INVITED EDITORIAL
Simple and Complex *ABCR:* Genetic Predisposition to Retinal Disease

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Diseases of the retina include a wide spectrum of photoreceptor-affecting phenotypes that have been mapped to >120 loci on the human genome (RetNet Retinal Information Network). Currently, less than half of the causal genes have been identified (RetNet Retinal Information Network), although substantial progress has been made in determining the genetic basis of monogenic eye disorders. Mutations in new genes that are responsible for some form of retinal degeneration are identified on a regular basis. The vast majority of these genes are involved in rare phenotypes in a limited numbers of patients.

When the ATP-binding cassette (ABC)–transporter gene, *ABCR,* was cloned and characterized in 1997 as the causal gene for autosomal recessive Stargardt disease (Allikmets et al. 1997*b*), it seemed as if just another missing link had been added to the extensive table of genetic determinants of rare monogenic retinal dystrophies. Now, >3 years later, mutations in the *ABCR* gene (also called "*ABCA4*") continue to emerge as one of the predominant determinants of a wide variety of retinal-degeneration phenotypes. *ABCR* has caused exciting and sometimes intense discussions among ophthalmic geneticists, resulting in >50 publications during this brief period of time. Now, in the current issue of the *Journal,* two more studies report interesting new data on *ABCR* genetics (Maugeri et al. 2000 [in this issue]; Rivera et al. 2000 [in this issue]).

In the following brief editorial, I attempt to summarize our current knowledge of the role of *ABCR* in retinal disease. Extensive genetic and functional studies allow the connection of many pieces of the *ABCR* puzzle into a picture that is emerging into focus.

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ABCR in Retinal Dystrophies

In 1997, several laboratories independently described *ABCR* as the causal gene for Stargardt disease/fundus flavimaculatus (STGD1 [MIM 248200]) (Allikmets et al. 1997*b;* Azarian and Travis 1997; Illing et al. 1997). Autosomal recessive STGD (arSTGD) is a juvenile-onset macular dystrophy associated with rapid central visual impairment, progressive bilateral atrophy of the foveal retinal pigment epithelium (RPE), and characteristic frequent appearance of orange-yellow flecks around the macula and/or the midretinal periphery. There is no definitive evidence of genetic heterogeneity of arSTGD; all families segregating the disorder have been linked to the *ABCR* locus on human chromosome 1p13-p22 (Kaplan et al. 1993; Anderson et al. 1995). Consequently, the role of the *ABCR* gene in arSTGD has not been disputed, even despite a relatively low (usually ~60%) mutation-detection rate in *ABCR* in patients with STGD (Lewis et al. 1999; Maugeri et al. 1999; Rivera et al. 2000 [in this issue]; Simonelli et al. 2000).

Subsequently, several cases were reported in which *ABCR* mutations segregated with retinal dystrophies of substantially different phenotype, such as autosomal recessive cone-rod dystrophy (arCRD) (Cremers et al. 1998; Rozet et al. 1998) and autosomal recessive retinitis pigmentosa (arRP) (Cremers et al. 1998; Martinez-Mir et al. 1998; Rozet et al. 1999). arCRD and arRP have been characterized as groups of genetically heterogeneous diseases in which several loci have been implicated by linkage (RetNet Retinal Information Network). Clinical heterogeneity of these disorders further complicates the assessment of genetic determinants of each disease entity. Cone-rod dystrophy is characterized by more prominent cone degeneration, which, in the electroretinogram (ERG), is distinguished by more-distinctive reduction of the photopic cone b-wave amplitude than the scotopic (rod b-wave) amplitude, compared with rod degeneration. Conversely, RP affects predominantly rod photoreceptors, the scotopic ERG is more severely reduced than the photopic ERG, and patients present with night blindness and loss of peripheral vision.

In all studies, disease-associated *ABCR* alleles have revealed an extraordinary heterogeneity (Allikmets et

al. 1997*b;* Rozet et al. 1998; Fishman et al. 1999; Lewis et al. 1999; Maugeri et al. 1999; Simonelli et al. 2000), as demonstrated also in the German population, reported by Rivera et al. (2000) in this issue of the *Journal.* The current tally of all *ABCR* alleles suggests ⩾350–400 *ABCR* variants (author's unpublished data), making the heterogeneity of *ABCR* comparable to that of another member of the ABC superfamily, the cystic fibrosis transmembrane conductance regulator (*CFTR*) (Riordan et al. 1989). What makes *ABCR* an even more difficult diagnostic target than *CFTR* is that, across all populations studied, the most-frequent disease-associated *ABCR* alleles—for example, G1961E, G863A/delG863, and A1038V—have been described in ~10% of patients with STGD, whereas the delF508 allele of *CFTR* accounts for close to 70% of all cystic fibrosis alleles (Zielenski and Tsui 1995).

On the basis of these findings, several investigators have proposed a model that suggests a direct correlation between the continuum of disease phenotypes and residual ABCR activity/function (van Driel et al. 1998; Allikmets 1999; Lewis et al. 1999; Maugeri et al. 1999; Shroyer et al. 1999). According to the predicted effect on the ABCR transport function, Maugeri et al. classified *ABCR* mutant alleles as "mild," "moderate," and "severe" (Maugeri et al. 1999). Different combinations of these were predicted to result in distinct phenotypes in a continuum of disease manifestations, the severity of disease manifestation being inversely proportional to the residual ABCR activity. On the basis of several assumptions, such as the predicted frequency of *ABCR* alleles in the general population and documented prevalences of different disease phenotypes, Maugeri et al. estimated the incidence of retinal degenerations caused by combinations of *ABCR* alleles (Maugeri et al. 1999). Several recent studies, including the two in this issue, confirm and expand the proposed model (Maugeri et al. 2000 [in this issue]; Rivera et al. 2000 [in this issue]).

These studies make three important points regarding the role of *ABCR* in retinal disease. First, in an extension of its earlier study, the laboratory of Frans Cremers has determined the major role of mutant *ABCR* alleles in arCRD (Maugeri et al. 2000 [in this issue]). This groundbreaking discovery of the major genetic component in a prominent fraction of retinal disease distinguishes arCRD as a disorder predominantly caused by genetic defects in one gene. This finding argues against the former assumption that arCRDs represent a genetically heterogeneous entity similar to arRPs (RetNet Retinal Information Network). It not only offers new possibilities for disease classification and diagnostics but also significantly advances possible approaches for treatment and prevention of this disorder. Second, both studies have identified a frequent, complex allele, L541P/A1038V, in patients of German origin who have both STGD and CRD (Maugeri et al. 2000 [in this issue]; Rivera et al. 2000 [in this issue]). Complex ABCR alleles are not uncommon in Stargardt disease (Lewis et al. 1998). This is a third instance in which an "ethnic group–specific" *ABCR* allele has been discovered. The earlier study by Maugeri et al. had defined the 2588G→C variant resulting in a dual effect, G863A/delG863, as a founder mutation in northern-European patients with STGD (Maugeri et al. 1999). Another *ABCR* allele, T1428M, which is very rare in populations of European descent, is apparently frequent (~8%) in the Japanese general population (Kuroiwa et al. 1999). These findings have a prominent influence on population-genetic studies that rely on *ABCR* allele frequencies (see below). In addition, the complexity of the genotype/phenotype–correlation studies in *ABCR*-related retinal dystrophies is underlined by the fact that two patients *homozygous* for the L541P/A1038V allele were diagnosed with CRD and STGD (Maugeri et al. 2000 [in this issue]; Rivera et al. 2000 [in this issue]). Third, the study by Maugeri et al. suggests that we revisit our current knowledge about the molecular genetics of arRP. The prediction that *ABCR* alleles are responsible for ~8% of arRP, making it the most prominent cause of the autosomal recessive form of RP, seems reasonable and warrants further investigation.

ABCR in Age-Related Macular Degeneration (AMD)

The summarized data listed above establish allelic variation in *ABCR* as the most prominent cause of retinal dystrophies with Mendelian inheritance patterns. The latest estimates suggest that the carrier frequency of *ABCR* alleles in the general population is close to 5% (Maugeri et al. 1999; J. R. Lupski, personal communication). This brings us to the hottest topic of ophthalmic genetics—the role of heterozygous *ABCR* alleles in a complex trait, AMD (also designated "ARMD2" [MIM 153800]). AMD, as a typical late-onset complex disorder, is caused by a combination of genetic and environmental factors. Its prevalence increases with age; among persons of age ⩾75 years, mild or early forms occur in nearly 30%, and advanced forms in ~7%, of the population (Klein et al. 1992; Vingerling et al. 1995). Consequently, various forms of AMD affect >10 million individuals in the United States alone. The Vision Research—A National Plan: 1999–2003 Web site has estimated that "with growth in the aged population, age-related macular degeneration (AMD) will become a more prevalent cause of blindness than both diabetic retinopathy and glaucoma combined." Not surprisingly, every effort is made to decipher the genetic components of AMD, and news from this research field gets particular attention.

In 1997, a joint study by four laboratories suggested

Table 1

Meta-analysis of Published Data on Two *ABCR* Alleles

	D2177N			G1961E[a]		
STUDY	No. of Cases	No of. Controls	*P* (OR [95% CI])[b]	No. of Cases	No. of Controls	*P* (OR [95% CI])[b]
Allikmets et al. (1997*a*)	7/167	1/220	.012	6/167	0/220	.006
ABCR Consortium 2000	21/1,189	8/1,258	.005	19/1,218	4/1,258	.0008
Stone et al. (1998)	2/182	0/96	.42	1/182	0/96	.65
De La Paz et al. (1999)	2/164	0/56	.55	NA	NA	NA
McNeil et al. (2000)	5/177	0/150	.045	2/177	0/150	.29
Overall	37/1,879	9/1,780	<.0001 (3.9 [1.9–8.0])	28/1,744	4/1,724	<.0001 (7.0 [2.5–20])

[a] NA = not applicable.

[b] OR = odds ratio; CI = confidence interval. *P* values were calculated from the one-sided Fisher's exact test, and odds ratios were calculated from the exact conditional hypergeometric distribution (Mehta and Patel 1995).

association of heterozygous *ABCR* alleles with the AMD phenotype (Allikmets et al. 1997*a*). This "classical" case-control study of 167 patients with AMD and 220 controls found, in 16% of patients, *ABCR* alterations that were interpreted as being associated with the disease phenotype, since they were found in <1% of controls. Most alterations resulted in rare missense mutations, some of which had also been found in patients with STGD1 (Allikmets et al. 1997*a*). Subsequently, several reports disputed the conclusions of that study, stating that they were unable to replicate these findings and, therefore, to confirm the association (Stone et al. 1998; De La Paz et al. 1999; Kuroiwa et al. 1999). Rivera et al. come to similar conclusions in their current article (Rivera et al. 2000 [in this issue]). Problems with replication of an association study of a complex disease are not unexpected, and discussion of the topic is beyond the scope of this editorial (e.g., see Long and Langley 1999; O'Donovan and Owen 1999). In short, difficulties arise mainly because of small sample size in studies of rare variants with modest effect on a complex trait.

Our hypothesis-generating finding that heterozygous *ABCR* mutations may increase susceptibility to AMD was recently tested by an expanded collaborative study that included 15 centers in Europe and North America (ABCR Consortium; Allikmets 2000). In that study, the two most common AMD-associated variants, G1961E and D2177N, were genotyped in 1,218 unrelated patients with AMD and in 1,258 reportedly unaffected, matched controls. Together, these two nonconservative amino acid changes were found in one allele of *ABCR* in 40 patients (~3.4%) and in 12 controls (~0.95%), a statistically significant difference ($P < .0001$) (Allikmets 2000). The risk of AMD was estimated to be increased approximately threefold in carriers of D2177N and approximately fivefold in carriers of G1961E. In the context of common complex disorders, this represents an important contribution to the disease load. Since AMD affects millions of people worldwide, and since the described mutations represent only 2 of 13 reported elsewhere (Allikmets et al. 1997*a*), the number of people at increased risk of developing age-related maculopathy, as carriers of variant *ABCR* alleles, is substantial.

To further explain and, it is hoped, resolve the "ABCR controversy," I offer the following meta-analysis of published data on the two most frequent *ABCR* variants (table 1). Data from Rivera et al.'s study of these variants are not separated in the table, since they had been included in the ABCR Consortium study (Allikmets 2000). It is apparent that the main reason for the controversial interpretation of the data is the *small sample size* in individual studies. If analyzed separately, none of the studies, with the exception of that by Allikmets et al. (1997*a*), yields statistically significant results. A substantial increase in the sample size, as in the consortium study or in the proposed meta-analysis, results in substantial increase of power of statistical analysis. Resulting *P* values, as well as relative risk estimates, leave no doubt that the association is statistically significant. Note that the relative risk estimates calculated on the basis of the meta-analysis are also increased compared with those in the consortium study (Allikmets 2000) and are estimated at ~4 for the D2177N mutation and at ~7 for the G1961E variant.

These analyses, once again, clearly demonstrate the need for large cohorts of cases and matched controls for association studies of rare alleles. When all available data are considered, heterozygous *ABCR* alleles are estimated to increase susceptibility to AMD in ~8%–10% of all cases. However, this estimate has to be assessed with caution, since the analysis of *ABCR* variation in AMD is far from complete. The reader has to be reminded that, even in Stargardt disease, ~30%–40% of disease-associated *ABCR* alleles go undetected (Allikmets 1999). In addition, as emphasized above, founder alleles in some ethnic groups can seriously affect the analysis, suggesting that large, multicenter-based studies of matched cases and controls are the only alternative method to achieve statistical significance. Consorted

study design also helps minimize the confounding effect of population stratification, the most serious reason for spurious associations (for an explanation, refer to Allikmets 2000). Until recently, designs with family-based controls, as in various modifications of transmission/disequilibrium tests (TDTs [Spielman et al. 1993]), were considered a panacea for association studies. However, not only are TDTs (even those using sibs instead of parents) very difficult to perform on late-onset disorders, but the effort may be of limited value, since the effect of population stratification may have been overestimated (Morton and Collins 1998) and since family-based control data lead to substantial loss of power for detection of linkage disequilibrium, compared with studies of case-control variety (Schaid and Rowland 1998). Instead, the genotyping of a sufficient number of independent single-nucleotide polymorphisms (SNPs) in matched cases and controls is likely to maintain the robustness of a more powerful, large, multiethnic case-control study (for review, see Risch 2000). Application of alternative approaches to decipher the genetic component of AMD, such as full genome scans by linkage, has yielded disappointing results, at least in the heterogeneous U.S. population (Weeks et al. 2000). These results were expected, since it was predicted quite some time ago (Risch and Merikangas 1996) that, in the absence of "major" loci, a likely scenario for AMD, linkage analysis would have limited power in comparison with association studies of candidate genes.

Functional Studies of ABCR

Data presented in the previous section, on the issue of allelic association of *ABCR* with AMD, should convince even the most skeptical reviewers. However, for those who are still not persuaded, supporting functional evidence is provided as follows. The ABCR protein was first described, in the 1970s, as a large component of photoreceptor outer-segment disk rims (Papermaster et al. 1976, 1978). Hence, it was called a "rim protein" (RimP) for the next 20 years. Only in 1997 was the encoding gene cloned and characterized as a member of the ABC-transporter superfamily, suggesting a transport function of some substrate in photoreceptor outer segments (Allikmets et al. 1997*b;* Illing et al. 1997). All-*trans* retinal, the isoform of rhodopsin chromophore, was identified as a potential substrate of ABCR, on the basis of its ability to stimulate ATP hydrolysis by reconstituted ABCR protein in vitro, suggesting that retinal could also be the in vivo substrate for ABCR (Sun et al. 1999). Studies of Abcr-knockout mice fully supported this hypothesis, proposing ABCR as a "flippase" of the protonated complex of all-*trans* retinal and phosphatidylethanolamine (N-retinylidene-PE) (Weng et al. 1999). Mice lacking the functional *Abcr* gene demonstrated delayed dark adaptation, increased all-*trans* retinal after light exposure, elevated phosphatidylethanolamine in rod outer segments, accumulation of the protonated N-retinylidene-PE complex, and striking deposition of a major lipofuscin fluorophore (A2-E) in the RPE. On the basis of these findings, it was suggested that the *ABCR*-mediated retinal degeneration may result from "poisoning" of the RPE because of A2-E accumulation, with secondary photoreceptor degeneration due to loss of the RPE support role (Weng et al. 1999). A2-E, a pyridinium bis-retinoid, which is derived from two molecules of vitamin A aldehyde and one molecule of ethanolamine, has been characterized as one of the major components of RPE lipofuscin. Accumulation of lipofuscin in the macular region of RPE is characteristic of aging eyes and is the hallmark of both STGD1 and AMD.

Together, these data define ABCR as the "rate keeper" of the transport of retinal in the visual cycle. ABCR is apparently not absolutely essential for this process, since individuals completely lacking the functional protein (e.g., some patients with arRP) maintain some eyesight for several years. Over time, however, even mild dysfunction of ABCR affects vision irreparably. Most recently, intriguing data were obtained, from studies of *Abcr*+/− heterozygous mice, that fully support *ABCR* involvement in AMD (Mata et al. 2000): a phenotype similar to that seen in Abcr knockouts (A2E accumulation in the RPE, etc.) was described in heterozygous mice, but its manifestation occurred at a slower, age-related, rate. The distinct, AMD-resembling phenotype in the *Abcr*+/− mouse model suggests that humans heterozygous for *ABCR* mutations may be predisposed to A2E accumulation and concomitant retinal or macular disease (Mata et al. 2000).

Remarkable allelic heterogeneity of the *ABCR* gene has substantially complicated genetic analysis of its involvement in retinal disease, especially in the AMD complex trait. In a situation in which a modest effect of a mutation can be estimated only by association analysis, the crucial question of the functional significance of a particular sequence variant often remains unanswered, generating sometimes unnecessary speculations. It is not enough to classify mutations as "possibly" or "probably" disease causing, only on the basis of circumstantial evidence—for example, on the basis of a predicted effect of an amino acid change on the protein function. Development of an ABCR mutagenesis system that allows the determination of the actual effect of *ABCR* variants have on the protein function has therefore been highly anticipated.

Most recent data from photoaffinity-labeling and ATPase-activity experiments in Jeremy Nathans' laboratory (Sun et al. 2000) have dramatically advanced our knowledge in this field. In addition to determining the

effect of ~40 *ABCR* mutations, these elegant experiments offer new evidence directly relevant to our discussion. For example, they demonstrate that both *ABCR* variants analyzed in the consortium study, G1961E and D2177N, affect the protein function in vitro. The mutant G1961E protein, produced after the transfection of human embryonic kidney (293) cells with cloned cDNA, exhibits both several-fold–lower binding of 8-azido-ATP and dramatic *inhibition* of ATPase activity by retinal, compared with the wild-type ABCR protein. The D2177N variant had no effect on 8-azido-ATP binding but exhibited a reproducible *elevation* in ATPase activity, relative to the wild type (Sun et al. 2000). Consequently, the *ABCR* variants deemed to be associated with the AMD phenotype are not anonymous SNPs but, rather, are mutations affecting ABCR function. These results will also challenge several suggestions (Fishman et al. 1999; Lotery et al. 2000) that G1961E, the mutation most frequently found in patients with STGD and/or AMD, is indeed a benign variant in linkage disequilibrium with another disease-causing mutation.

Another issue that has been clarified is that of the functional significance of the G863A/delG863 variant. This variant is the most common single allele among northern-European patients with STGDand is also present in ~3% of the general population (Maugeri et al. 1999). Although Maugeri et al. classified this variant as a "mild" mutation, its role in retinal pathology has been disputed because of its high (>1%) frequency in the general population. Data by Sun et al. clearly demonstrate a profound biochemical defect caused by either version of this mutation, effectively supporting the conclusions of the earlier study. Last, both mutations found on the "German" complex allele, L541P and A1038V, even if analyzed separately, render the ABCR protein defective (Sun et al. 2000). In summary, functional studies fully support the proposed genotype/phenotype model of *ABCR* and offer several tools to advance our knowledge about the role of *ABCR* in chorioretinal disease.

Outlook

The scientific progress in the determination of the role of the *ABCR* gene in retinal pathology has been remarkable. We have significantly expanded our knowledge of the extensive range of phenotypes caused by various combinations of *ABCR* mutations. *ABCR* research has led to the formation of multicenter studies, encompassing large cohorts of ethnically diverse samples. It is now known that ABCR functions as the transporter of N-retinylidene-PE, and there is an in vitro system(s) to study functional implications of all mutations. Finally, there is a mouse model that accurately reproduces many features of human disorders. Considering all the above, I see no reason why the scientific excitement that has followed *ABCR* research cannot transform into an equal excitement for people who would benefit the most—those affected with different forms of retinal disease. Our efforts should now be moved to the next stage of research, directed toward the finding of therapeutic solutions for *ABCR*-mediated chorioretinal disease, by either improving the transport function of ABCR or preventing accumulation of toxic products resulting from ABCR malfunction.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim/ (for STGD1 [MIM 248200]; and ARMD2 [MIM 153800])

RetNet Retinal Information Network, http://www.sph.uth.tmc.edu/Retnet/home.htm

Vision Research—A National Plan: 1999–2003, http://www.nei.nih.gov/publications/plan/plan.htm

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