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ARTICLE

Microarray-based mutation analysis of the *ABCA4* (*ABCR*) gene in autosomal recessive cone–rod dystrophy and retinitis pigmentosa

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Mutations in the *ABCA4* gene have been associated with autosomal recessive Stargardt disease (STGD1), cone–rod dystrophy (CRD), and retinitis pigmentosa (RP). We employed a recently developed genotyping microarray, the ABCR400-chip, to search for known *ABCA4* mutations in patients with isolated or autosomal recessive CRD (54 cases) or RP (90 cases). We performed detailed ophthalmologic examinations and identified at least one *ABCA4* mutation in 18 patients (33%) with CRD and in five patients (5.6%) with RP. Single-strand conformation polymorphism (SSCP) analysis and subsequent DNA sequencing revealed four novel missense mutations (R24C, E161K, P597S, G618E) and a novel 1-bp deletion (5888delG). Ophthalmoscopic abnormalities in CRD patients ranged from minor granular pigmentary changes in the posterior pole to widespread atrophy. In 12 patients with recordable electroretinogram (ERG) tracings, a cone–rod pattern was detected. Three patients demonstrated progression from a retinal dystrophy resembling STGD1 to a more widespread degeneration, and were subsequently diagnosed as CRD. In addition to a variable degree of atrophy, all RP patients displayed ophthalmologic characteristics of classic RP. When detectable, ERG recordings in these patients demonstrated rod–cone patterns of photoreceptor degeneration. In conclusion, in this study, we show that the *ABCA4* mutation chip is an efficient first screening tool for arCRD.
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Introduction

The *ABCA4* (ABCR) gene was identified as the gene underlying autosomal recessive Stargardt disease (STGD1).[1] Since the cloning of the *ABCA4* gene, other studies have implicated this gene also in autosomal recessive cone–rod dystrophy (arCRD) and in autosomal recessive retinitis pigmentosa (arRP).[2–17] In addition, heterozygous *ABCA4* mutations were found in 16% of cases with age-related macular degeneration.[18] The high prevalence of heterozygous *ABCA4* mutations in the general population, and the inability of relatively small-sized studies to replicate this result shed doubt on the significance of the molecular findings in patients with AMD.[19–24] In a larger

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independent study, two *ABCA4* mutations were significantly associated with AMD.[25]

The *ABCA4* gene encodes the ABCR protein, previously identified as the rim protein (RmP), a retina-specific ATP-binding cassette transporter. This protein is thought to act as a flippase for *N*-retinylidene phosphatidylethanolamine (*N*-retinylidene-PE), thereby facilitating the transport of all-*trans*-retinal from the disk lumen to the photoreceptor cytoplasm.[26,27] In Abcr(−/−) mice, *N*-retinylidene-PE is converted, through various intermediates, into A2E, a major component of lipofuscin. A2E accumulates in toxic levels in the retinal pigment epithelium (RPE), eventually resulting in the degeneration of the RPE and the overlying neuroretina. Interestingly, Abcr(−/−) mice raised in complete darkness do not accumulate A2E, suggesting that avoiding excessive light might be beneficial for humans with *ABCA4*-associated retinal dystrophy.[28] Recently, Radu *et al*[29] were able to rescue retinal dystrophy in Abcr(−/−) mice by treating them with isotretinoin (Accutane or 13-*cis*-retinoic acid), a drug commonly used in dermatology for the treatment of severe acne. Both studies show that therapeutic approaches for humans with *ABCA4*-associated retinal dystrophies are feasible, which underlines the importance to identify patients with CRD and RP caused by mutations in *ABCA4*.

Numerous mutation analysis studies in patients with STGD1 have yielded more than 400 different *ABCA4* mutations.[1,6,8,9,22,30–35] Unlike STGD1, arCRD and arRP are not genetically homogeneous disorders (http://www.sph.uth.tmc.edu/RetNet).[2,36–38] Studies investigating the role of *ABCA4* mutations in arCRD estimate involvement of this gene in 24–75% of all cases.[5–9,11,16] *ABCA4* mutation analysis in arRP patients thus far was restricted to families in which STGD1 and RP segregated.[4,10,14,15,17] Therefore, it has not been possible to make an accurate prediction regarding the contribution of *ABCA4* mutations in arRP pathology. Recently, a genotyping microarray (ABCR400 chip) was developed containing all currently known *ABCA4* mutations, which robustly identifies 98% of the known *ABCA4* mutations in patients with STGD1.[39]

In this study, we utilized the ABCR400 array to systematically screen for mutations in patients with isolated or autosomal recessive RP (90 patients) and CRD (54 patients). We re-evaluated the clinical features in patients with *ABCA4* mutations and provide for the first time an assessment of the contribution of *ABCA4* gene mutations as a cause for arRP in the Caucasian population.

Materials and methods

Patients and controls

The charts of 90 patients with isolated (56) or autosomal recessive (34) RP and 54 patients with isolated (35) or autosomal recessive (19) CRD were collected from the centers collaborating in this study. RP patients were ascertained in Nijmegen (74), Rotterdam (10), and Heidelberg (6). CRD patients were ascertained in Heidelberg (36), Nijmegen (14), and Rotterdam (4). Some of these cases might be due to X-linked or autosomal dominant mutations. In the remainder of this manuscript, the patient groups will be designated as CRD and RP.

This study was approved by the institutional review board (CCMO). After informed consent was obtained, blood samples were taken. Samples from 93 healthy Dutch blood donors were used as controls. The clinical data of all patients were examined and, when clinical data were incomplete or obtained with obsolete methods, patients were clinically re-evaluated. Kinetic perimetry was performed with the Goldmann perimeter. A recent electroretinogram (ERG), recorded in accordance with the ISCEV protocol,[40] was available for all patients, except for patient 9444. The employed ERG methods for this patient, as well as the earlier ERGs of three other patients, were performed as described by Thijssen *et al*[41] (patients 12608 and 15680) and Alexandridis *et al* (patient 15730).[42] When possible, colour vision was tested with the Ishihara and Panel D15 tests. The diagnosis of CRD was based on initial complaints of decreased or blurred central vision, without a history of night blindness. Maculopathy, characterized by a bull's eye pattern or granular alterations of the macular RPE, with or without relatively mild peripheral retinopathy was considered typical. Visual field testing usually shows a central scotoma, while the peripheral fields are either normal or show a mild to moderate constriction. In addition, ERG recordings in CRD either show reduction or absence of cone responses in the presence of quantitatively less reduction in rod responses, or an equal impairment of both photoreceptor systems.[43–46] The initial symptom in RP patients is night blindness. Visual field defects typically originate in the midperiphery, with gradual enlargement to both the periphery and the center of the retina. Typically, the ERG recordings demonstrate photoreceptor degeneration in a rod–cone pattern.[47] In selected cases, especially in the later stages of both CRD and RP, rod and cone ERGs may be equally impaired or may even become nonrecordable, which makes a correct diagnosis at this stage more difficult. In such cases, the nature of the initial complaints, the aspect of the fundus and – if available – ERG recordings of an earlier stage of the retinal dystrophy are used to discriminate between RP and CRD.

Mutation screening

The microarray mutation analysis of the *ABCA4* gene with the ABCR400 chip was performed as described earlier. [39]In patients with one *ABCA4* mutation, we searched for additional mutations using single-strand conformation polymorphism (SSCP) analysis and DNA sequence analysis

of aberrantly migrating fragments as described elsewhere (Maugeri *et al*[5] and references therein). The ABI PRISM Big Dye Terminator Cycle Sequencing V2.0 Kit was used for sequencing and the reactions were analyzed with the ABI PRISM 3700 DNA analyzer (Applied Biosystems). Four novel missense mutations were tested in 93 control DNA samples. The presence of R24C (70C>T) was analyzed using *Hin*fI restriction fragment analysis of PCR-amplified exon 2. The normal PCR product (191 nts) was cut into fragments of 167 and 24 nts; the mutant PCR product with the 70C>T alteration was not digested. E161K (481G>A) was tested using *Mbo*II, which cuts the normal PCR product of exon 5 (240 nts) into three fragments (170, 40, 30 nts) and the mutant PCR product into two fragments (200 and 40 nts). The P597S (1789C>T) mutation was analyzed using *Alw*I, which cuts the normal PCR product of exon 13 (280 nts) into fragments of 100, 90, 60, and 30 nts and the mutant PCR product into fragments of 160, 90, and 30 nts. An amplification-refractory mutation-specific (ARMS) assay[48] was performed to test G618E (1853G>A). For specific amplification of the mutant sequence, the SSCP reverse primer and a mutation-specific forward primer (5′ GCAGGACATGGTTGAACAGCA 3′) were used. ARMS cycling parameters consisted of 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 1 min, and a final extension of 5 min at 72°C using 2.0 mM $MgCl_2$.

Results

Mutation analysis

Genotyping was performed on DNA isolated from blood samples of 54 CRD patients and 90 RP patients with the ABCR400 chip. Employing the ABCR400 microarray chip, we identified *ABCA4* mutations in 18 patients (33%) with CRD (Table 1) and in five patients (5.6%) with RP (Table 2). Nine of 54 CRD patients were compound heterozygous (7) or homozygous (2) for *ABCA4* variants; nine were heterozygous. Five out of 90 RP patients were heterozygous for *ABCA4* variants. Although the segregation of the L541P and A1038V mutations could not be tested in the respective families, we have grouped them as complex alleles, based on previous observations that these alterations invariably occur in *cis* configuration in German patients with CRD (Table 1).[5,34] Indeed, all four patients carrying these variants (14488, 14752, 16242, and 16582) were from Germany. Likewise, the 2588C and 2828A variants are presumed to be located in the same allele since the 2588C allele in previous studies was always found together with 2828A (see Discussion).[33,49]

Next, we employed SSCP analysis and DNA sequencing in patients with one *ABCA4* mutation and identified five novel *ABCA4* mutations that were not present on the microarray, that is, R24C, E161K, P597S, G618E, and 5888delG. None of the four new missense mutations could be identified in a panel of 93 healthy control individuals.

Table 1 *ABCA4* sequence variants in CRD patients

CRD patient number	*Inheritance*	*Allele 1*		*Allele 2*		*Mutations segregate*
		Nucleotide change	*Effect*	*Nucleotide change*	*Effect*	
12608	Isolated	IVS38-10T>C	Unknown[a]	IVS38-10T>C	Unknown[a]	Yes
14488	Isolated	1622T>C; 3113C>T	L541P; A1038V	Not identified		NA
14750	Isolated	4918C>T	R1640W	Not identified		NA
14752	Isolated	1622T>C; 3113C>T	L541P; A1038V	IVS38-10T>C	Unknown[a]	?
15105	Isolated	IVS36+2T>C	Splicing	IVS40+5G>A	Splicing	?
15428	Isolated	1622T>C; 3113C>T	L541P; A1038V	2300T>A	V767D	?
15429	Isolated	52C>T	R18W	**70C>T**	**R24C**	?
15680	Isolated	5882G>A	G1961E	Not identified		NA
15730	Isolated	2588G>C; 2828G>A	ΔG863/G863A; R943Q	2588G>C; 2828G>A	ΔG863/G863A; R943Q	?
16242	Isolated	1622T>C; 3113C>T	L541P; A1038V	Not identified	NA	
16243	Isolated	5381C>A **481G>A**	A1794D **E161K**	**1789C>T**	**P597S**	?
16569	Aut. rec.	3259G>A	E1087K	Not identified		NA
16582	Isolated	1622T>C; 3113C>T	L541P; A1038V	IVS38-10T>C	Unknown[a]	?
16583	Isolated	194G>A	G65E	768G>T	Splice site	?
16697	Isolated	2588G>C; 2828G>A[b]	ΔG863/G863A; R943Q	**1853G>A**; 4297G>A	**G618E**; V1433I	Yes
16755	Isolated	2588G>C; 2828G>A	ΔG863/G863A; R943Q	Not identified		NA
16887	Isolated	768G>T	Splicing	IVS38-10T>C	Unknown[a]	Yes
17906	Aut. rec.	768G>T	Splicing	Not identified		NA

[a]Mutation which is presumed to be in linkage disequilibrium with unknown pathologic *ABCA4* mutation.
[b]Polymorphic variants 4203A, 5603T, and 5682C also present. ? = segregation analysis not possible or not determined. NA = not applicable.
Novel variants in bold lettering.

Table 2 *ABCA4* sequence variants in RP patients

RP patient number	*Inheritance*	*Allele 1*		*Allele 2*	
		Nucleotide change	*Effect*	*Nucleotide change*	*Effect*
9304	Aut. Rec.	2588G>C; 2828G>A[a]	ΔG863/G863A; R943Q	**5888delG**	**R1963fs**
9444	Aut. Rec.	6529G>A	D2177N	Not identified	
9545	Isolated	6529G>A	D2177N	Not identified	
14753	Isolated	1622T>C; 3113C>T	L541P; A1038V	Not identified	
17597	Isolated	6148G>C	V2050L	Not identified	

[a]Polymorphic variants 4203A, 5603T, and 5682C also present.
Novel variant in bold lettering.

The 5888delG mutation is predicted to result in the truncation of the second nucleotide-binding domain of the ABCR protein and can thus be considered a null mutation. The four novel missense mutations significantly alter the charge or hydrophobicity of the respective amino-acid residues, which invariably are conserved in mouse Abcr and, with the exception of R24, also in human ABCA1 (data not shown). R24 is located in the N-terminal cytoplasmic domain, one residue next to the first transmembrane domain of ABCR. E161 is located in the first lumenal loop; P597 and G618 are both located in the first cytoplasmic loop.

In three cases with isolated CRD (12608, 16697, and 16887), we were able to perform segregation analysis of the mutations. The mutations in patients 12608 and 16887 were shown to be located on different chromosomes. In family members of patient 16697, the 2588C; 2828A variants segregated from the G618E; V1433I mutations. For the other patients with two or more *ABCA4* variants, no parents or unaffected siblings were available for genetic analyses. The three variants identified in patient 16243 were arbitrarily indicated in the table since we were unable to test their segregation.

Ophthalmologic analysis CRD patients

An overview of the clinical findings in the patients with *ABCA4*-associated CRD is given in Table 3. All patients experienced a loss of central visual acuity as the initial symptom; in most patients a central scotoma was present, varying from 10 to 40 degrees in size. The visual acuity in most of these patients is 20/125 or lower. An exception is patient 15429 with a visual acuity of 20/32 at 53 years, which cannot be attributed to the early stage of the disease progression. Color vision tests could be performed in 11 patients, who all demonstrated a red–green defect. In 10 of the 18 patients, the photopic (cone) responses on the ERG are more severely decreased compared to the scotopic (rod) responses. In patients with nonrecordable or equally reduced cone and rod responses, the diagnosis CRD was based on earlier ERG recordings, the initial symptoms, and the overall aspect of the fundus.

Three patients (14752, 16697, and 16887) presented with yellow flecks, located at the posterior pole and midperiphery (Figure 1b, patient 16887). Two of these individuals were initially diagnosed with STGD1 and demonstrated characteristic blocking of choroidal background fluorescence. However, in the later stage of their disease, these patients developed full-field ERG abnormalities in a cone–rod pattern. These patients should therefore be classified either as STGD1 with peripheral involvement or as CRD.

Ophthalmologic analysis RP patients

The clinical data of the five RP patients with *ABCA4* mutations are summarized in Table 4. Typical RP features like peripheral bone spicules and progressive attenuation of the retinal vasculature were invariably present. Only patient 9304 demonstrated extensive chorioretinal atrophy. Color vision tests could be performed in three patients: all had blue–yellow type defects. In patients 9304 and 14753, no scotopic and photopic ERG responses could be elicited, the other three patients demonstrated a rod–cone pattern of photoreceptor degeneration.

Discussion

In previous *ABCA4* mutation analysis studies, RP patients were ascertained because of their familial relationship with STGD1 patients. In this study, we describe the first systematic search for *ABCA4* mutations in patients with isolated or autosomal recessive RP. In addition, this is the first CRD mutation analysis study that is primarily based on a genotyping microarray. The *ABCA4* gene has shown an extraordinary allelic heterogeneity and most of the sequence variants have been observed in only a few cases. Therefore, the interpretation of the pathologic nature of sequence variants, in particular missense mutations and apparently benign variants, is problematic.

Pathogenicity of *ABCA4* variants

In Table 5, the functional consequences of *ABCA4* missense mutations, that is ABCR protein expression, ATP binding, and ATPase activity, are summarized. Likewise, the known

Table 3 Clinical data of CRD patients with sequence changes identified

No	Sex	Age[a] (years)	Visual acuity Right	Visual acuity Left	Fundus	Perimetry	ERG Photopic	ERG Scotopic
12608	M	40	HM	HM	Peripheral bone spicules and extensive chorioretinal atrophy. Very pale optic disc	Central scotoma of 40 deg.	ND	ND
14488	F	48	CF	20/400	Diffuse, granular RPE changes in macula, peripheral pigmentations	Central scotomas of 25 deg in right eye and 10 deg in left eye	↓↓	↓
14750	M	17	CF	CF	Central hyper- and hypopigmentation of the RPE	Large paracentral scotomas	ND	ND
14752	F	17	20/400	20/400	Yellow spots in the posterior pole. Central RPE changes	Central scotoma of 15–20 deg.	↓↓/ND	↓
15105	M	25	20/200	20/125	Granular aspect of macular RPE	Central scotoma of 10–15 deg	↓↓	↓
15428	F	53	CF	CF	Chorioretinal atrophy at the macula with extensive hypo- and hyperpigmentation	Central scotoma of 10–15 deg. Peripheral constriction (50–60 deg)	↓↓	↓
15429	F	53	20/32	20/32	Large central chorioretinal atrophy with small island	Central scotoma of 15–20 deg	↓↓	↓
15680	F	51	HM	HM	Chorioretinal atrophy, Bull's eye maculopathy	Central scotoma and bad fixation	↓↓ (1985) ↓↓	↓ (1985) ↓↓
15730	M	30	CF	CF	Granular RPE changes at the macula	Right eye: only island 20–40 deg in superior field. Left eye: central scotoma of 20 deg	↓↓ (1985) ND	↓ (1985) ND
16242	F	23	20/200	20/200	Bull's eye with central chorioretinal atrophy	Central scotoma and ringscotoma 20–50 deg	ND	↓↓
16243	M	40	20/125	20/100	Initially granular RPE atrophy at the macula, later geographical atrophy	Central scotoma of 10 deg	↓↓	↓
16569	M	11	20/400	20/400	Granular RPE changes at the macula. No bone spicules and no attenuated retinal vessels	Large central scotomas	↓↓/ND	↓↓
16582	F	19	20/400	20/400	Mild granular changes at the macula	Central scotoma of 20 deg	↓	↓
16583	M	17	20/200	20/400	Granular changes at the macula	Central scotoma of 10–20 deg	↓↓	↓
16697	F	24	20/125	20/125	Bull's eye maculopathy. Mild flecks at posterior pole	(Para)central scotomas	↓	↓
16755	F	62	CF	20/400	Granular RPE changes at the macula	Central scotoma of 30–40 deg	↓↓/ND	↓↓/ND
16887	F	17	20/400	20/400	Central chorioretinal atrophy. Granular changes at midperiphery	Central scotoma	↓	↓
17906	F	14	20/63	20/63	Dark pigmentations at the macula, surrounded by hypopigmented halo	(Para)central scotomas	ND	↓

[a]Age at last visit; CF, counting fingers; HM, hand movements; ND, not detectable; ↓ = decreased; ↓↓ = severely decreased.

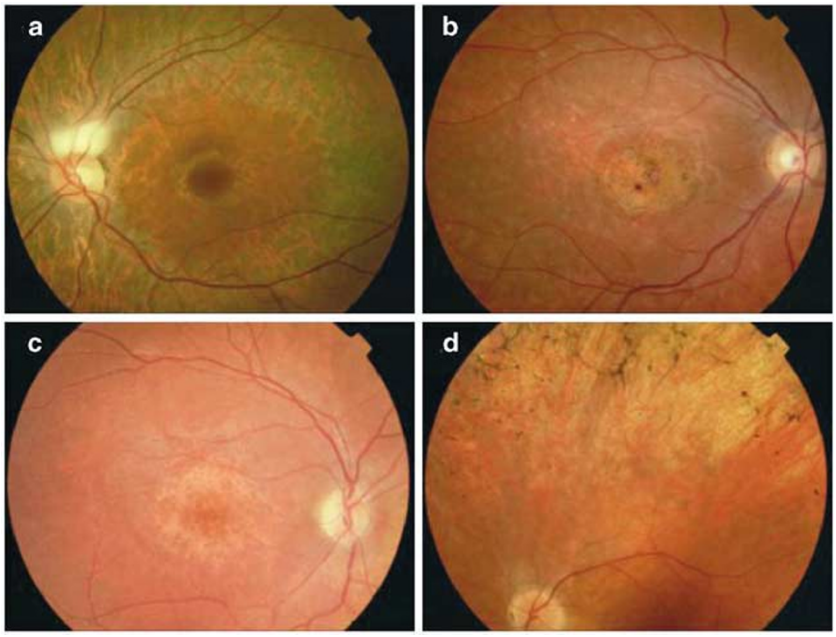

Figure 1 Fundus pictures of patients with CRD and RP. (**a**) CRD patient 15680 with bull's eye maculopathy and temporal pallor of the optic disc (with myelinated nerve fibers). In the periphery (not visible), there are minor hyperpigmentations but the retinal vessels are of normal size. (**b**) Chorioretinal atrophy in the posterior pole of CRD patient 16887. Mild granular changes located at midperipheral retina (not visible). This patient was initially diagnosed as STGD1, in view of the yellow flecks, which are still faintly visible. (**c**) CRD patient 16569, taken at 12 years, with an obvious pallor of the temporal optic nerve head and atrophic changes in the macula. At that time, the photopic ERG is already nonrecordable and the scotopic ERG is severely decreased. (**d**) RP patient 17597 shows typical RP features such as bone spiculas in the periphery and attenuated retinal vessels. Large choroidal vessels can be seen in the midperiphery indicative of atrophic changes.

Table 4 Clinical data of RP patients with sequence changes identified

No	*Sex*	*Age*[a] *(years)*	*Visual acuity* *Right*	*Left*	*Fundus*	*Perimetry*	*ERG* *Photopic*	*ERG* *Scotopic*
9304	F	87	CF	CF	Severe chorioretinal atrophy	Presently, impossible to perform. Ringscotomas in the past	ND	ND
9444	M	61	20/63	20/63	Bone spicules and attenuated vessels. Minor excavation of disc	Ringscotomas	↓ (1982) ND	↓↓ (1982) ND
9545	F	60	20/63	20/200	Bone spicules and attenuated vessels. Glaucomatous aspect of left optic disc	Initially ringscotomas, later progressive constriction of visual field. Also, Bjerrum scotomas, more pronounced in the left eye than in the right eye	↓	↓↓
14753	F	25	20/63	20/63	Gliosis at the macula, attenuated retinal vessels	Central island of 10–15 deg Ringscotoma of 50 deg	ND	ND
17597	F	48	20/20	20/20	Peripheral bone spicule pigmentations, mild attenuation of vessels, midperipheral atrophy	Partial ringscotoma	↓↓/ND	ND

[a]Age at last visit; CF, counting fingers; HM, hand movements; ND, not detectable; ↓ = decreased; ↓↓ = severely decreased.

or predicted effects of the splice site mutations are indicated. For two conservative missense mutations (V1433I and V2050L), the pathologic nature can be questioned. The IVS38-10T>C variant is a splice acceptor variant that has no detrimental effect on splicing, but has been found in 27 of 518 STGD1 patients compared to one of 316 ethnically matched control individuals.[34,35] Therefore, it is very well possible that the IVS38-10C variants

Table 5 Functional assessment of missense (A) and splice site (B) mutations

(A) *Missense mutation*	*Nature of amino-acid change*	*Effect on ABCR function*[Ref]
R18W	Nonconservative	Unknown
R24C	Nonconservative	Unknown; adjacent to first transmembrane domain
G65E	Nonconservative	Unknown
E161K	Nonconservative	Unknown
L541P	Conservative	Decreased ATP binding and ATPase activity[50]
P597S	Nonconservative	Unknown
G618E	Nonconservative	Unknown
V767D	Nonconservative	Decreased ABCR expression[10]
G863A	Nonconservative	Decreased ATPase activity[50, 51]
R943Q	Nonconservative	Decreased ATPase activity[51]
A1038V	Conservative	Decreased ATP binding and ATPase activity[50]
E1087K	Nonconservative	Decreased ATP binding[50]
V1433I	Conservative	Unknown
R1640W	Nonconservative	Unknown
A1794D	Nonconservative	Introduction charged aa in 10th transmembrane domain
G1961E	Nonconservative	Decreased ATP binding and ATPase activity [50]
V2050L	Conservative	Unknown
D2177N	Nonconservative	Increased ATPase activity[50]
(B) *Splice site mutation*	*Effect on mRNA*[Ref]	*Predicted effect on ABCR protein*
768G>T	Nonsense-mediated decay[33]	No protein
IVS36+2T>C	Unknown	Truncation of exon 36 resulting in V1673fs?
IVS38-10T>C	No effect[34]	Variant in linkage disequilibrium with unknown mutation
IVS40+5G>T	350-bp insertion in ~50% of mRNA[34]	Insertion of aberrant amino-acid stretch?

observed in five CRD alleles in our patient cohort are in linkage disequilibrium with an unidentified pathologic *ABCA4* mutation. In two RP patients, we identified the D2177N mutation heterozygously. The D2177N mutation has never been found in patients with STGD1 but was found to be associated with age-related macular degeneration at a statistically significant level.[25] As shown by Sun *et al*,[50] this mutation, contrary to other mutations, results in increased ATP hydrolysis when compared to the wild-type protein. These data do not allow us to draw a definitive conclusion regarding the pathologic nature of D2177N.

An unexpected finding is the detection of the 2588C variant in four patients; homozygous in one CRD patient and heterozygous in three CRD/RP patients. Based on a genotype–phenotype correlation model proposed by us and others, individuals carrying two 2588C alleles would not be expected to show retinal pathology since this variant was deemed a mild allele.[33,49] In three of the 2588C carrying haplotypes (in patients 15730 and 16755, Table 1), the 2588C and 2828A variants are not accompanied by the polymorphic variants 4203A, 5603T, and 5682C (data not shown), but might have been linked to a more severe mutation in the 3′ part of the gene. Also, the 2588C variant has been found in *cis* with an intragenic deletion spanning exon 14 of the *ABCA4* gene in a French RP patient.[4] Secondly, other genetic factors might have a significant effect on the phenotypic outcome of *ABCA4* mutations. This was recently demonstrated in one of two siblings with autosomal dominant STGD3-associated macular dystrophy in which a heterozygous *ABCA4* mutation aggravated the retinal dystrophy.[52] Finally, in view of the high carrier frequency of the 2588C allele in the Dutch and German populations, patients 15730 and 16755, who only carry the 2588C variant(s), might do so by chance.

Phenotypic spectrum of CRD and RP patients with *ABCA4* mutations

CRD patients present with a substantial clinical heterogeneity as observed in other studies.[7,13,16,17] This variability is expressed in the rate of visual loss, the extend of the visual field defects, and the ophthalmoscopic appearance. Three of the 18 *ABCA4*-associated CRD patients in this study represent a subtype that initially resembles STGD1 but, contrary to the classic juvenile macular degeneration of Stargardt, progresses to a more widespread loss of cone and rod photoreceptors. Ideally, improved genotype–phenotypes correlations in the future would enable the early detection of STGD1 patients that are at risk for progression to this CRD phenotype.

Thus far, patients with RP and *ABCA4* mutations have demonstrated a remarkably homogeneous phenotype, characterized by severe loss of visual functions, extensive atrophy, and early loss of ERG responses.[2–4,10,12,15,17] In this study, only one of five RP patients with *ABCA4* variants (9304) demonstrates this characteristic atrophy. The RP phenotype in the remaining patients is moderately severe, with variable atrophy; in addition, ERG responses can often still be elicited. Given this clinical presentation and the fact that homozygous null mutations were not found

in these patients, it is possible that the *ABCA4* mutations did not contribute to the RP phenotype in some or all of these four patients. If, on the other hand, alterations in the *ABCA4* gene are responsible for these RP phenotypes, the phenotypic variability of *ABCA4*-related RP is higher than assumed.

ABCA4 involvement in CRD and RP patients

Genotyping of 90 RP patients revealed sequence variations in the *ABCA4* gene in five individuals. As discussed above, only one of these patients shows the ophthalmologic features seen in other RP patients with *ABCA4* mutations. Taken into consideration the high heterozygosity frequency of *ABCA4* mutations in the general population, these data strongly suggest that *ABCA4* mutations are only a minor cause (2–5%) of arRP not exceeding the contribution of most other arRP genes (http://www.sph.uth.tmc.edu/Retnet).

We also identified 27 putative pathologic *ABCA4* alleles in 18 of 54 (33%) patients with CRD. Four additional missense mutations in three of these patients were identified using SSCP and sequence analysis. Besides the *ABCA4* gene, only one other gene (retinol dehydrogenase 5 – *RDH5*) and two loci (CORD8 on 1q12–q24 and CORD9 on 8p11) have been implicated in arCRD.[36–38] If earlier data are combined with the results of this study, *ABCA4* mutations are found in 40% of the arCRD cases (Table 6). It can be estimated that, on average, the mutation detection efficiency for *ABCA4* mutations is 60% (Jaakson *et al*.[39] and references therein), suggesting that *ABCA4* mutations will be present in approximately 67% of arCRD cases.

Microarray analysis as a tool for DNA diagnostics in CRD and RP

The analysis of the *ABCA4* gene is of importance to establish the mode of disease inheritance in CRD families, which is associated with very different recurrence risks in the offspring of mutation carriers. In the future, genotyping may also be helpful to accurately predict the development of *ABCA4*-associated retinal dystrophies, especially for the subgroup of patients, initially diagnosed as STGD1 with subsequent progression to CRD. In addition, identification of patients with causal *ABCA4* mutations might become very important if novel insights regarding *ABCA4*-associated pathology and treatment of Abcr(−/−) mice develop into rational therapeutics for human patients. It is likely that mutation chip technology, which enables fast, reliable, and cost-efficient mutation analysis, will play an important role in these future developments.

Table 6 Incident of *ABCA4* mutations in different cohorts of CRD patients

Study	*Number of CRD patients analysed*	*Number of patients with ABCA4 mutations*
Maugeri *et al*[5]	20	13
Papaioannou *et al*[6]	8	4
Birch *et al*[7]	30	11
Briggs *et al*[8]	8	6
Paloma *et al*[9]	2	2
Ducroq *et al*[11]	55	13
Fishman *et al*[16]	30	16
Current study	54	18
Total	207	83 (40%)

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