An Analysis of *ABCR* Mutations in British Patients with Recessive Retinal Dystrophies

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Purpose. Several reports have shown that mutations in the *ABCR* gene can lead to Stargardt disease (STGD)/fundus flavimaculatus (FFM), autosomal recessive retinitis pigmentosa (arRP), and autosomal recessive cone-rod dystrophy (arCRD). To assess the involvement of *ABCR* in these retinal dystrophies, the gene was screened in a panel of 70 patients of British origin.

Methods. Fifty-six patients exhibiting the STGD/FFM phenotype, 6 with arRP, and 8 with arCRD, were screened for mutations in the 50 exons of the *ABCR* gene by heteroduplex analysis and direct sequencing. Microsatellite marker haplotyping was used to determine ancestry.

Results. In the 70 patients analyzed, 31 sequence changes were identified, of which 20 were considered to be novel mutations, in a variety of phenotypes. An identical haplotype was associated with the same pair of in-*cis* alterations in 5 seemingly unrelated patients and their affected siblings with STGD/FFM. Four of the aforementioned patients were found to carry three alterations in the coding sequence of the *ABCR* gene, with two of them being in-*cis*.

Conclusions. These results suggest that *ABCR* is a relatively polymorphic gene. Because putative mutations have been identified thus far only in 25 of 70 patients, of whom only 8 are compound heterozygotes, a large number of mutations have yet to be ascertained. The disease haplotype seen in the 5 patients carrying the same "complex" allele is consistent with the presence of a common ancestor. (*Invest Ophthalmol Vis Sci.* 2000;41:16–19)

Stargardt disease (STGD; MIM No. 248200) is the most frequent cause of inherited macular dystrophy in childhood, with an estimated prevalence of 1:10,000.[1] Fundus flavimaculatus (FFM) presents with a similar phenotype to STGD, and linkage analysis suggests that the two are allelic autosomal recessive conditions mapping to the short arm of chromosome 1 (1p21–p22.1).[2,3] Homozygosity mapping has demonstrated additional loci for autosomal recessive retinitis pigmentosa (arRP) and autosomal recessive cone-rod dystrophy (arCRD), which colocalize with the STGD/FFM locus.[4,5] More recently, the *ABCR* gene, which encodes a retina-specific ATP-binding cassette (ABC) transporter, has also been mapped to the same chromosomal location.[6,7] Mutations in the *ABCR* gene have now been implicated in all the above conditions.

Previous studies have demonstrated *ABCR* mutations in 30% to 50% of the STGD/FFM patients screened.[7–9] Of these, a lesser percentage has been shown to carry mutations on both alleles of the gene.[8] We set out to determine the mutation spectrum of the *ABCR* gene in recessive retinal dystrophies by screening all 50 exons of the gene in 70 unrelated British patients with a STGD/FFM, arRP, or arCRD phenotype.

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Methods

Ascertainment of Patients

Patients were from small nuclear families showing a recessive mode of inheritance of disease, with both parents being unaffected. Patients were British residents of a variety of ethnic origins (European Caucasian, and Asian from the Indian subcontinent). All 70 patients were identified through the retinal dystrophy clinics at Moorfields Eye Hospital using standard techniques and were clinically categorized into three groups: (1) patients with a diagnosis of Stargardt disease/fundus flavimaculatus ($n = 56$) based on macular dysfunction on pattern electroretinogram (ERG) and the presence of orange/yellow flecks at the posterior pole, sometimes extending anterior to the vascular arcades; (2) one affected member of each of six families with arRP, with the diagnosis based on typical peripheral pigmentary retinal degeneration and markedly reduced scotopic ERG; and (3) one affected member of each of eight families with arCRD, with the diagnosis based on the finding of abnormal cone (30-Hz flicker) and rod (scotopic) ERGs and the absence of retinal flecks. For the arRP and arCRD families, all affected siblings showed identical haplotypes for the microsatellite markers tested from the 1p21–p22.1 region, thus providing evidence in support of linkage to the STGD/FFM locus (data not shown). Informed consent for genetic studies in adherence to the Declaration of Helsinki was obtained by the examining clinicians. For comparison, 96 ethnically matched individuals

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TABLE 1. List of Mutations Found in 70 Patients of British Origin

Nucleotide Change	Amino Acid Change	No. of Patients (/70)	Phenotype	No. of Controls (/96)
G161A	Cys-54-Tyr	1	STG/FFM	NF
A286G	Asn-96-Asp	1	STG/FFM	NF
A286C	Asn-96-His	1	STG/FFM	NF
A466G	Ile-156-Val	1	STG/FFM	NF
C1220T	Ala-407-Val	6	STG/FFM, arCRD	NF
T1271C	Val-424-Ala	2	STG/FFM, arRP	NF
C1335G	Ser-445-Arg	1	STG/FFM	NF
C1804T	Arg-602-Trp	1	STG/FFM	NF
C2337A	Cys-779-Ter	1	STG/FFM	NF
*G2588C	Gly-863-Ala	5	STG/FFM	2/176
3392delC	1147 Ter	1	STG/FFM	NF
T4286C	Val-1429-Ala	1	STG/FFM	NF
4774-2A→C	Splice acceptor	2	STG/FFM	NF
†C4918T	Arg-1640-Trp	1	STG/FFM	NF
C5107G	Gln-1703-Lys	1	STG/FFM	NF
5161delAC	Frameshift	1	STG/FFM	NF
C5337G	Tyr-1779-Ter	1	STG/FFM	NF
C6088T	Arg-2030-Ter	1	arCRD	NF
6282+7G→A	Splice donor	1	STG/FFM	NF
G6449A	Cys-2150-Tyr	2	arCRD	NF
A6479G	Lys-2160-Arg	1	STG/FFM	NF

* Independently reported by Allikmets et al.[6]
† Independently reported by Rozet et al.[8]
NF, not found in 96 ethnically matched control individuals.

with no personal or family history of retinopathy were selected to serve as controls.

Heteroduplex and Haplotype Analyses

Genomic DNA was extracted using the Nucleon II extraction kit (Scotlab Bioscience) and amplified using primers that allowed amplification of the complete coding region (50 exons) under standard conditions.[6,10] Amplified exons were analyzed by electrophoresis on MDE Flowgen gels run at 180 V overnight using Hoeffer 600S apparatus. Microsatellite markers described in Table 3 were used for genotyping with polymerase chain reaction (PCR). Amplified products were fractionated on 8% nondenaturing polyacrylamide gels and visualized by ethidium bromide staining.

Direct Sequencing

PCR products that demonstrated a heteroduplex pattern were sequenced using the PRISM Ready Reaction Sequencing Kit (Perkin–Elmer Cetus) and analyzed on an ABI 373 automated sequencer.

RESULTS

A total of 20 novel sequence changes were identified and thought to be mutations in 20 of 56 STGD/FFM, 1 of 6 arRP, and 4 of 8 arCRD patients. The majority of the mutations were single nucleotide substitutions at conserved amino acid positions, but deletion events were also observed (Table 1). It is notable that the four truncating mutations (at amino acid positions Cys779, Leu1147, Tyr1779, and Arg2030) were identified in three patients with STGD/FFM and one with arCRD. Only eight patients were found to be compound heterozygotes, whereas the majority were heterozygous for a single mutation (13 STGD/FFM, 1 arRP, and 3 arCD). Although none of these changes was detected in 96 control samples, their pathogenicity has yet to be determined either biochemically or using animal models. Twenty-eight patients had at least one sequence variant identified as a "polymorphism," whereas 14 patients had more than 2 (Table 2). Interestingly, most of these polymorphisms appear to concentrate toward the 3′-end of the gene, namely in exon 42, and introns 43, 48, and 49. By contrast, the observed mutations are distributed evenly throughout the entire coding sequence of the *ABCR* gene, and the phenomenon of clustering within specific regions such as the ATP-binding domains was not detected.

In the family of a STGD/FFM patient, three sequence alterations Cys-54-Tyr, Gly-863-Ala, and Arg-943-Gln in *ABCR* exons 3, 17, and 19, respectively, were found to be present. Both the Gly-863-Ala and Arg-943-Gln substitutions were present in the unaffected mother who had no clinical evidence of the disease at the age of 58 years. Neither of these putative

TABLE 2. List of Polymorphisms Found in 70 Patients of British Origin

Nucleotide Change	Exon	Amino Acid Change	No. of Patients (/70)	No. of Controls (/96)
1356+11delG	10 intron	—	2	NF
*G2828A	19	Arg-943-Gln	8	16/176
3815-82G→C	25 intron	—	1	NF
G5682C	40	Leu-1894-Leu	1	30
C5842T	42	Pro-1948-Leu	4	7
G5844A	42	Pro-1948-Pro	12	22
T5885C	42	Val-1962-Val	9	NF
6006-16G→A	43 intron	—	19	NF
6729+21C→T	48 intron	—	2	NF
6816+27G→C	49 intron	—	4	NF

* Independently reported by Allikmets et al.[6]
NF, not found.

TABLE 3. Ancestral Haplotype Shared by the 5 Families Carrying the Two Amino Acid Alterations Gly-863-Ala and Arg-943-Gln in-*cis*

		Family 1				Family 2				Family 5	
Marker	**Distance**	**1a**	**1b**	**1c**	**1d**	**2a**	**2b**	**Family 3**	**Family 4**	**5a**	**5b**
D1S198		**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
	6.2										
D1S216		**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
	10.4										
D1S207		**3**	**3**	**3**	**3**	**3**	**3**	—	**3**	**3**	**3**
	11.9										
D1S2813		**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
ABCR gene	2.9										
D1S236		**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
	10.9										
D1S248		4	4	4	4	**5**	**5**	**5**	**5**	2	2
	11.8										
D1S252		1	1	1	1	**1**	**1**	—	**1**	2	2
	9.0										
D1S305		1	1	1	1	1	1	2	—	2	2

Column 1 denotes the markers used for haplotyping analysis, and column 2 shows the genetic distance between the respective markers (in cM). Markers span a total of 63.1 cM on chromosome 1. Bold numbers denote ancestral haplotype.

mutations was detected in the unaffected father, in whom the third alteration (Cys-54-Tyr) was identified. All three alterations were present in the three affected siblings but not in the unaffected ones. We therefore assumed that the two sequence changes Gly-863-Ala and Arg-943-Gln were in-*cis* on the maternally inherited chromosome, comprising a "complex" allele. Moreover, because the mother and the unaffected siblings reported no symptoms of the disease, these two changes on their own were not enough to produce the STGD/FFM phenotype when found in-*cis*.

When screening additional patients, we discovered that four more individuals affected with STGD/FFM carried the same two changes in-*cis* (Gly-863-Ala and Arg-943-Gln). Haplotype analysis by means of eight microsatellite markers, distributed over a 63-cM interval around the *ABCR* locus, revealed conservation of alleles between these five families, with affected individuals sharing at least five of them (Table 3). In addition, three of these affected individuals also carried a third sequence variation (Asn-96-His, Ala-407-Val, or Val-424-Ala) derived from the other parental chromosome. A third mutation on the other parental chromosome has not been detected in the fourth individual so far.

DISCUSSION

This study has demonstrated mutations in 45% of the STGD/FFM patients, with only 14% being compound heterozygotes; therefore, a great number of mutations have yet to be ascertained. The detection of *ABCR* changes in our patients is comparable with that of other studies, and it is possible that allelic mutations reside in parts of the gene (e.g., the promoter region or the introns) that have not yet been screened. Because no sequence changes in this gene have been investigated for deleterious effects to protein function or RNA splicing, some of the variants classified as polymorphisms could represent mutations. For example, although an exonic sequence alteration might not introduce an amino acid substitution, it may introduce an ectopic splice site and thus have a detrimental effect on RNA splicing. Similarly, it can be speculated that an intronic sequence change could also affect proper splicing even if it occurs outside the splice consensus sequences.[11]

Microheterogeneity in this region of chromosome 1 with a second as yet unidentified gene lying in the vicinity of the *ABCR* gene could explain the lack of observed mutations in 5 of 6 and 4 of 8 of the arRP and arCRD families, respectively, that are linked to this locus. A similar occurrence of heterogeneity has been noticed in other disorders where mutational screening has excluded candidate genes from linked families.[12]

In general, there is a limited number of reports demonstrating founder effects in human genetic disorders. Here we report such a phenomenon in the *ABCR* gene, even though it accounts for a small proportion (9%) of the STGD/FFM patients investigated. The allele in question is the "complex" one carrying two sequence changes, Gly-863-Ala and Arg-943-Gln, in-*cis*. The absence of the disease haplotype in 50 control samples tested is consistent with the presence of a founder effect in the 5 patients and their families.

In this study, 7% of the STGD/FFM patients were found to carry three sequence variations in the *ABCR* gene. Of the two amino acid alterations in-*cis*, Gly-863-Ala besides being a putative missense mutation could also affect proper RNA splicing as it occurs at the acceptor splice site of exon 17.[13] The second alteration, Arg-943-Gln, has been classified as a "polymorphism."[6] It could be that both changes contribute to the disease phenotype, having an additive effect as has been reported in other diseases.[14] The *ABCR* gene and its protein have only recently been characterized; however, another "relative" from the ABC family, the *CFTR* gene, has been more thoroughly investigated. In the case of the *CFTR* gene and cystic fibrosis (CF), "complex" alleles appear to be relatively frequent and modulate the phenotype of CF patients.[13] Further biochemical and/or animal model studies are needed to establish the effect of single and multiple sequence changes in the *ABCR* protein.

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