ABCR Gene and Age-Related Macular Degeneration

Stargardt disease is a recessively inherited degeneration of the macula of the retina arising in youth (usually in teenagers and young adults) that is caused by defects in the ABCR gene (1). Rando Allikmets et al. investigate (2) the idea that heterozygote carriers of ABCR mutations might develop age-related macular degeneration (AMD) late in life. We wish to point out methodological deficiencies that call into question their interpretation of their results (2).

Allikmets et al. comprehensively surveyed a set of 167 unrelated patients with AMD exon by exon for DNA sequence variants (2). If at least one patient was found to have a variation affecting the encoded protein sequence in a particular exon, a set of control individuals was evaluated for variations in the same exon. Thirteen variants that were found at greater frequency in AMD patients than in normal individuals were listed in table 1 of the report; those that were found at greater frequency in controls were placed in table 2 in the report. (The polymorphism S2255I present in approximately equal frequency in the two groups was also shown in table 2).

Almost all variants in both tables were missense changes that are not known to be causes of Stargardt disease, and most were rare alleles found only in one individual each. On inspection of table 1, Allikmets et al. conclude that variant alleles are more common in AMD patients than in the control group, and that the variants have a pathogenic role in AMD. However, not accounting for the data in table 2 at this point creates a bias tending to support the report’s conclusions. Perhaps more importantly from a methodological standpoint, the controls were not evaluated equally intensively, because it appears that only 15 of the 51 exons were screened in that group. In this sort of study, it is essential to provide objective evidence documenting that the controls are drawn from the same population as the patients with AMD. The “racial matching” of the controls and AMD groups is insufficient because, even among Caucasians, there may be risk factors for AMD that vary between different ethnic groups within the same “race.”

The discovery of new sequence anomalies in a gene that is already a known cause of a disease does not mean that that the newly discovered anomalies are pathogenic. The data supporting a pathogenic role for each variant encountered must be individually examined. Missense mutations are especially problematic unless there is a basic understanding of the structure and function of the encoded protein, which there is not for the protein product of the Stargardt gene, ABCR. Supporting evidence could come from a statistically significant abundance of a particular sequence change in a disease group as opposed to a control group. Only three of the sequence anomalies in tables 1 and 2 in the report (2) appear to be nonrandomly distributed between the AMD and control groups. Two of these, G1961E and D2177N, are in excess in the AMD patients (P = 0.006 and 0.012, respectively, with the use of the right tail of Fisher’s exact test), and the other, R943Q, is in excess in the controls (P = 0.006, with the use of the left tail). However, since 18 comparisons are being made in tables 1 and 2, the chance that observations for some of them are not randomly distributed among the control and AMD groups is quite likely. With the use of the Bonferroni adjustment (3), significance would have to be at the 0.05/18 = 0.003 level. With the use of this criterion for statistical significance, none of the DNA sequence alterations in tables 1 or 2 is significantly different in abundance between the AMD and control groups. Additional data would be required before one concluded that there is a causative connection between AMD and any of the DNA sequence changes. For example, observing cosegregation of the AMD and a sequence anomaly in a large family would be strong supportive evidence. Allikmets et al. did not show (2) cosegregation of any of these sequence variants with AMD in any multiplex families.

More problems arise in the report’s projection of the incidence of Stargardt-gene mutations in AMD. The introduction of the report (2) states that the incidence of AMD is 30% and that they found AMD-associated alterations in 16% of AMD patients. Variants causing AMD would then be predicted to have a frequency in the general population of 0.048. If all of the sequence anomalies listed in table 1 do in fact predispose a person to AMD, one would expect 4.8% of the control group to be carriers, yet only 0.45% are. Furthermore, if all ABCR mutations causing AMD in heterozygotes cause Stargardt disease in homozygotes, then the expected incidence of Stargardt disease would be 1/1651, which is about six times the observed frequency of 1/10,000 (4). Allikmets et al. address this last discrepancy in note 24 of their report, where they assume a lower incidence of AMD (20%) and a lower incidence of ABCR mutations in AMD patients (15%). These “adjustments” decrease the expected incidence of Stargardt disease to 1/4311, still about 2.5 times the observed incidence.

Either Stargardt disease is much more frequent than currently appreciated (almost equal in frequency to cystic fibrosis, which is highly unlikely), or many of the “mutations” discovered by Allikmets et al. do not cause Stargardt disease. Which of them, if any, predispose a person to AMD also remains unanswered.

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Allikmets et al. (1) report that 13 variations in the “Stargardt disease gene” ABCR are associated with age-related macular degeneration (AMD). ABCR is the first identified AMD gene and will be an important starting point for further research. There is a flaw in the interpretation of the data. Table 1 in the report (1) summarizes alterations that were found either significantly more often in AMD patients than in controls (normal individuals) or that were only present in AMD patients (n = 13), while all other variations (n = 5) are presented in table 2 in the report. Allikmets et al. consider the former “AMD-associated,” but this conclusion is a direct consequence of the data representation and lacks statistical relevance. When all 18 variations are combined, AMD patients do not show a higher frequency of variations than controls (36% as opposed to 31%; P = 0.30). If one accepts a type 1 error of 0.05 when studying 18 loci, then the D2177N missense mutation is indeed found significantly more often in AMD patients than in controls (4.2% as opposed to 0.45%; P = 0.023). However, the second statis-
cal significant finding is a lower frequency of the variant at R943Q locus in AMD patients (4.7% as opposed to 16.25%; \(P = 0.01\)). It is not clear why this finding is not discussed.

The diagnosis of AMD (1) is not based on the internationally accepted grading system (2), and we question whether the patient with “a few tiny juxtafoveal drusen” has AMD. Finally, we are uncertain about the interpretation of figure 2 in the report. The clinical end-stages of Stargardt disease and AMD may look similar, and because the paternal ABCR mutation was not identified, the Stargardt disease patient could well be a “compound heterozygote.” Consequently, the “AMD” in the older heterozygote mother may be a mild expression of Stargardt disease.

To fully comprehend the relevance of the ABCR gene for the etiology and diagnosis of AMD, further studies are needed in well-defined AMD patients and controls.

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Response: Dryja *et al.* raise several concerns about our report (1), including the suggestion that the data do not support our main conclusion—that alterations in the ABCR gene play a role in AMD. They object to our selection of controls, question the severity of the alterations that we described, and raise statistical issues. We answer their four main objections by reviewing the data from our report and by providing further clarification.

1) Controls. Dryja *et al.* state that risk factors for AMD in Caucasians may differ between ethnic groups. While confounding factors are a potential problem with case-control designs, we have controlled for this by matching Utah AMD patients with an equal number of unrelated individuals from Utah families in the Centre d’Étude du Polymorphisme Humain study. We observed no substantial differences in the selection of AMD patients in our study. Among the Utah and Boston AMD patients, similar numbers of variants found in AMD patients were observed (13/71 as opposed to 13/96) and there was a similar distribution of the common alleles G1961E (2 and 4) or D2177N (5 and 2).

We found a large number of variant alleles; for each exon in which we found a variant, we screened a set of 220 racially matched control individuals. The vast majority of alterations were not found in the control group. We agree that rare variants might be present in the control group in those exons in which we did not identify variants in AMD patients. In addition to the 15 exons screened in the control group (1), we published data on control samples (40 to 85) screened in 6 additional exons (2). To date, we have analyzed a total of 29 exons for 220 controls and 10 other exons in 40 to 85 controls and have identified no additional amino acid altering variants in these control samples. For the 12 exons that have not been analyzed in control individuals, we have examined 167 AMD and 150 patients with Stargardt disease (STGD1) and have not seen an alteration. It is unlikely that there are sufficient variants in these exons in control individuals to alter our conclusion.

2) Severity of alterations. In the absence of a functional assay for the ABCR/Rim protein, we cannot state explicitly how any of the alleles that we have identified will affect the protein. However, three variants found in AMD patients either affect the first base of a splice donor site or cause a frameshift. These alleles are highly likely to produce an ABCR protein that is null or has severely impaired function. Four alleles found in AMD patients are also STGD1 mutations (R1898H, G1961E, 6519del11, and G863A), and five others (E471K, R1129L, R1517S, G1578R, and D2177N) result in the gain or loss of charged residues. It is possible that these alterations also have an effect on ABCR function.

3) Statistical issues. We reported that 16% of the AMD patients in our sample (1) had ABCR alterations that were not found in a sample of control individuals. Because this was a hypothesis-generating investigation, we did not report detailed statistical analysis. Dryja *et al.* correctly state that most of these alterations were uncommon and, if a multiple test correction is used, none is statistically significant when considered alone. However, a Bonferroni correction is often overly conservative and can mask true associations (3). In no place did we apply a statistical test to the data in table 1 in (1) alone. We have completed typing of all variants on 220 control individuals (Table 1). Variants S2255I and R943Q are frequent enough to test alone and were not significantly different in either group. For the remaining 16 alleles, we applied the CLUMP test of Sham and Curtis (4). This method collapses into a 2 × 2 table and assesses significance with Monte Carlo simulations to generate tables having the same marginal totals as the table in question. In several runs, each with 10,000 different simulations, we did not find any tables that had a higher value than ours by chance. Adding in the data from S2255I and R943 gave the same result. This supports an association (\(P < 0.0001\)) between ABCR variants and AMD. As we stated explicitly in note 22 of our report (1), when we pool the alleles that are known to be associated with STGD1 (R1898H, G1961E, 6519del11, and G863A), those alleles are found in 9/167 AMD and 2/220 controls. This comparison is significant (\(P = 0.0098\) with a left-tailed Fisher’s exact test).

4) Segregation of ABCR alleles in AMD. For a complex clinical disorder such as AMD, segregation of a susceptibility locus may not be observed in all pedigrees (5). We agree that evidence of segregation of ABCR alterations in AMD pedigrees would provide additional evidence for a role of the gene in this disorder, and we are pursuing this line of research actively. However, in our elderly AMD cohort, few parents of the AMD subjects are alive to study and most of their children are not yet old enough to manifest the phenotype. We did report that a higher proportion of patients with an AMD-associated variant had a close relative (mostly first-degree relatives) with the disorder, further suggesting a familial component in these families.

We have recently found STGD1 patients

### Table 1. Prevalence of ABCR variants in patients with AMD and in population control individuals.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Cases (n = 167)</th>
<th>Controls (n = 220)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E471K</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>V643G</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>G818E*</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>G863A</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>R1129L</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>T1428M</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>R1517S</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>H1562T</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>G1578R</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>S196+1G-&gt;A</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>R1898H</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>G1961E</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>L1970F</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>6519del11</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>D2177N</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>6588delC</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>R943Q</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td>S2255I</td>
<td>24</td>
<td>34</td>
</tr>
</tbody>
</table>

*This allele was mistakenly identified in our report (1) as D846H; D846H has been found in one patient with STGD and in no patients with AMD or control individuals to date. \(P > 0.3\).
with the E471K and L1970F alleles, bringing to six the number of alterations found in both AMD and STGD1. We have also identified an ABCR mutation (P1380L) in a grandparent with AMD who transmitted the altered allele to independent sets of STGD1 grandchildren born to two of her children (6). Furthermore, we have ascertained 25 additional pedigrees in which both AMD (confirmed by an ophthalmologist in at least 14 of the cases) and STGD1 segregate within the family, further supporting a connection between these two conditions.

We did not state that "all ABCR mutations causing AMD in heterozygotes cause Stargardt disease in homozygotes"; We did state that our data "suggest(s) that some mutations that cause recessive STGD1 may enhance susceptibility to AMD in the heterozygous state." In note 24, we commented that the estimated population frequency of STGD1 and the estimated frequency of AMD are not inconsistent with this concept. That is, the estimates are of the same order of magnitude. The penetrance of ABCR alleles for AMD and the fraction of STGD1 alleles that might cause AMD, both crucial variables needed for these estimates, are as yet unknown. In our first paragraph we cited the work of Klein et al. that "mild forms of AMD occur in nearly 30% of those 75 years and older" (7).

The points raised by Klaver et al. are mostly addressed above. We can see no justification for pooling data from frequent polymorphisms (R943Q and S2255I) with rare variants. However, even when we do, the results are significant. All our AMD subjects were graded according to an established system (8), and nearly all (96%) had stage 3 disease or greater. As to international diagnostic criteria, there is no universally accepted definition of the early stages of AMD, which may include small drusen formation and minimal retinal pigment epithelial changes. The term "age-related macular degeneration" encompasses a wide spectrum of disease, only a small proportion of which is currently treatable. Some definitions include an age criterion and degrees of loss of visual acuity (9).

As stated in the legend of figure 1 in our report (1), we have not yet identified the ABCR mutation in the STGD1 patient shown in figure 2 of our report, and we do believe that he is a compound heterozygote. The pedigree is consistent with the STGD patient's disease being a result of recessive alterations in ABCR. As an observation relative to the hypothesis of that paper, the patient's mother (a carrier for the 6519del11 allele) has AMD. Her husband, a presumed obligate carrier for a different ABCR allele, also had AMD. The paternal uncle also suffers macular degeneration and would have a 0.5 chance of inheriting the same unidentified ABCR allele.

In summary, a reader could reach two possible conclusions from our work. The more conservative would be that we have an interesting "hypothesis-generating finding," that ABCR mutations may confer an increased risk to AMD. The second, which we support, is that ABCR mutations are in fact involved in a fraction of AMD cases. We look forward to the dissemination of data relevant to our hypotheses.

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