ABCA4 gene analysis in patients with autosomal recessive cone and cone rod dystrophies

Veronique B.D. Kitiratschky[1], Tanja Grau[1], Antje Bernd[2], Eberhart Zrenner[2], Herbert Jägle[2], Agnes B. Renner[3], Ulrich Kellner[3,4], Günther Rudolph[5], Samuel G. Jacobson[6], Artur V. Cideciyan[6], Simone Schaich[1], Susanne Kohl[1], and Bernd Wissinger[1]

1 *Molecular Genetics Laboratory, Institute for Ophthalmic Research, Centre for Ophthalmology, University Tübingen, Tübingen, Germany*

2 *Institute for Ophthalmic Research, Centre for Ophthalmology, University Tübingen, Tübingen, Germany*

3 *Department of Ophthalmology, Charité Campus Benjamin Franklin, Berlin, Germany*

4 *RetinaScience, Bonn, Germany*

5 *Department of Ophthalmology, Ludwig Maximilians University, Munich, Germany*

6 *Scheie Eye Institute, Department of Ophthalmology, University of Pennsylvania, Philadelphia, USA*

Abstract

The ATP-binding cassette (ABC) transporters constitute a family of large membrane proteins which transport a variety of substrates across membranes. The ABCA4 protein is expressed in photoreceptors and possibly functions as a transporter for N-retinylidene-phosphatidylethanolamine (N-retinylidene-PE), the Schiff base adduct of all-*trans*-retinal with PE. Mutations in the *ABCA4* gene have been initially associated with autosomal recessive Stargardt disease. Subsequent studies have shown that mutations in *ABCA4* can also cause a variety of other retinal dystrophies including cone rod dystrophy and retinitis pigmentosa. To determine the prevalence and mutation spectrum of *ABCA4* gene mutations in non-Stargardt phenotypes we have screened 64 unrelated patients with autosomal recessive cone (arCD) and cone rod dystrophy (arCRD) applying the Asper Ophthalmics ABCR400 microarray followed by DNA sequencing of all coding exons of the *ABCA4* gene in subjects with single heterozygous mutations.

Disease-associated *ABCA4* alleles were identified in 20 of 64 patients with arCD or arCRD. In four of 64 patients (6%) only one mutant *ABCA4* allele was detected and in 16 patients (25%), mutations on both *ABCA4* alleles were identified. Based on these data we estimate a prevalence of 31% for *ABCA4* mutations in arCD and arCRD, supporting the concept that the *ABCA4* gene is a major locus for various types of degenerative retinal diseases with abnormalities in cone or both cone and rod function.

Introduction

The ATP-binding cassette (ABC) transporters constitute a family of large membrane proteins which transport a variety of substrates across membranes [1]. One member of the family, the ABCA4 protein, is expressed in cone and rod photoreceptors [2] and possibly functions as a transporter for N-retinylidene-phosphatidylethanolamine (N-retinylidene-PE), the Schiff base adduct of all-*trans*-retinal with PE [3]. It has been proposed that a failure in the transport of all-

Corresponding Author: Bernd Wissinger, Molecular Genetics Laboratory, Röntgenweg 11, 72076 Tübingen, Germany, Phone: +49 7071 2985032, Fax: +49-7071-295725, E-mail: wissinger@uni-tuebingen.de.

trans retinal from the luminal to the cytosolic side of photoreceptor discs may disrupt the visual cycle. Finally, it may lead to accumulation of N-retinylidene-PE and toxic lipofuscin A2-E in the RPE upon phagocytosis of photoreceptors. *ABCA4* gene mutations have been initially identified in patients with autosomal recessively inherited Stargardt disease and fundus flavimaculatus (both OMIM ID 248200) [4,5] and subsequently also in autosomal recessively inherited cone rod dystrophy (arCRD, OMIM ID 604116) [6] and retinitis pigmentosa (OMIM ID 601718) [6–8]. Moreover, single heterozygous *ABCA4* sequence variants have been proposed as a susceptibility factor for age-related macular degeneration (OMIM ID 153800) [9]. Consequently, a genotype-phenotype correlation based on the residual function of the ABCA4 protein has been suggested [10]. Mutations in *ABCA4* are the principal cause of Stargardt disease. However, predictions of the prevalence of *ABCA4* gene mutations in arCRD have varied considerably between studies, ranging from 23 to 65% [11–14]. Moreover, it has not yet been investigated whether *ABCA4* mutation may also be involved in autosomal recessive cone dystrophy (arCD) with no detectable impairment of the rod system. In order to address these questions we investigated the prevalence of *ABCA4* gene mutations in a large and clinically well defined group of patients affected by arCD or arCRD.

Methods

Mutation analysis

Patients diagnosed with CD or CRD and a family history consistent with an autosomal recessive mode of inheritance were included in the study and recruited in four different centers (Centre for Ophthalmology, University Tübingen, Tübingen, Germany; Scheie Eye Institute, Department of Ophthalmology, University of Pennsylvania, Philadelphia, USA; Department of Ophthalmology, Charité Campus Benjamin Franklin, Berlin, Germany; Department of Ophthalmology, Ludwig Maximilians University, Munich, Germany). The study was performed according to the tenets of the Declaration of Helsinki and approved by the ethical committees of the participating institutions. Informed consent was obtained from all patients.

Sixty-four patients met the inclusion criteria, forty-six patients had arCRD, eighteen patients had arCD. All patients were subsequently screened for *ABCA4* gene mutations. The initial mutation screening was performed with the 10th version of the ABCR400 microarray (AsperBiotech, Tartu, Estonia) which queries >450 known sequence variants. The genotyping procedure included PCR amplification of all *ABCA4* exons, fragmentation of amplification products, and solid-phase mini-sequencing, also termed arrayed primer extension technology (APEX), which has been described in detail elsewhere [15]. Mutations identified by microarray genotyping were confirmed by conventional DNA sequencing of the relevant exon(s). For patients with only one mutation identified via chip analysis, all coding exons of the *ABCA4* gene were amplified and sequenced employing the VariantSEQr Resequencing System (Applied Biosystems, Darmstadt, Germany) according to manufacturer's instructions. DNA sequencing was done with BigDye Sequencing Chemistry and products were separated on a 3100 capillary sequencer (both Applied Biosystems).

Segregation analysis and exclusion of a novel sequence variant in healthy controls

Segregation analysis in family members was performed by direct DNA sequencing of the respective exon(s). The novel sequence variant c.4848+2T>C was evaluated in 123 healthy control individuals (246 chromosomes) by means of PCR/RFLP with the restriction enzyme Csp6I (Fermentas, Glen Burnie, Maryland). PCR products were digested overnight and the RFLP pattern evaluated by agarose gel electrophoresis.

Patients and evaluation of clinical phenotype

The clinical records of patients with one or two mutant *ABCA4* alleles were analysed retrospectively. The diagnoses of arCD and arCRD were mainly based on the results of full field electroretinography (ERG). Patients with reduced cone ERGs and normal rod ERGs were diagnosed as arCD, whereas patients with reduced cone and rod ERGs were defined as arCRD. Characteristic symptoms and signs, fundus appearance and visual field results were used to corroborate the diagnosis [16–18]. A standardised spreadsheet was used and the patient records were evaluated for the following information: onset age; disease duration; best corrected visual acuity (BCVA); color vision tested with saturated or desaturated Panel D15 test, or Arden color vision test; glare sensitivity; night vision symptoms; funduscopic appearance; kinetic and/or static perimetry and full field ERGs according to ISCEV standard (except for patient RCD63/3402 who received an ERG recording before introduction of ISCEV standards). Details of the ERG methodology and the normal data used by the contributing clinical authors have been published [18,19]. Mean visual acuities were calculated by determining the logarithmic mean [20] with qualitative visual acuity measures scaled as follows: counting fingers - 0,0014; hand movements - 0,0052 according to [21]; light perception - 0,0005 and no light perception - 0,0001 according to [22].

Results

Prevalence and mutation spectrum of ABCA4 mutations in patients with arCD and arCRD

Among the 64 unrelated patients, 46 patients with arCRD, 18 patients with arCD, who were screened with the ABCR400 gene chip, *ABCA4* gene mutations were identified in 20 (31%) patients. Homozygous or compound heterozygous mutations were identified in 13 of the 64 (20%) patients, and single heterozygous mutations were identified in another 7 (11%) patients. All mutations identified by microarray analysis were confirmed by DNA sequencing. By design, the chip only detects known sequence variants and thus misses novel mutations which have not yet been integrated onto the chip. In order to identify the second mutant allele, we sequenced all coding exons of the *ABCA4* gene in those 7 patients in whom the ABCR400 chip detected only a single disease associated allele. This resulted in the identification of three novel sequence variants (c.700C>T, c.3539_3554del, and c.4848+2T>C) in three unrelated patients.

Genotypes for all patients with one or two disease-associated alleles are given in Table 1. In total, microarray and the "all-exon-sequencing" approach together identified 36 mutant *ABCA4* alleles in 64 patients, representing 24 different sequence variants, including the complex allele [c.1622T>C;c.3113C>T] (Table 2).

In our study group missense mutations were most prevalent (17 of 37 mutations; 46%), followed by intronic and splice site mutations (12/37; 32%), nonsense mutations (4/37; 11%), and small deletions (4/37; 11%). Most sequence variants appeared only once. In contrast, the c.5461-10T>C allele was found in 8 patients and thus was the most prevalent sequence variant in this study.

The *ABCA4* gene mutation prevalence was higher in arCRD (16/46, 35%) than in arCD (4/18, 22%).

Classification of newly identified sequence alterations

Based on our "all-exon-sequencing" strategy in subjects in whom only a single heterozygous mutation was detected by DNA chip analysis we identified three new sequence alterations that most likely represent disease-causing mutations. Two of the sequence variants are predicted to result in a truncated ABCA4 protein: c.700C>T constitutes an early stop mutation (p.Q234X), while the c.3539_3554del deletion leads to a frameshift and subsequent

introduction of a premature termination codon after eight altered amino acid residues (p.S1181PfsX8). Furthermore, we observed a concordant segregation of c.3539_3554del in family ZD291 (Fig. 1). The third novel sequence alteration c.4848+2T>C affects the canonical GT-dinucleotide of the donor splice site of intron 34 and thus most likely impairs splicing of mutant transcripts. In addition, this sequence variant was not found in 246 chromosomes of healthy control individuals.

Segregation analysis in families affected by arCD and arCRD

We performed segregation analysis in all families in which samples from additional family members were available (Fig. 1). For an autosomal recessive mode of inheritance homozygous or compound heterozygous mutations in affected patients, and single heterozygous or no mutations in unaffected relatives is required, which we will call concordant segregation. Furthermore, independent segregation of mutations in *trans* (in subjects carrying two heterozygous mutations) is needed. A number of complex alleles (i.e. two sequence variants in *cis*) has been described in *ABCA4* [23,24]. In total, independent and concordant segregation of the two mutations on different alleles could be demonstrated in 9 families: RCD79, ZD289, RCD9, ZD291, ZD85, ZD290, RCD113, RCD337, RCD143. Three families did not fulfill all of the above stated segregation criteria. In family RCD 157 both affected siblings were concordant in being heterozygous for two mutations but we could not prove independent segregation because no other family members were available for segregation analysis. Only one single heterozygous sequence variant was identified during screening of the index patient in RCD63 and could be followed in that family. However, the presence of this mutation in all three affected siblings at least supports a causal relationship with the *ABCA4* locus in this family. Pedigree RCD141 involves the marriage of two affected subjects (II:2 and II:3) and affected offspring. For II:3 concordant and independent segregation of two identified mutations can be demonstrated. The second *ABCA4* mutation in the pedigree remains unidentified. Yet the segregation pattern is fully compatible with an autosomal recessive inheritance and linkage with the *ABCA4* locus.

Clinical description of patients with arCD and arCRD and ABCA4 gene mutations

The clinical data of the 20 index patients with at least one identified *ABCA4* gene mutation were evaluated in detail (Table 3). Most patients had arCRD (16/20). Four patients had arCD, one of those with a differential diagnosis of arCRD. For both disease groups together, arCD and arCRD, the median age of onset was 9 years (range 2 to 27 years) and the mean visual acuity was 0,04 (range: light perception [digitised as 0.0005 according to [22]] to 0,06) after a median disease course of 22 years (range: six months to 44 years). Color vision testing performed in 14 patients indicated minor to severe color vision abnormalities. Six patients showed minor confusions or confusions along the red-green or blue-yellow axis, while five patients, all affected by arCRD, had severe color vision impairment (i.e. chaotic arrangements in the PD15 test) or were unable to discriminate colors. In contrast, two patients, one of them with the diagnosis of arCD (RCD102/6810, RCD92/6809), had normal color vision with the color vision tests we used. Signs of retinal dystrophy such as pigment clumping and atrophy of RPE or choroid were present in almost all patients. All patients had a central scotoma. Peripheral visual field of patients with arCD was normal, whereas about half of the patients with arCRD had constricted visual fields. Two patients (RCD63/3402, RCD143/8805), both affected by arCRD, had extensive central and peripheral visual field defects with only islands of vision remaining. Patients affected by arCD had abnormal cone ERGs, and patients affected by arCRD had both abnormal rod and cone ERGs. Cone ERGs were not detectable in two patients (ZD289/781, RCD143/8805) and in another two patients (RCD185/11721, RCD147/9136) both rod and cone ERGs were abolished. Patients diagnosed with arCRD normally presented with both cone and rod photoreceptor dysfunction at their first visit. But in one patient (ZD289/781/F) who had a diagnosis of arCD even after 16 years of disease

duration, a late occurring rod dysfunction was finally observed, resulting in the change of the diagnosis to arCRD.

Discussion

Prevalence of ABCA4 gene mutations in arCRD and arCD

In this study we evaluated the prevalence and spectrum of *ABCA4* gene mutations in 64 patients affected by arCD and arCRD, using a microarray-based approach and subsequent "all-exon-sequencing". To our knowledge this is the largest number of arCD and arCRD patients assessed by a microarray-based *ABCA4* mutation screening and the first time that *ABCA4* gene mutations have been identified to cause arCD.

The reported prevalence of *ABCA4* gene mutations in patients affected by arCRD varies among different studies, ranging from one fourth to almost two thirds of the studied populations [11, 12,14,25–27]. A recent investigation which combined previously published data with their own data calculated that *ABCA4* gene mutations are found in 40% of the arCRD patients [13]. Our findings are consistent with previous reports as we identified *ABCA4* gene mutations in 31% of our cohort. The different prevalences observed between studies might be attributed to ascertainment bias due to the relatively small sample sizes in previous reports, ranging from 8 to 54 patients. The careful ascertainment of patients is also an important issue for exact determination of *ABCA4* gene mutation prevalence in arCRD, since inclusion of patients with Stargardt disease, a phenotype with a relatively high *ABCA4* gene mutation prevalence of 58 to 75 % [23,28], or arRP with an *ABCA4* gene mutation prevalence of around 5% [13], will bias the result.

In the current study we performed the "all-exon-sequencing" only in selected patients who already had one identified mutant allele after microarray analysis. Thus, the actual prevalence of *ABCA4* mutations in patients with arCD and arCRD could be higher. We also cannot exclude that we might have missed genomic rearrangement mutations based on the applied methods. Taken together, the ABCR400 microarray is rapid, cost-effective and useful both in genetic diagnostics and research as a first screening tool.

The c.5461-10T>C mutation was the most common sequence variant in our patient sample, present both in patients with adCD and adCRD. It has been questioned whether this sequence variant is pathogenic on its own because heterologous expression of this mutation failed to show a splicing defect [23]. Therefore it has been suggested that the c.5461-10T>C is in linkage disequilibrium with another yet unknown pathogenic mutation. We found that 6 of 8 patients carrying the c.5461-10T>C variant were also heterozygous for known pathogenic *ABCA4* mutations, a much higher number than expected by chance.

Grading of ABCA4 mutations

A model which correlates phenotypes of retinal diseases with the residual activity of the mutant ABCA4 protein has been suggested [10]. Depending on a frequent founder mutation, c. 2588G>C, which has been assumed to be mild, a model has been proposed that categorises mutations as null, moderately severe, or mild. A combination of two null mutations should lead to retinitis pigmentosa, whereas a combination of a null and a moderately severe mutation should cause CRD. Our data, however, do not support such a clear cut genotype-phenotype correlation. For example, two of our arCRD patients (RCD79/6184 and RCD113/3668) are homozygous for putative null alleles (p.Y245X and p.V1973X). Another arCRD patient (RCD9/1989) harbours two mutations (p.P1380L and p.R2077W) which are both characterised by substantially impaired ATP-binding [29]. A fourth arCRD patient (RCD92/6809) is homozygous for the missense mutation p.L244P. Interestingly, a patient who is also a

homozygote for p.L244P, but suffers from Stargardt disease has been described in the literature [28]. Moreover, there is no higher proportion of nonsense or truncating mutations in our sample of CRD and CD patients compared with that of a cohort comprised of Stargardt patients only [23]. Yet, we noted that certain mutations which are highly prevalent in Stargardt patients (recruited from a comparable population [23]), for example, the c.5882G>A, the c.3113C>T, and c.2588G>C mutations were rare in our sample; in contrast, the c.5461-10T>C variant is less common in Stargardt patients. Thus, although the model is attractive, it may not accurately predict the expression of the retinal disease. It has become clear in recent years that not only complex but also Mendelian diseases are shaped by modifying genetic or environmental factors [30,31]. A model which includes the effect of modifying factors may increase understanding of the connection between genotype (at potentially various genetic loci), environment, and phenotype.

In conclusion, we performed mutation analyses and provided data on the prevalence of *ABCA4* gene mutations in patients with arCD and arCRD. Our data confirm and extend previous investigations that identified the *ABCA4* gene as the major locus responsible for autosomal recessive retinal dystrophies, including arCD and arCRD.

Acknowledgements

References

1. Borst P, Zelcer N, van Helvoort A. ABC transporters in lipid transport. Biochim Biophys Acta 2000;1486:128–144. [PubMed: 10856718]

2. Molday LL, Rabin AR, Molday RS. ABCR expression in foveal cone photoreceptors and its role in Stargardt macular dystrophy. Nat Genet 2000;25:257–258. [PubMed: 10888868]

3. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. Cell 1999;98:13–23. [PubMed: 10412977]

4. Allikmets R, Singh N, Sun H, et al. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat Genet 1997;15:236–246. [PubMed: 9054934]

5. Rozet JM, Gerber S, Souied E, et al. Spectrum of ABCR gene mutations in autosomal recessive macular dystrophies. Eur J Hum Genet 1998;6:291–295. [PubMed: 9781034]

6. Cremers FP, van de Pol DJ, van Driel M, et al. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. Hum Mol Genet 1998;7:355–362. [PubMed: 9466990]

7. Martinez-Mir A, Paloma E, Allikmets R, et al. Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene ABCR. Nat Genet 1998;18:11–12. [PubMed: 9425888]

8. Rudolph G, Kalpadakis P, Haritoglou C, Rivera A, Weber BH. Mutations in the ABCA4 gene in a family with Stargardt's disease and retinitis pigmentosa (STGD1/RP19). Klin Monatsbl Augenheilkd 2002;219:590–596. [PubMed: 12353176]

9. Allikmets R, Shroyer NF, Singh N, et al. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science 1997;277:1805–1807. [PubMed: 9295268]

10. Maugeri A, van Driel MA, van de Pol DJ, et al. The 2588G-->C mutation in the ABCR gene is a mild frequent founder mutation in the Western European population and allows the classification of ABCR mutations in patients with Stargardt disease. Am J Hum Genet 1999;64:1024–1035. [PubMed: 10090887]

11. Ducroq D, Rozet JM, Gerber S, et al. The ABCA4 gene in autosomal recessive cone-rod dystrophies. Am J Hum Genet 2002;71:1480–1482. [PubMed: 12515255]

12. Fishman GA, Stone EM, Eliason DA, Taylor CM, Lindeman M, Derlacki DJ. ABCA4 gene sequence variations in patients with autosomal recessive cone-rod dystrophy. Arch Ophthalmol 2003;121:851–855. [PubMed: 12796258]

13. Klevering BJ, Yzer S, Rohrschneider K, et al. Microarray-based mutation analysis of the ABCA4 (ABCR) gene in autosomal recessive cone-rod dystrophy and retinitis pigmentosa. Eur J Hum Genet 2004;12:1024–1032. [PubMed: 15494742]

14. Maugeri A, Klevering BJ, Rohrschneider K, et al. Mutations in the ABCA4 (ABCR) gene are the major cause of autosomal recessive cone-rod dystrophy. Am J Hum Genet 2000;67:960–966. [PubMed: 10958761]

15. Jaakson K, Zernant J, Kulm M, et al. Genotyping microarray (gene chip) for the ABCR (ABCA4) gene. Hum Mutat 2003;22:395–403. [PubMed: 14517951]

16. Hamel C. Cone rod dystrophies. Orphanet Journal of Rare Diseases 2007;2:7. [PubMed: 17270046]

17. Szlyk JP, Fishman GA, Alexander KR, Peachey NS, Derlacki DJ. Clinical subtypes of cone-rod dystrophy. Arch Ophthalmol 1993;111:781–788. [PubMed: 8512479]

18. Yagasaki K, Jacobson SG. Cone-rod dystrophy. Phenotypic diversity by retinal function testing. Arch Ophthalmol 1989;107:701–708. [PubMed: 2719580]

19. Kellner U, Wissinger B, Tippmann S, Kohl S, Kraus H, Foerster MH. Blue cone monochromatism: clinical findings in patients with mutations in the red/green opsin gene cluster. Graefes Arch Clin Exp Ophthalmol 2004;242:729–735. [PubMed: 15069569]

20. Bach M, Kommerell G. Determining visual acuity using European normal values: scientific principles and possibilities for automatic measurement. Klin Monatsbl Augenheilkd 1998;212:190–195. [PubMed: 9644663]

21. Schulze-Bonsel K, Feltgen N, Burau H, Hansen L, Bach M. Visual acuities "hand motion" and "counting fingers" can be quantified with the freiburg visual acuity test. Invest Ophthalmol Vis Sci 2006;47:1236–1240. [PubMed: 16505064]

22. Paunescu K, Preising MN, Janke B, Wissinger B, Lorenz B. Genotype-phenotype correlation in a German family with a novel complex *CRX* mutation extending the open reading frame. Ophthalmology 2007;114:1348–1357. [PubMed: 17320181]

23. Rivera A, White K, Stohr H, et al. A comprehensive survey of sequence variation in the ABCA4 (ABCR) gene in Stargardt disease and age-related macular degeneration. Am J Hum Genet 2000;67:800–813. [PubMed: 10958763]

24. Shroyer NF, Lewis RA, Yatsenko AN, Lupski JR. Null missense ABCR (ABCA4) mutations in a family with stargardt disease and retinitis pigmentosa. Invest Ophthalmol Vis Sci 2001;42:2757–2761. [PubMed: 11687513]

25. Papaioannou M, Ocaka L, Bessant D, et al. An analysis of ABCR mutations in British patients with recessive retinal dystrophies. Invest Ophthalmol Vis Sci 2000;41:16–19. [PubMed: 10634594]

26. Birch DG, Peters AY, Locke KL, Spencer R, Megarity CF, Travis GH. Visual function in patients with cone-rod dystrophy (CRD) associated with mutations in the ABCA4(ABCR) gene. Exp Eye Res 2001;73:877–886. [PubMed: 11846518]

27. Valverde D, Riveiro-Alvarez R, Aguirre-Lamban J, et al. Spectrum of the ABCA4 gene mutations implicated in severe retinopathies in Spanish patients. Invest Ophthalmol Vis Sci 2007;48:985–990. [PubMed: 17325136]

28. Briggs CE, Rucinski D, Rosenfeld PJ, Hirose T, Berson EL, Dryja TP. Mutations in ABCR (ABCA4) in patients with Stargardt macular degeneration or cone-rod degeneration. Invest Ophthalmol Vis Sci 2001;42:2229–2236. [PubMed: 11527935]

29. Sun H, Nathans J. ABCR, the ATP-binding cassette transporter responsible for Stargardt macular dystrophy, is an efficient target of all-trans-retinal-mediated photooxidative damage in vitro. Implications for retinal disease. J Biol Chem 2001;276:11766–11774. [PubMed: 11278627]

30. Nadeau JH. Modifier genes in mice and humans. Nat Rev Genet 2001;2:165–174. [PubMed: 11256068]

31. Weatherall DJ. Phenotype-genotype relationships in monogenic disease: lessons from the thalassaemias. Nat Rev Genet 2001;2:245–255. [PubMed: 11283697]
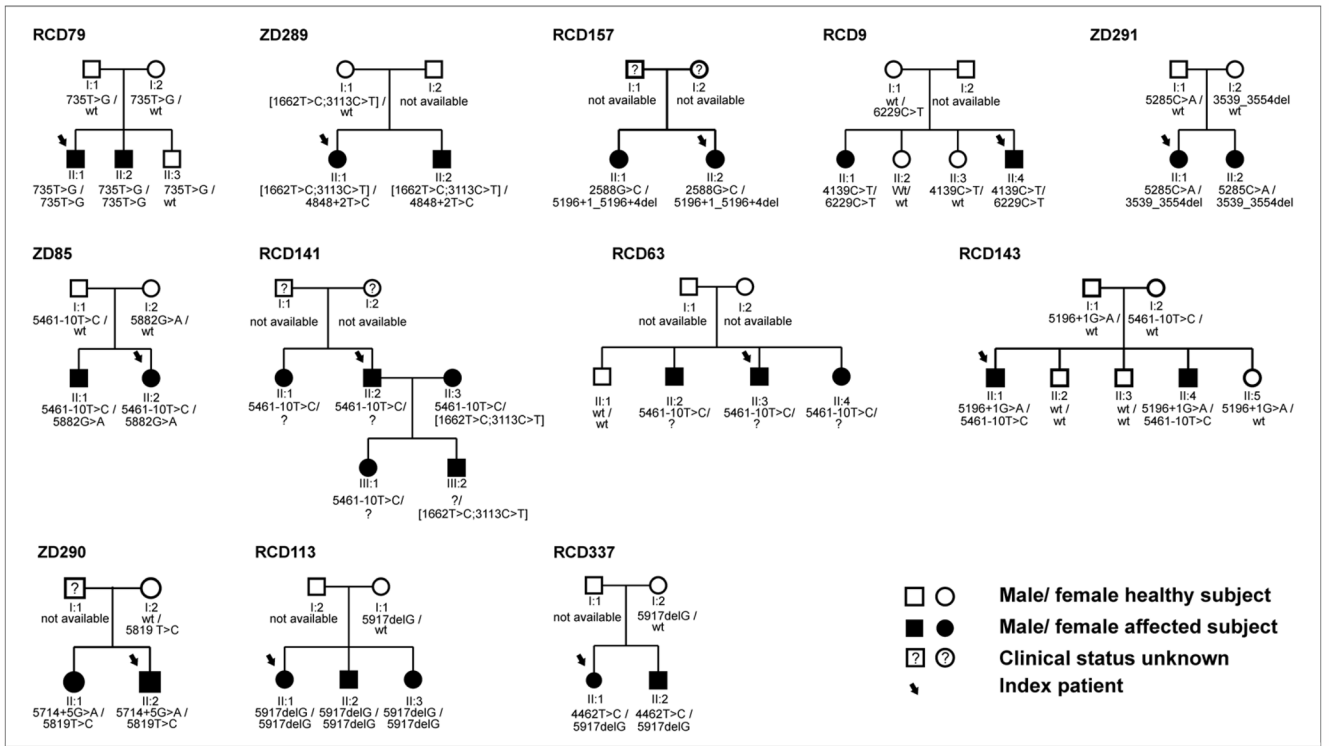
Figure 1.
Pedigrees of arCD and arCRD families segregating ABCA4 gene mutations. Index patients
used for the ABCA4 microarray screening and subsequent DNA sequencing are marked by an
arrow. Genotypes of all available family members are listed below the respective subject. A
suspected but not identified second mutation in the ABCA4 gene is indicated with a question
mark. Pedigrees are arranged from left to right and top to bottom in the same order as patients
are listed in Table 1. The pedigree number is given above left to each pedigree.

Table 1

Genotypes of arCRD and arCD index patients screened for *ABCA4* mutations.

Family ID/Patient ID/Gender	Allele 1	Allele 2	Segregation in family	Diagnosis
RCD92/6809/F	c.731 T>C [a]	c.731 T>C	n.a.	CRD
RCD79/6184/M	c.735 T>G	c.735 T>G	yes	CRD
ZD289/781/F	[c.1622T>C;c.3113C>T]	c.4848+2T>C	yes	CRD
RCD157/10492/F	c.2588G>C	c.5196+1_c.5196+4del	(yes)	CRD
RCD51/4984/F	c.3608G>A	n.d.	n.a.	CRD
RCD9/1989/M	c.4139C>T	c.6229C>T	yes	CRD
RCD42/2509/M	c.4139C>T	c.1928T>G	n.a.	CRD
ZD291/5781/F	c.5285C>A	c.3539_c.3554del	yes	CD
ZD85/5862/F	c.5461-10T>C	c.5882G>A	yes	CD
RCD147/9136/F	c.5461-10T>C	c.700C>T	n.a.	CRD
RCD141/8594/M	c.5461-10T>C	n.d.	(yes)	CRD
RCD63/3402/M	c.5461-10T>C	n.d.	(yes)	CRD
RCD143/8805/M	c.5461-10T>C	c.5196+1G>A	yes	CRD
ZD146/10313/F	c.5461-10T>C	c.2588G>C	n.a.	CD, DD: CRD
RCD98/3474/F	c.5461-10T>C	c.4234C>T	n.a.	CRD
RCD194/10840/M	c.5461-10T>C	c.4457 C>T	n.a.	CRD
ZD290/6810/M	c.5714+5G>A	c.5819T>C	yes	CD
RCD113/3668/F	c.5917delG	c.5917delG	yes	CRD
RCD337/8019/F	c.5917delG	c.4462T>C	yes	CRD
RCD185/11721/F	c.6148G>C	n.d.	n.a.	CRD

DD, differential diagnosis

Table 2

Spectrum of *ABCA4* gene mutations observed in patients with arCRD and arCD.

Type of Mutation/Exon	Nucleotide change	Effect	No. of alleles	Reference
Missense:				
6	c.731T>C [a]	p.L244P	2	28
12	c.1622T>C [b]	p.L541P	1	5
13	c.1928T>G	p.V643G	1	9
17	c.2588G>C	p.G863A and p.G863del	2	4
21	c.3113C>T [b]	p.A1038V	1	4
25	c.3608G>A	p.G1203E	1	32
28	c.4139C>T	p.P1380L	2	33
30	c.4457C>T	p.P1486L	1	33
30	c.4462T>C	p.C1488R	1	33
37	c.5285C>A	p.A1762D	1	32
41	c.5819T>C	p.L1940P	1	34
42	c.5882G>A	p.G1961E	1	9
45	c.6148G>C	p.V2050L	1	33
45	c.6229C>T	p.R2077W	1	33
Nonsense:				
6	c.700C>T	p.Q234X	1	This study
6	c.735T>G	p.Y245X	2	32
28	c.4234C>T	p.Q1412X	1	10
Deletion:				
24	c.3539_3554del	p.S1181PfsX8	1	This study
43	c.5917delG	p.V1973X	3	23
Splice site/intronic:				
26	c.5196+1G>A	splicing	1	9
34	c.4848+2T>C	splicing	1	This study
36	c.5196+1_5196+4del	splicing	1	15
39	c.5461-10T>C	unknown	8	14
40	c.5714+5G>A	splicing?	1	6

Table 3

Clinical data of arCD and arCRD patients with *ABCA4* gene mutations.

Family ID/ Patient ID/ Gender	Diagnosis	Onset age	Dis. dur.	BCVA (OD/OS)	Color vision	Glare sensitivity	Night vision	RPE atrophy	Choroidal atrophy	RPE clumping	Additional fundus findings	Visual Field [1]	Rod GF-ERG	Cone GF-ERG
RCD92/ 6809/ F	CRD	8	16	0.05	PD15 sat: normal	n.i.	Normal	M		X		C — central scotoma	abnormal	abnormal
RCD79/ 6184/ M	CRD	5	27	0.05 / 0.05	PD15 sat: chaotic	increased	Normal	P	X			P — slight concentric narrowing; C — central scotoma	abnormal	abnormal
ZD289/ 781/ F	CRD	10	31	0.05 / 0.02	PD15 sat: red-green defect	increased	abnormal	P / M	X	X / X	white dots	P — slight concentric narrowing; C — n.i.	severely reduced	extinguished
RCD157/ 10492/ F	CRD	14	32	0.02 / 0.05	PD15 sat: scotopic defect / PD15 desat: unable to discriminate colors	increased	abnormal	P / M	X	X	chorioidal sclerosis	P; C — central scotoma	abnormal	abnormal
RCD51/ 4984/ F	CRD	2	20	HM / 0.05	PD15 desat: unable to discriminate colors	n.i.	n.i.	P / M		X		P — concentric narrowing; C — central scotoma	abnormal	abnormal
RCD9/ 1989/ M	CRD	10	18	0.05 / 0.02	PD15 sat: red-green defect	increased	abnormal	P / M	X	X / X		P — concentric narrowing; C — central scotoma	amplitudes abnormal	abnormal
RCD42/ 2509/ M	CRD	13	44	0.05 / 0.02	PD15 desat: unable to discriminate colors	n.i.	n.i.	P / M	X	X / X		P — normal; C — central scotoma	latencies normal	
RCD94/ 5781/ F	CD	10	10	0.05 / 0.02	PD15 sat: red-green defect	increased	Normal	P / M	X	X		P — concentric narrowing; C	normal	abnormal
ZD85/ 5862/ F	CD	6	8	0.07 / 0.05	PD15 sat: red-green defect	Normal	Normal	P / M	X / X			P — normal; C — central scotoma	normal	abnormal
RCD147/ 9136/ F	CRD	13	24	0.1 / HM	subjectively abnormal	increased	abnormal	P / M	X / X	X	bone spicules	P — n.i.; widely extinguished visual field	extinguished	extinguished
				0.015				P	X	X	bone spicules	C / P — field with remaining central and ring-shaped peripheral island		
RCD141/ 8594/ M	CRD	7	32	0.02	subjectively abnormal	increased	abnormal	M	X	X		C — n.i.	abnormal	abnormal
RCD63/ 3402/ M	CRD	12	43	0.02 / CF	n.i.	Normal	abnormal	P / M	X / X	X	bone spicules	P — widely extinguished visual field	abnormal	abnormal

Family ID/ Patient ID/ Gender	Diagnosis	Onset age	Dis. dur.	BCVA (OD/OS)	Color vision	Glare sensitivity	Night vision	RPE atrophy	Choroidal atrophy	RPE clumping	Additional fundus findings	Visual Field [1]	Rod GF-ERG	Cone GF-ERG
RCD143/ 8805/ M	CRD	9	34	CF / 0.02	PD15 desat: unable to discriminate colors	increased	abnormal	P / M	X / X	X / X	severe panretinal	C/ P: field with remaining peripheral island / widely extinguished visual	abnormal	extinguished
ZD146/ 10313/ F	CD	18	0.5	LP / 0.6	n.i.	increased	Normal	P / M	X		bone spicules	C/ P: field with remaining central and sickle-shaped peripheral island / central scotoma	n.i.	abnormal
	DD: CRD CRD			0.6				P				P: n.i.		
RCD98/ 3474/ F	CRD	6	23	0.02	PD15 sat: red-green defect	increased	abnormal	M	X	X		C: central scotoma	abnormal	abnormal
RCD194/ 10840/ M	CRD	27	2	0.03 / 0.6	PD15 desat: minor unspecific confusions	slightly increased	Normal	P / M	X	X	fundus flavimaculatus	P: concentric narrowing / C: central scotoma	abnormal	abnormal
RCD102/ 6810/ M	CD	7	23	0.6 / 0.1	PD15 sat: normal	Normal	Normal	P / M	X	X		P: normal / C: central scotoma	normal	abnormal
RCD113/ 3668/ F	CRD	7	1	0.1 / 0.03	Arden: red-green and blue-yellow defect	increased	n.i.	P / M	X	X / X	central tapetoid C reflex	P: normal / central scotoma	abnormal	abnormal
ZD124/ 8019/ F	CRD	6	13	0.03 / 0.02	PD15 sat: red-green defect	increased	abnormal	P / M	X	X / X		P: concentric narrowing / C: central scotoma	abnormal	abnormal
RCD185/ 11721/ F	CRD	5	13	0.02 / 0.1	subjectively normal	Normal	Normal	P / M	X			P: concentric narrowing / C: n.i.	extinguished	extinguished
				0.1				P	X	X		P: concentric narrowing		