Abstract  Based on recent studies of the photoreceptor-specific ABC transporter gene \textit{ABCR} (\textit{ABCA4}) in Stargardt disease (STGD1) and other retinal dystrophies, we and others have developed a model in which the severity of retinal disease correlates inversely with residual ABCR activity. This model predicts that patients with late-onset STGD1 may retain partial ABCR activity attributable to mild missense alleles. To test this hypothesis, we used late-onset STGD1 patients (onset: \(\geq 35\) years) to provide an in vivo functional analysis of various combinations of mutant alleles. We sequenced directly the entire coding region of \textit{ABCR} and detected mutations in 33/50 (66\%) disease chromosomes, but surprisingly, 11/33 (33\%) were truncating alleles. Importantly, all 22 missense mutations were located outside the known functional domains of ABCR (ATP-binding or transmembrane), whereas in our general cohort of STGD1 subjects, alterations occurred with equal frequency across the entire protein. We suggest that these missense mutations in regions of unknown function are milder alleles and more susceptible to modifier effects. Thus, we have corroborated a prediction from the model of ABCR pathogenicity that (1) one mutant ABCR allele is always missense in late-onset STGD1 patients, and (2) the age-of-onset is correlated with the amount of ABCR activity of this allele. In addition, we report three new pseudodominant families that now comprise eight of 178 outbred STGD1 families and suggest a carrier frequency of STGD1-associated ABCR mutations of about 4.5\% (~1/22).

Introduction

Stargardt disease (STGD1, OMIM 248200) is an autosomal recessive retinal dystrophy classically observed in juveniles or young adults before the age of 30 years. The estimated frequency of STGD1 in the United States is between 1:8,000–10,000 (Blacharski 1988). STGD1 is characterized by (1) acquired impairment of central vision, (2) progressive bilateral atrophy (“beaten metal” or “bull’s eye”) of the foveal retinal pigment epithelium (RPE), and (3) orange-yellow flecks frequently located around the macula and/or midretinal periphery (Blacharski 1988).

Late age-of-onset (\(\geq 20\) years) has been reported in 21\% (Lewis et al. 1999) to 36\% (Noble and Carr 1979) of STGD1 families. These late-onset patients are often diagnosed as having fundus flavimaculatus (FFM, OMIM 248200), a clinically similar disorder, historically given a separate nosology. Classically, FFM manifests at a later age-of-onset (20–64 years) and has a slower progression and thus a better visual prognosis. Despite this clinical segregation, FFM is allelic with STGD1 (Anderson et al. 1995; Gerber et al. 1995; Lewis et al. 1999). The causative gene for STGD1/FFM has previously been mapped to chromosome 1p21-p13 (Kaplan et al. 1993; Anderson et al. 1995; Gerber et al. 1995) and has been cloned (Allikmets et al. 1997b). This gene, \textit{ABCR} (recently known as \textit{ABCA4}), contains 50 exons and encodes a photoreceptor-specific ATP-binding cassette transporter (Allikmets et al. 1997b). Several reports have localized the ABCR protein...
(also known as rim protein, RmP) in both cone photoreceptors and the outer segment disks of the rod photoreceptors (Papamastorou et al. 1982; Azarian and Travis 1997; Sun and Nathans 1997; Mata et al. 2000; Molday et al. 2000). ABR appears to function as an outwardly directed flippase for all-trans-retinaldehyde conjugated to phosphatidylethanolamine (PE; Sun et al. 1999; Weng et al. 1999; Mata et al. 2000). Mice homozygous for null mutations in the abcr gene have a complex retinal phenotype including slow photoreceptor loss, elevated PE in outer segments, and accumulation of retinoids in the RPE (Weng et al. 1999; Mata et al. 2000). These aggregate data suggest that the development of STGD1 involves the accumulation of retinoids in the RPE from phagocytosed outer segment disks that contain these retinoids. RPE cells are poisoned by an increased quantity of the toxic retinoid A2E (Mata et al. 2000), with eventual death of the RPE and overlying photoreceptors, thus resulting in macular atrophy (Allikmets et al. 1997b; Weng et al. 1999; Lewis and Lupski 2000; Mata et al. 2000; Molday et al. 2000; Lewis et al. 2001).

Previous studies have shown a range of different types of mutant ABR alleles in early-onset STGD1 families (Allikmets et al. 1997b; Rozet et al. 1998; Lewis et al. 1999; Maugeri et al. 1999; Shroyer et al. 1999, 2000; Souied et al. 1999b; Papaioannou et al. 2000; Rivera et al. 2000; Simonelli et al. 2000). The ABR missense mutations identified in this general cohort are located with equal frequency in regions of known function compared with other regions (Lewis et al. 1999). One investigation of FFM patients (>17 years at onset) reported seven ABR missense mutations (four paired and three singles) in 30 disease chromosomes (Rozet et al. 1998). Other recent studies that included 55 late-onset Stargardt subjects (age-of-onset: ≥20 years) identified 35 families with paired mutant ABR alleles (Lewis et al. 1999; Souied et al. 1999b; Rivera et al. 2000; Simonelli et al. 2000). However, different hypotheses have been espoused for genotype/phenotype correlations: (1) that late-onset is associated with paired missense mutations (Rozet et al. 1998), or (2) that late-onset is associated with the absence of mutations in the amino terminal one-third of ABR (Lewis et al. 1999).

ABCR mutations also occur in clinically diverse disorders including recessive retinitis pigmentosa (RP19; Cremers et al. 1998; Martinez-Mir et al. 1998; Klevering et al. 1999; Rozet et al. 1999; N. F. Shroyer et al., submitted), recessive cone-rod dystrophy (CRD; Cremers et al. 1998; Rozet et al. 1999; Maugeri et al. 2000) and age-related macular degeneration (AMD; Allikmets et al. 1997a; Souied et al. 1999a; Allikmets and the International ABR Screening Consortium 2000). From these observations, we and others have proposed a model for ABR-associated pathogenicity in which the severity of retinal disease and thus the age-of-onset or presentation is correlated inversely with residual ABR activity (Cremers et al. 1998; Allikmets 1999; Maugeri et al. 1999; Shroyer et al. 1999; Lewis and Lupski 2000; Lewis et al. 2001). In this model, a combination of null alleles leads to recessive RP19, a combination of severe/moderate mutations causes CRD, and a combination of either severe/mild or moderate/moderate alleles gives STGD1 in which the age-of-onset is related to the activity of the individual combination of mutant alleles. Individuals heterozygous for some ABR mutant alleles are susceptible for developing AMD. One prediction of this model is that late-onset STGD1 may be associated with two mild missense ABR alleles. Here, we test this hypothesis with patient samples as an in vivo functional assay of the selected combination of mutant alleles by examining the type and the location of ABR mutations in late-onset STGD1 subjects.

Materials and methods

Patients

DNA from 25 subjects from our general cohort of 278 STGD1 families who manifest late-onset STGD was analyzed completely by sequence analysis of ABR. Of these 25 subjects, eight were reported previously by Lewis et al. (1999) but here were analyzed independently by complete sequence analysis rather than a mutation screening approach. All affected individuals from families with late-onset STGD1 had both ophthalmoscopic examinations and fluorescein angiograms characteristic of STGD1/FFM. Each family had: (1) onset of Stargardt disease of 35 years or older in at least one family member; (2) a pattern of inheritance consistent with an autosomal recessive trait; (3) ophthalmoscopically characteristic retinal disorder; (4) bilateral central vision loss; (5) “dark choroid” documented by fluorescein angiography in at least one affected individual in the family. We determined the “age-of-onset” of STGD as the age-of-onset of visual impairment based on anamnestic medical records and interviews with selected family members. Families were North Americans of Western European ancestry except for AR723, which is African-American. Enrollment criteria were as published previously (Anderson et al. 1995; Allikmets et al. 1997b; Lewis et al. 1999).

We selected a subset of STGD1 families in which the proband had an age-of-onset of 35 years and older (2 standard deviations (SDs) later than average, i.e., 15.8 years+35 years; Lewis et al. 1999). We investigated three additional late-onset STGD1 subjects (AR140-03, AR632-04, and AR662-03), because they were affected offspring in families manifesting pseudodominant inheritance of STGD1. We also used DNA samples from four patients (AR129-05, AR311-03, AR343-04, and AR387-04) with an age-of-onset of less than 35 years, assuming that they had identical genotypes with their later onset proband sibling. Sequence analysis for subject AR336-03 was performed independently from the report of Shroyer et al. (2000). Control individuals were over the age of 65 years and had normal retinal examination (R.A.L.). This study was approved by the Institutional Review Board for Human Subject Research at Baylor College of Medicine.

Molecular analysis

DNA was extracted from peripheral leukocytes by standard methods (Tilzer et al. 1989). Tailed primers were used for the polymerase chain reaction (PCR) amplification of exons and flanking intronic regions of ABR from DNA of STGD1 subjects and relatives. Primer tails consist of M13–21 or reverse universal primer sequence (Allikmets et al. 1997b). PCR products were purified with the QIAquick 96-well PCR purification kit (Qiagen). Purified PCR products were used for bi-directional dye primer cycle sequencing with BigDye Primer M13–21 or Reverse Sequencing Ready Reaction kits (PE Applied Biosystems). Sequencing products were analyzed on an ABI 377 automated sequencer, and chromatograms were analyzed by Sequencher 3.1 software (Gene Codes Corporation).