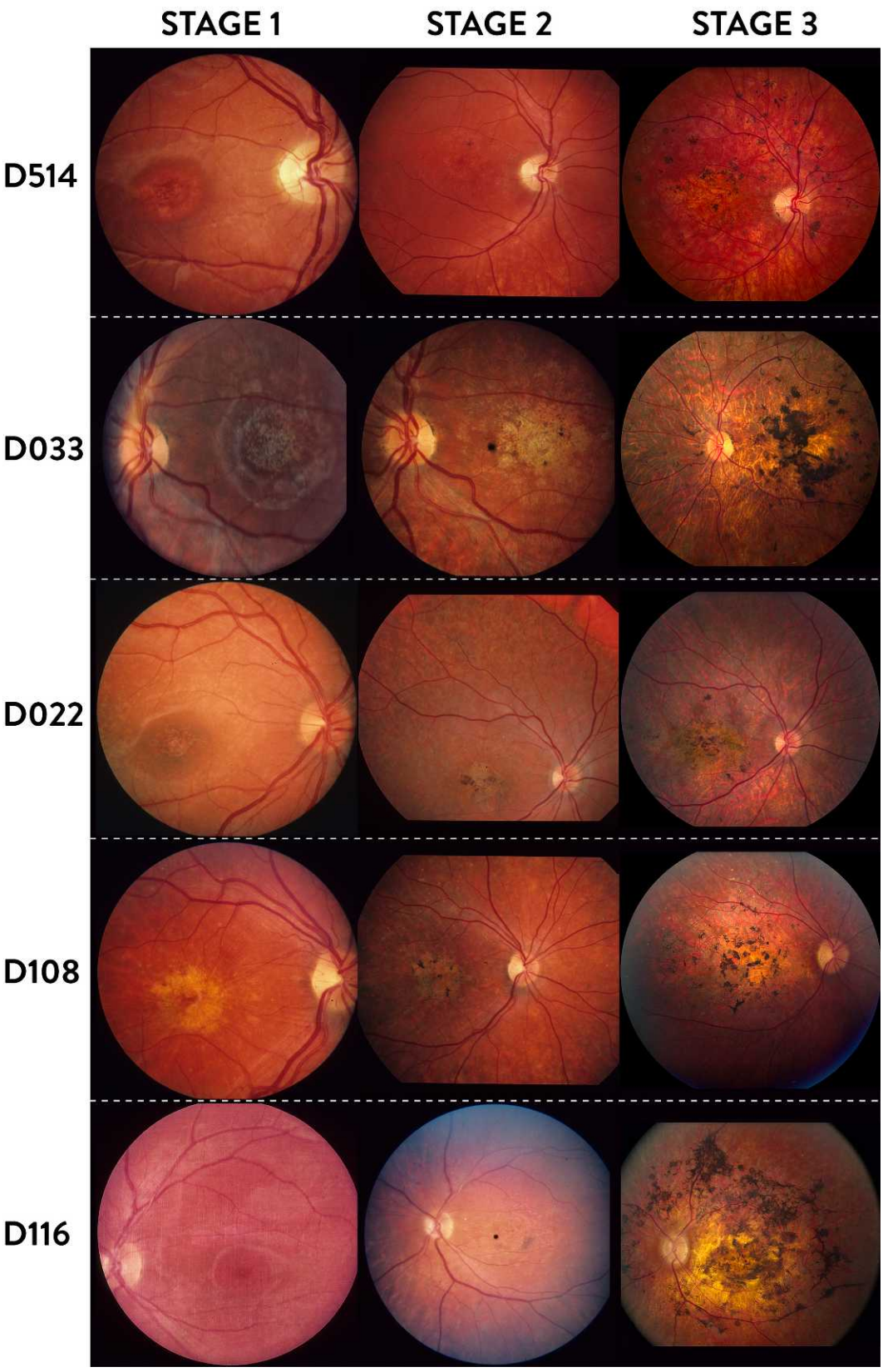

FIGURE 1. Three stages of generalized choriocapillaris dystrophy in 5 patients. Stage 1: Bull's-eye maculopathy. Stage 2: Expanding maculopathy with indistinct margins, central pigmentations, and early peripheral atrophy. Stage 3: End-stage disease with central atrophy and coarse round pigmentations plus widespread peripheral chorioretinal atrophy. The *dark spot* in the maculae of patients D514 and D116 at Stage 2 is a camera artifact.

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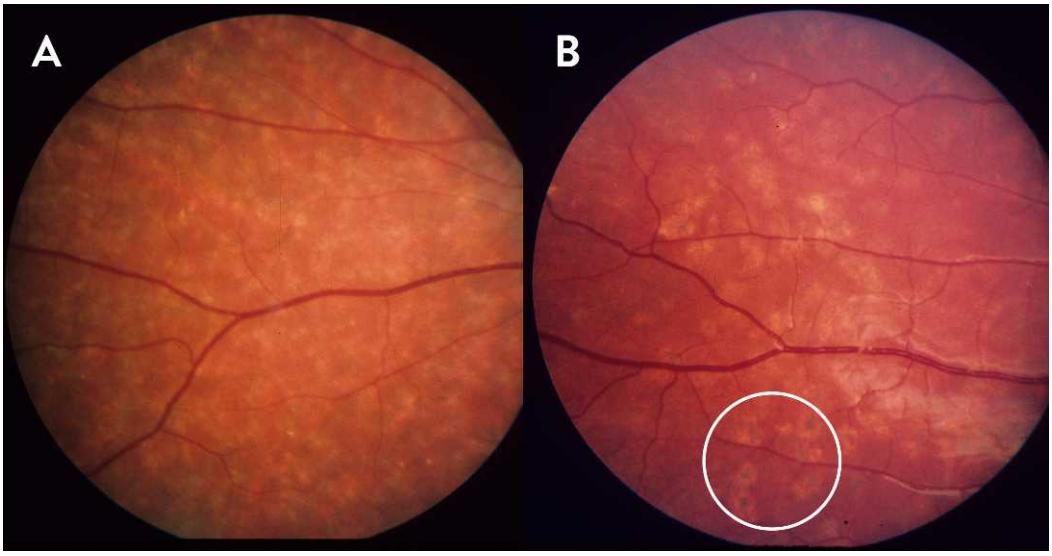

Figure 2. Peripheral/mid peripheral atrophy in the initial/middle stages of generalized choriocapillaris dystrophy. (**A**) Patient D117 at the age of 18, with small, partially confluent, round and oblong patches of unpigmented atrophy, and (**B**) patient D112 at the age of 14, with less confluent peripheral round patches of atrophy, some of which have a small, round central pigmentation (*white circle*).

mutation (21%), p.G863A, c.2408delG, and p.L541P/A1038V. It should be noted that the segregation and phase of the mutations were evaluated in only a few cases that had relatives available to analyze. Predicted severity and consequences of each mutation are provided in Table 3.

Family GCCD0801

A single large family with generalized choriocapillaris dystrophy contributed 6 cases to this study (Fig. 5). The affected family members in generation III reached the end-stage of the

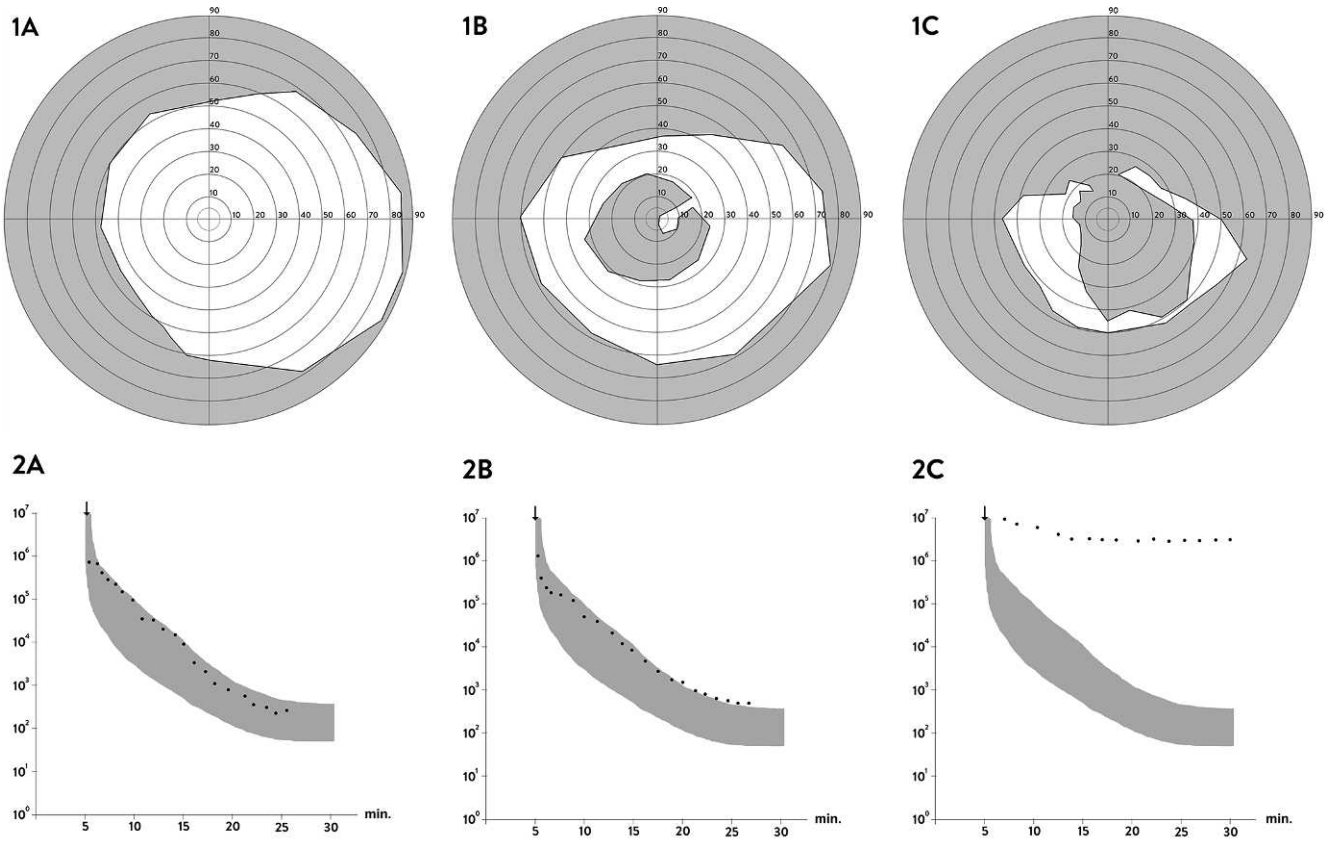

Figure 3. Goldman visual fields for object IV of patient D112 (10 years old) in stage 1 (**1A**), and patient D514 in stage 2 ([**1B**], 20 years old) and stage 3 ([**1C**], 36 years old). Dark adaptations for patient D107 in stage 1 ([**2A**], 15 years old), patient D514 in stage 2 ([**2B**], 20 years of age), and patient D804 in stage 3 ([**2C**], 58 years of age).

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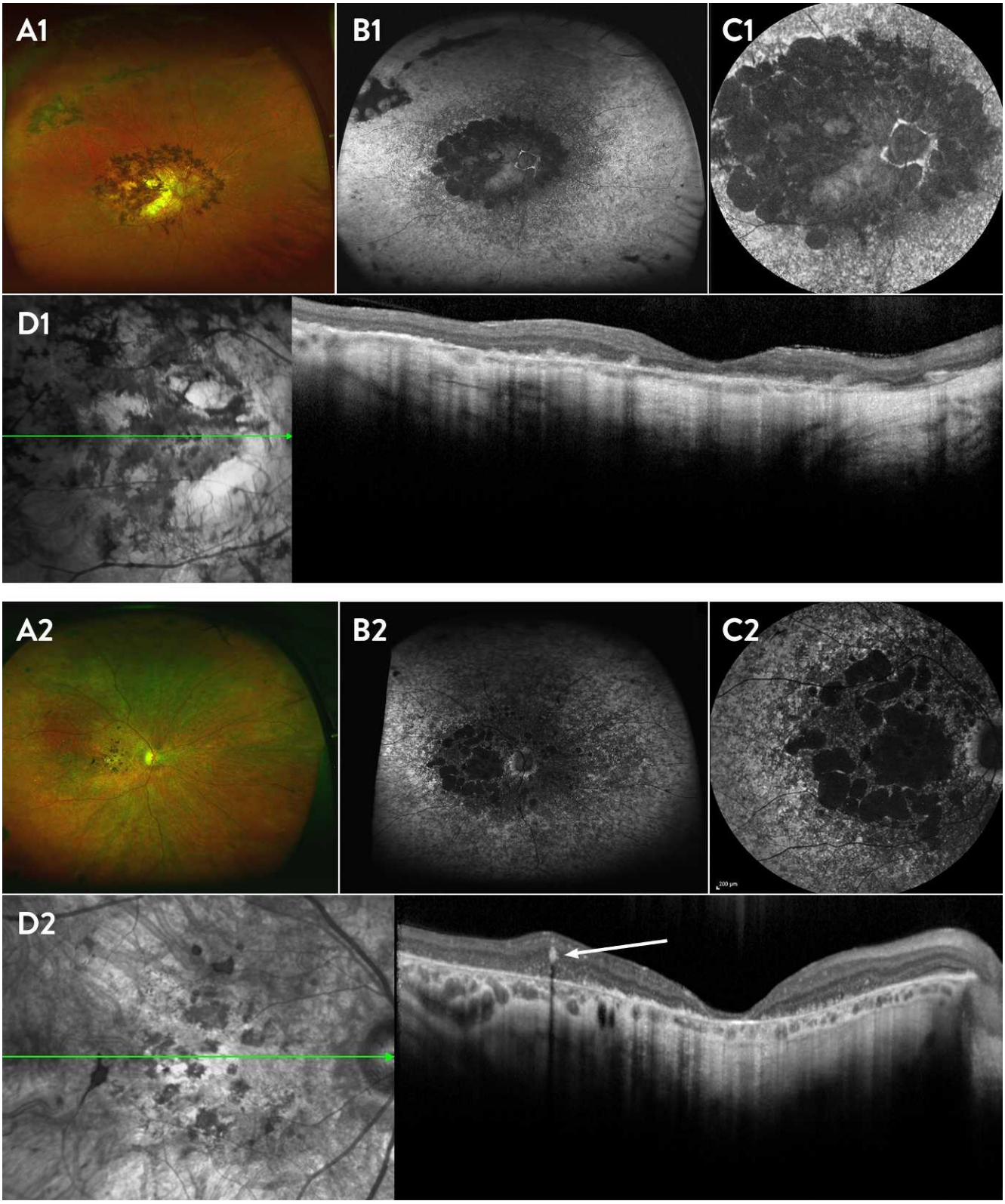

Figure 4. Fundus photographs in color (**A**), autofluorescence (**B**, **C**), and infrared ([**D**], *left*), and OCTs ([**D**], *right*) from two patients (*upper block* No. 1, D116 at the age of 47; *lower block* No. 2, D023 at the age of 39) with end-stage generalized choriocapillaris dystrophy characterized by semiconfluent multifocal areolar outer retinal atrophy, the severity of which decreases with increasing eccentricity. Central patches of visible sclera are surrounded by coarse confluent hyperpigmentation. The periphery shows isolated or clustered hyperpigmentation. In and around the fovea there is pronounced atrophy of the retinal pigment epithelium, the photoreceptor layer, and the outer nuclear layer and intraretinal hyperreflective material corresponding to the hyperpigmentation ([**D2**], *white arrow*).

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Table 2. Summary of Detected Potential Pathogenic Variants (Known and Novel [in Bold Face]) Found in the *ABCA4* Gene of Patients With Generalized Choriocapillaris Dystrophy

		Mutation 1		Mutation 2	
Patient	**Method**	**Nucleotide**	**Protein**	**Nucleotide**	**Protein**
D513	NGS	c.203C>T	p.P68L	c.2894A>G	p.N965S
D514	Microarray, NGS	c.2894A>G	p.N965S	c.5461-10T>C	-
D516	NGS	c.4926C>G	p.S1642R	c.5041_5055del	p.V1681_C1685del
D517	NGS	**c.5169C>G**	**p.Y1723***	c.6079C>T	p.L2027F
D137	Microarray, NGS	c.2894A>G	p.N965S	c.2894A>G	p.N965S
D801	Microarray, NGS	c.6386+1G>A	Aberrant splicing	c.4234C>T	p.Q1412*
D109	Microarray	c.2894A>G	p.N965S	c.4234C>T	p.Q1412*
D040	Microarray	c.6229C>T	p.R2077W	c.6229C>T	p.R2077W
D159	Microarray	c.3113C>T	p.L541P/A1038V	c.3113C>T	p.L541P/A1038V
D129	Microarray	c.2894A>G	p.N965S	c.3322C>T	p.R1108C
D115	Microarray	c.2894A>G	p.N965S	c.3113C>T	p.L541P/A1038V
D033	Microarray	c.2894A>G	p.N965S	c.2041C>T	p.R681*
D023	Microarray	c.203C>T	p.P68L	c.3329-2A>G	Aberrant splicing
D001	Microarray	c.666_678del	p.K223_R226delfs	c.4667+2T>C	Aberrant splicing
D147	Microarray, HRM	c.2894A>G	p.N965S	c.2408delG	p.G803fs
D162	Microarray	c.3329-2A>G	Aberrant splicing	c.6089G>A	p.R2030Q
D022	Microarray, HRM	c.4462T>C	p.C1488R	c.4102C>T	p.R1368C
D112	Microarray, HRM	c.2894A>G	p.N965S	c.1529T>G	p.L510R
D108	Microarray, HRM	c.1648G>A	p.G550R	c.4102C>T	p.R1368C
D107	Microarray	c.666_678del	p.K223_R226delfs	c.2588G>C	p.G863A
D070	Microarray	c.2588G>C	p.G863A	c.2588G>C	p.G863A
D116	Microarray	c.2300T>A	p.V767D	c.5461-10T>C	-
D135	Microarray, HRM	c.2894A>G	p.N965S	c.2408delG	p.G803fs
D117	Microarray, HRM	c.3191-2A>G	Aberrant splicing	c.2408delG	p.G803fs
D186	Microarray, HRM	c.3322C>T	p.R1108C	c.6386+1G>A	Aberrant splicing
D173	Microarray, HRM	c.4469G>A	p.C1490Y	c.2915C>A	p.T972N

disease between 40 and 50 years of age with visual acuities reduced to 1/60, reduced cone and rod responses on ERG recordings, prolonged dark adaptation times, large central scotomas, and fundi with widespread chorioretinal atrophy and coarse hyperpigmentations. Both of the affected brothers in generation IV had an early onset at 7 years of age, with visual acuity loss as the initial symptom and with fundi showing bull's eye maculopathy. The oldest brother (Fig. 5, 4:8) was last seen in his early 20s with a visual acuity reduced to 1/60, prolonged dark adaptation and ERG recordings showing severely reduced cone and rod responses. His fundus at that time was characterized by widespread chorioretinal atrophy and coarse, round hyperpigmentations in the center and periphery. The younger brother suffered from congenital brain damage in addition to generalized choriocapillaris dystrophy, and advanced examinations, such as ERG, had not been possible. He was last seen at the age of 25, and his visual acuity was reduced to finger counting. His fundus examination revealed widespread chorioretinal atrophy with hyperpigmentations in the entire retina.

Mutational screening was performed with ABCR600 microarray and NGS sequencing of the *ABCA4* gene. Two *ABCA4* mutations were detected in patient D109 from generation III; a common Danish missense mutation p.N965S and a nonsense mutation p.Q1412*. In the fourth generation, the array screening identified only the p.Q1412* mutation, which was presumably inherited from the unaffected father. We found the second, at that time novel, splice mutation c.6386+1G>A with NGS, suggesting that the mother of the two affected brothers must have been a carrier of this third mutation, explaining the occurrence of this recessive disorder in two successive generations and a pseudo-dominant inheritance pattern.

Discussion

This retrospective review outlines a particular, hitherto poorly described disease phenotype within the group of generalized choriocapillaris dystrophies.[23] The clinical picture develops from an early bull's eye type of maculopathy into a severe central and peripheral chorioretinal dystrophy with a profound visual loss. The characteristic end-stage was described before the era of molecular genetics within the mixed group of "diffuse choroidal atrophies,"[23] "primary choroidal sclerosis,"[24] and "diffuse choroidal sclerosis."[25]

Given the wide variation of phenotypes associated with *ABCA4* and the length of time that is required to gain an overview of the stages covered during the development of the disease, it is not surprising that the longitudinal characteristics of this particular form of generalized choriocapillaris dystrophy have not been described in detail before. The end-stage is likely to have been reported in single cases under the clinical definitions of CRD,[5–8] RP-like,[9–11] and retinal dystrophy,[12] and a family-based study has shown fundus images that resemble the earlier stages.[13] The phenotype, however, differs from the photoreceptor dystrophies RP, cone-dystrophy, and CRD, by the primary and significant involvement of the choriocapillaris and retinal pigment epithelium layers, emphasized by a normal or slightly subnormal ERG at the initial stage, and the severe fundus appearance at later stages. While generalized choriocapillaris dystrophy in its advanced stage may resemble fundus flavimaculatus stage IV,[4] none of our patients ever demonstrated the characteristics of early Stargardt disease or fundus flavimaculatus flecks. The small, rounded, peripheral atrophies that can be present in the initial stages were mistaken in some of our patients, though, for flavimaculatus flecks. Because most of our cases presented several decades ago, we do not have modern image studies that can tell whether patients with

Table 3. In Silico Analysis of *ABCA4* Variants Detected in This Study Using Alamut 2.2 Software

cDNA Variant	Protein Variant	Effect on Protein Function	AGVGD Class	SIFT Prediction	Effect on Protein PPH2 Prediction	Effect on Protein TASTER Prediction	Effect on Splicing
Missense variants							
c.203C>T	p.P68L		C65	Deleterious	Probably damaging	Disease causing	
c.1529T>G	p.L510R		C65	Deleterious	Benign		Polymorphism
c.1622T>C	p.L541P	Reduced ATP binding mislocalization[26,27]	C65	Deleterious	Probably damaging	Disease causing	
c.1648G>A	p.G550R		C65	Deleterious	Possibly damaging	Disease causing	New acceptor site
c.2300T>A	p.V767D	Reduced protein[28]	C65	Deleterious	Benign	Disease causing	
c.2588G>C	p.G863A	Reduced protein level, reduced ATP binding, reduced ATPase activity[26]	C55	Deleterious	Possibly damaging	Disease causing	Predicted change at acceptor site 1 bp upstream: −11.1%, creating a new stronger acceptor 3 bp downstream
c.2894A>G	p.N965S	Reduced ATP binding[26]	C45	Deleterious	Probably damaging	Disease causing	New acceptor site
c.2915C>A	p.T972N		C55	Deleterious	Probably damaging	Disease causing	
c.3113C>T	p.A1038V	Reduced ATP binding, reduced ATP hydrolysis[26]	C65	Deleterious	Benign	Disease causing	
c.3322C>T	p.R1108C	Reduced ATP binding[26]	C65	Deleterious	Probably damaging	Disease causing	
c.4102C>T	p.R1368C		C65	Deleterious	Probably damaging	Disease causing	
c.4462T>C	p.C1488R		C65	Deleterious	Possibly damaging	Disease causing	
c.4469G>A	p.C1490Y	Misfolding, mislocalization[27]	C65	Deleterious	Probably damaging	Disease causing	Cryptic donor strongly activated
c.4926C>G	p.S1642R		C25	Deleterious	Benign	Disease causing	
c.6079C>T	p.L2027F	Reduced ATP binding[26,29]	C15	Deleterious	Probably damaging	Disease causing	
c.6089G>A	p.R2030Q		C35	Deleterious	Probably damaging	Disease causing	
c.6229C>T	p.R2077W	Reduced ATP binding[26]	C65	Deleterious	Probably damaging	Disease causing	
Deletion/frame-shift/stop variants							
c.666_678del	p.K223_R226delfs						
c.2041C>T	p.R681*						
c.2408delG	p.G803fs						
c.4234C>T	p.Q1412*						
c.5041_5055del	p.V1681_C1685del						
c.5169C>G	p.Y1723*						
Splicing affecting variants							
c.3191-2A>G							Predicted change at acceptor site 2 bps downstream: −100%
c.3329-2A>G							Predicted change at acceptor site 2 bps downstream: −100%
c.4667+2T>C							Predicted change at donor site 2 bps upstream: −100%

Investigative Ophthalmology & Visual Science

Table 3. Continued

cDNA Variant	Protein Variant	Effect on Protein Function	AGVGD Class	SIFT Prediction	Effect on Protein PPH2 Prediction	Effect on Protein TASTER Prediction	Effect on Splicing
c.4773+3A>G							Predicted change at donor site 3 bps upstream: −46.5%
c.6386+1G>A							Predicted change at donor site 1 bp upstream: −100%
Variant of unknown effect							
c.5461-10T>C							Predicted change at acceptor site 10 bps downstream: −4.3%

Nucleotide positions and protein translation correspond to CCDS747.1 and NP_000341.2, respectively. References to the effect on ABCA4 protein function are given in parentheses. bp, base pair; cDNA, complementary DNA; AGVGD, Align Grantham Variation Grantham Deviation; PPH2, polymorphism phenotyping 2.

generalized choriocapillaris dystrophy have the occasional hallmarks of early Stargardt disease, such as vermillion fundus, fundus hyperautofluorescence, and a dark choroid on fluorescein angiograms. The differential diagnoses include CRD, RP, central areolar atrophy, pattern dystrophy, and serpiginous choroiditis.

In all but two patients with the typical phenotype of generalized choriocapillaris dystrophy, we found at least one, and in most patients two, severe mutations in the *ABCA4* gene. The Danish p.N965S founder mutation was among the most frequent mutations found in our patients, and this mutation is believed to lead to moderate-to-severe retinal phenotypes, because ABCA4 protein dysfunction results in impaired ATP hydrolysis. The variant also is deemed pathogenic by all predictive programs and, in addition to a protein defect, it also may affect splicing (Table 3).[15,26] This cohort harbored a higher than usual fraction (10/60, ~17%) of deleterious variants (deletions and stop codons), which are postulated to result in a completely dysfunctional protein. In addition, most other, missense, mutations are deemed severe either by in silico or indirect functional analyses (Table 3), or from data presented in previous studies even if the exact effect of the variant on the protein is unknown (e.g., the c.5461-10T>C variant).[26–29] The p.G863A mutation found in some of our patients is a common mutation in Stargardt disease patients of mainly Dutch descent,[30] and it also has been observed in patients with CRD.[31] This mutation is considered to be relatively mild and claimed not to be disease-causing in a homozygous state[32]; however, the patient D070 (Table 2) who is homozygous for this mutation, is one of a few examples of two mild mutations leading to generalized choriocapillaris dystrophy. Since the entire *ABCA4* gene was not sequenced in

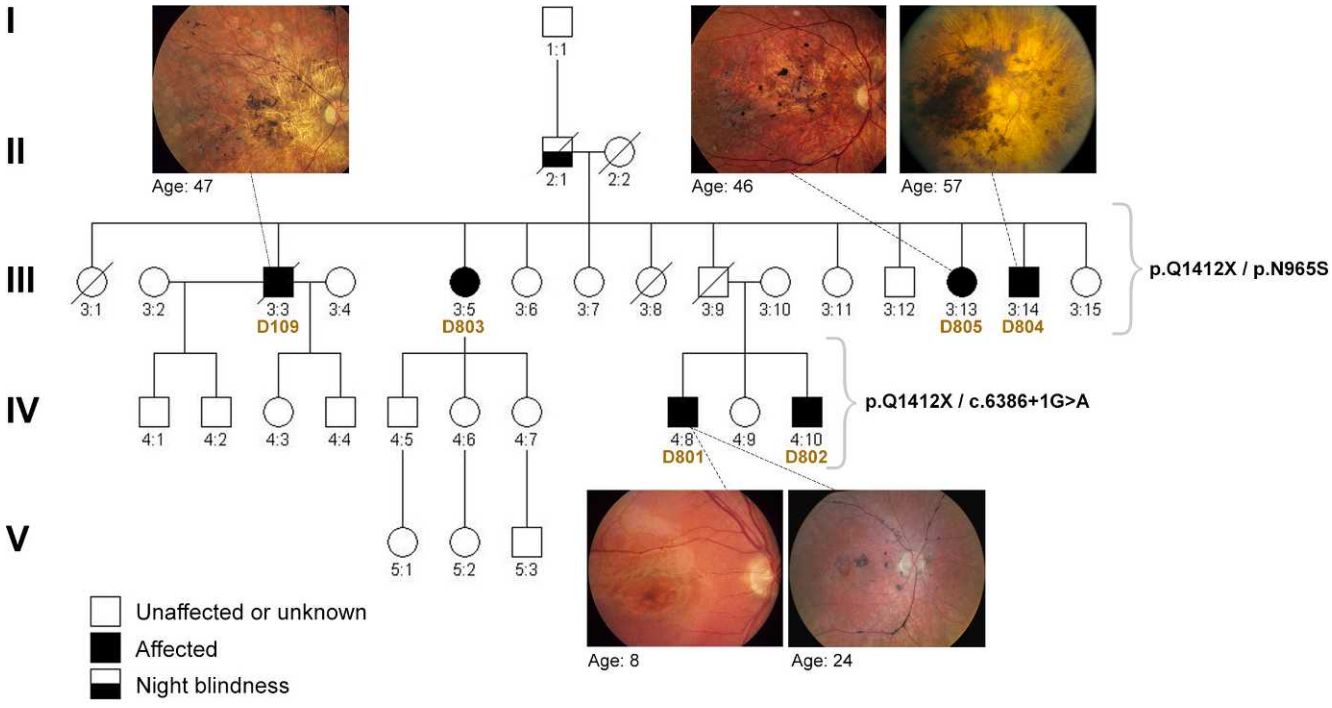

Figure 5. Family GCCD0801 with *ABCA4* mutations and fundus photographs of affected family members from two generations.

this patient, we cannot exclude the possibility that allelic severe *ABCA4* mutation(s), including a large deletion, remained undetected. It also may be possible that these patients have additional modifier alleles, or epigenetic or environmental factors that have a particularly prominent effect. Whole exome or genome sequencing, or genome-wide association studies (GWAS) would be needed to determine if the patients in this cohort share other common or rare variants in other genes that may influence the phenotype.

In summary, we delineated a relatively large cohort of patients (26 of a mixed group of 108 patients (24%) with both pathogenic *ABCA4* alleles identified)[14,15] presenting a severe *ABCA4*-associated phenotype characterized by three stages with early-onset central retinopathy that disperses and expands to widespread end-stage generalized choriocapillaris dystrophy. This phenotype, which apparently is not rare among the *ABCA4*-associated retinopathies in Denmark, is caused by mostly deleterious mutations in the *ABCA4* gene, including the high prevalence of the p.N965S mutation (Table 3), which likely explains the early onset, panretinal degeneration, and fast progression of the disease. A combination of early onset of symptoms, a characteristic fundus appearance, and specific severe mutations can be used to improve the prediction of the development of generalized choriocapillaris dystrophy in a large number of cases.

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