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Molecular and phenotypic analysis of a family with autosomal recessive cone-rod dystrophy and Stargardt disease

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Purpose: To identify the causative gene mutations in three siblings with severe progressive autosomal recessive cone-rod dystrophy (arCRD) and their fifth paternal cousin with Stargardt disease (STGD1) and to specify the phenotypes.

Methods: We evaluated eight sibs of one family, three family members displayed arCRD, and one STGD1. All of them were screened for mutations using a new microarray for autosomal recessive retinitis pigmentosa.

Results: We found a new pathologic ATP-binding cassette transporter (ABCA4) splice-site mutation, c.3523-2A>T and the previously reported c.5327C>T (p.P1776L) missense mutation in the arCRD patients. The three siblings shared these two ABCA4 mutations and showed similar phenotypes. An unusual aspect was nystagmus which presented in one of the arCRD patients. In the STGD1 patient we found the c.5327C>T (p.P1776L) missense mutation and a novel c.868C>T (p.R290W) missense mutation.

Conclusions: Two new ABCA4 mutations were identified in a family with arCRD and STGD1. A new finding was nystagmus associated with arCRD in one of the patients.

Retinal dystrophies display a high degree of clinical and genetic heterogeneity. Frequently, a single disease may be caused by mutations in a multitude of different genes, and in some cases, mutations in a single gene may lead to clinically distinct diseases. One such gene is the retina specific ATP-binding cassette transporter (ABCA4) gene. Mutations in the ABCA4 gene have been shown to cause most cases of autosomal recessive Stargardt disease (STGD1; OMIM number 248200), a significant fraction of cases of autosomal recessive cone-rod dystrophy (arCRD; OMIM number 604116), and in some cases mutations in ABCA4 were found in patients suffering from autosomal recessive retinitis pigmentosa (arRP) [1-16]. ABCA4 has also been suggested to be a susceptibility factor for age-related macular degeneration (AMD) [17,18].

ABCA4 is a member of the ATP-binding cassette (ABC) transporter gene superfamily and encodes the ABCR protein. ABCR is located at the rim of the outer segment disks of rod and cone photoreceptors [19,20] and is involved in the transport of all-trans-retinaldehyde across photoreceptor disk membranes from the lumen to the photoreceptor cytoplasm through a flip-flop activity [21-23]. Mutations in ABCA4 lead to an accumulation of all-trans-retinal inside the photoreceptor disk lumen. This free all-trans-retinal is unfavorable and therefore Schiff-bonded to phosphatidyl ethanolamine. This bonding leads to toxic levels of N-retinylidene-N-retinylethanolamine (A2E) in the retinal pigment epithelium (RPE), which results in RPE cell apoptosis, followed by irreversible photoreceptor cell death [23-25].

The variability of severity in the different diseases associated with ABCA4 mutations has led to a genotype-phenotype model in which the residual activity of the mutated ABCR protein is inversely correlated with the severity of the retinal dystrophy [2,12]. This model predicts that two severe (null) mutations may lead to arRP, a combination of a severe with a moderately severe mutation may result in cone-rod dystrophy (CRD), and two moderate or a severe and a mild mutation may lead to STGD1 [4].

We present a pedigree displaying both arCRD and STGD1 in which some of the causative mutations in ABCA4 were identified with the Affymetrix Gene Chip® CustomSeq™ Resequencing Array (arRP-I) [26]. This technique allowed us to identify two novel ABCA4 mutations. Further, we show the ABCA4-associated CRD and STGD1 phenotypes and reveal a new clinical feature, nystagmus.

METHODS

This study was approved by the Institutional Review Board of the Montreal Children’s Hospital in Montreal and the protocol adhered to the Declaration of Helsinki. The three arCRD patients were patients in our clinic and were enrolled in this study. We were able to collect blood samples from five additional individuals. In total we recruited five women (1 arCRD and 1 STGD1 patient) and three men (2 arCRD patients). All persons signed informed consent.
Methods: DNA was isolated using the Qiagen DNA isolation kit. DNA samples were then analyzed with the arRP-I array. This newly developed custom designed array contained oligonucleotides created from the exons and 5 bp of flanking intronic sequences from 11 of the 19 currently known AR genes: ABCA4, CNGA1, CRB1, MERTK, PDE6A, PDE6B, RGR, RHO, RLBP1, RPE65, and TULP1 [26]. DNA from patient VI-1 was sequenced bidirectionally for all coding exons using gene-specific PCR primers as described in Table 1 [26]. The PCR products were purified using the Millipore purification system and thereafter analyzed on ABI3730 or ABI3100 DNA analyzers. Automatic analysis was done by ABI basecaller.

The c.868C>T (p.R290W) sequence change was tested in DNA from 92 anonymous healthy Dutch blood donors and 95 healthy blood donors from the São Miguel island (Azores, Portugal). This was done, by amplifying exon 8 of the ABCA4 gene and, followed by restriction fragment length polymorphism (RFLP) analysis using EagI. The amplicon consisted of 400 bp, which in the case of a wild-type allele was cut by EagI into fragments of 80 and 320 bp fragments. EagI does not cut the mutated allele.

Patients: The pedigree consisted of three siblings affected with arCRD and a fifth paternal cousin affected with STGD1 (Figure 1). Genealogic studies revealed that IV:1, IV:2, V:7, and V:8 originated from São Miguel, which is part of the Azores Archipelago. All clinical data were analyzed retrospectively, and additional information was collected through ophthalmic examination including best corrected visual acuity, slit-lamp examination, funduscopy, electroretinography (ERG), and Goldmann perimetry.

RESULTS

Several likely benign sequence variants in the ABCA4 gene were identified in patient V:6 with the arRP-I chip: c.141A>G (p.Q47P), c.1268A>G (p.H423R), c.5603A>T (p.N1868I), c.5682C>G (p.L1894L), c.6069T>C (p.I2023I) and several intronic sequence changes were then identified (c.302+26A>G, c.859-32T>C, c.1239+18>A, c.1239+28>C, and c.1356+11delG, c.4352+54A>G, c.5585-51A and c.6817-49C>G). Indeed, sequence analysis confirmed the presence of the two likely pathologic variants. Sequence analysis of V:2 and V:5 revealed the same mutations. The three unaffected siblings and the mother of the patients only carried the c.3523-2A>T mutation.

Further investigation of the family revealed a fifth paternal cousin with STGD1.

Direct sequence analysis of the DNA of patient VI:1 identified the c.5327C>T (p.P1776L) missense mutation and revealed a novel ABCA4 sequence change, c.868C>T.

![Figure 1](http://www.molvis.org/molvis/v13/a174/)
(p.R290W). The c.868C>T (p.R290W) sequence change was not detected in the DNA from 92 healthy Dutch controls or from 95 healthy individuals from São Miguel island.

Clinical evaluation: Unfortunately, no early clinical data were available for our CRD patients. The siblings affected with CRD were in their early 40s at the time they first visited our ocular genetics clinic. On history, however, all siblings reported visual acuity difficulties since early childhood followed by peripheral field loss in the second decade. Night blindness occurred in the third decade.

Initial visual acuity test results ranged from light perception to counting fingers. Patient V:2 showed distinct pendular nystagmus. Anterior segments were normal in all three patients. Funduscopy revealed pale optic disks with severely attenuated retinal vessels in all three patients. Individuals V:2 and V:5 showed distinct atrophy of the RPE in the macular area (Figure 2 and Figure 3). Bone spicule pigmentation were evident throughout the retina in V:2, and limited to the posterior pole and midperiphery in patient V:5. Patient V:6 showed heavy bone spicule pigmentation throughout the entire retina with extensive macular involvement. On ERG, no detectable signals were found in all three patients. Goldmann kinetic perimetry revealed small temporal islands with target V4-e in the arCRD patients.

Patient VI:1 was 11 years old when she received the diagnosis of STGD1 at another institution. At 52 years, visual acuity of the right eye was counting fingers whereas visual acuity of her left eye was hand movements. Funduscopy revealed normal optic disks, mild attenuation of the vessels, and large atrophic lesions in both maculae. Lobular atrophy of the RPE was seen in the mid and peripheral regions (Figure 4).

DISCUSSION
A family with arCRD and STGD1 was investigated using a new arRP-I array designed to detect mutations in 11 arRP genes including ABCA4. In hindsight, the use of the arRP-I chips in this particular pedigree was not logical given the indication of ABCA4 involvement through the ascertainment of the fifth paternal cousin with STGD1. Instead, a much cheaper technique, arrayed primer extension (APEX)-based analysis of the known ABCA4 variants (ABCR500) could have been used. The ABCR500 array would also have identified one of the alleles in both branches of the pedigree.

In three siblings with arCRD, both mutations in the ABCA4 gene were found, i.e., the c.5327C>T (p.P1776L) mutation previously described in a STGD1 patient [10] and a new splice site mutation; c.3523-2A>T. Direct sequencing of DNA of a fifth paternal cousin with STGD1 from Bermuda (but from São Miguel island origin) revealed the c.5327C>T (p.P1776L) mutation and a new variant, c.868C>T (p.R290W).
The arginine at position 290 resides in the first intradiskal loop of ABCR and is conserved in human, mouse, rat, dog, and Xenopus. The change from a basic to neutral/hydrophobic residue is likely to have functional implications. The proline residue at position 1776 resides in the middle of a stretch of hydrophobic residues constituting the ninth transmembrane domain of ABCR.

Biochemical analysis of recombinant ABCR bearing these mutations was not performed. The previously presented genotype-phenotype model suggest that the residual ABCR protein activity is inversely correlated to disease severity. Therefore, the previously identified p.P1776L [10] mutation is likely a mild or moderately severe mutation, since both the arCRD and STGD1 sibs shared this mutation. Difference in phenotype would have to be explained by the difference in severity of the c.3523-2A>T (splice site mutation) and the p.R290W (missense) mutations. Most likely, this splice acceptor site mutation preceding exon 24 of ABCA4 results in the skipping of exon 24, which leads to a frameshift and a translational stopmutation in the third triplet following the exon 23/exon 25 splice junction.

It was difficult to determine the exact clinical diagnosis (especially the issue of RP versus CRD) in our three patients as no early ERGs were available. The occurrence of nystagmus, which is a new finding in a CRD patient with ABCA4 mutations, supports the history of early loss of central vision. The loss of visual acuity, followed by night blindness and peripheral field loss, suggest the diagnosis of CRD. In our three siblings, the retinal degeneration led to complete loss of the central retina and almost complete loss of the peripheral retina with an RP-like appearance by the time they were 40 years old. This is consistent with the results from Lorenz and Preising, who suggested that RP caused by ABCA4 mutations is a severe progressive cone-rod disease [15].

It was not surprising to find some peripheral involvement in our STGD1 patient since ABCA4 is expressed in both cones and rods. If there were an ERG available on this patient, one might assume it would show a cone-rod pattern as seen in a significant fraction of STGD patients [27].

Several studies are ongoing to design new treatment strategies for retinal dystrophies, some of which are specific for retinal diseases caused by ABCA4 mutations. Studies with administration of isotretinoin and N-(4-hydroxyphenyl)retinamide (HPR) to Abcr -/- mice showed reduction of accumulation of the toxic lipofuscin fluorophores [28,29].

Given these developments, it is important to identify patients with ABCA4 mutations, as they may be eligible for future therapeutic interventions. Detailed clinical description of these types of retinal dystrophy patients is essential in order to facilitate the search for the causal gene.

In conclusion, mutations in the ABCA4 gene should be considered in patients with arCRD and in older patients presenting with a severe RP-like phenotype and a history of early central visual acuity loss.