

# Molecular Genetic Analysis of ABCR Gene in Japanese Dry Form Age-Related Macular Degeneration

Nobuo Fuse,\* Takefumi Suzuki,\* Yuko Wada,\* Madoka Yoshida,\*  
Masahiko Shimura,\* Toshiaki Abe,\* Mitsuru Nakazawa,<sup>†</sup> and Makoto Tamai\*

\* *Department of Ophthalmology, Tohoku University School of Medicine, Sendai, Miyagi, Japan;* <sup>†</sup>*Department of Ophthalmology, Hirosaki University School of Medicine, Hirosaki, Aomori, Japan*

**Purpose:** To explore whether the mutation in the retina-specific ATP-binding cassette transporter (ABCR) gene, the Stargardt's disease gene, contributes to the prevalence of the dry form of age-related macular degeneration (dry AMD) in Japanese unrelated patients.

**Methods:** Twenty-five Japanese unrelated patients with dry AMD who were diagnosed by fluorescein angiography and indocyanine green angiography were chosen as the dry AMD group. None of these cases had apparent choroidal neovascularization. To detect the mutations in the ABCR gene, genomic DNA was extracted from leukocytes of peripheral blood, and 26 exons of the ABCR gene were amplified by polymerase chain reaction (PCR). All the PCR products were then directly sequenced. When a mutation was detected, the occurrence of a mutation was compared between these AMD patients and the control group.

**Results:** After direct sequencing, a point mutation in exon 29 was found in one of the 25 dry AMD patients. In addition, a polymorphism in exon 45 was found in two other patients, and three sequence variations in exon 23 were detected in all patients. The incidence in AMD patients in whom a mutation in exon 29 (4%) was detected was less than that in controls (5%). Screening of the intron-exon boundaries also led to the identification of intronic mutation in intron 33.

**Conclusion:** In this study we found no relationship between allelic variation in the ABCR gene and the prevalence of dry AMD in Japanese unrelated patients. **Jpn J Ophthalmol 2000;44:245-249** © 2000 Japanese Ophthalmological Society

**Key Words:** Dry form age-related macular degeneration, retina-specific ATP-binding cassette transporter, Stargardt's disease.

## Introduction

Age-related macular degeneration (AMD) can cause acquired visual impairment that has been estimated to affect as many as 30% of the population over age 65.<sup>1,2</sup> Age-related macular degeneration is a multifactorial disorder that is associated with environmental risk factors.<sup>3</sup> Genetic factors may also contribute to AMD.<sup>4,5</sup> Clinically, AMD is divided into two subtypes: dry AMD and wet AMD.<sup>9</sup> At present, there is no reliable prevention or therapy for dry AMD. One approach to identifying the genes responsible for multifactorial disorders is to survey

inherited eye diseases that resemble the phenotypes of AMD. There are several hereditary retinal dystrophies that resemble AMD; however, no mutation in retina-specific genes has been reported in dry or wet AMD.

Recently, mutations in a retina-specific ATP-binding cassette transporter (ABCR) gene have been associated with Stargardt's disease.<sup>7</sup> The locus of this disease was originally mapped to markers on chromosome 1p13-p21.<sup>8</sup> The ABCR protein is a member of the ATP-binding cassette transporter superfamily.<sup>7,9</sup> Immunohistochemical analysis of macaque, bovine, and mice retinas showed that the ABCR/Rim protein is restricted to the rim and incisures of the rod outer-segment discs (ROS).<sup>8-10</sup> The ABCR protein is involved in the transport of specific substrates, such as retinal derivatives, metabolites, anti-oxi-

Received: March 3, 1999

Correspondence and reprint requests to: Nobuo FUSE, MD, Department of Ophthalmology, Tohoku University School of Medicine, Sendai-shi, Miyagi-ken 980-8574, Japan

dants, lipids or peptides, to and from the discs. Stargardt's disease is a common form of autosomal recessive macular dystrophy characterized by juvenile onset, central visual dysfunction, perimacular yellow deposits, and atrophy of the retinal pigment epithelium.<sup>13</sup> Stargardt's disease has phenotypic similarities to AMD. Recently, Allikmets et al<sup>14</sup> hypothesized that mutations of the gene that cause Stargardt's disease may enhance susceptibility to AMD in the heterozygous state. They reported that mutations in ABCR were associated with 16% (26/167) of AMD cases, while 0.45% (1/220) of a racially matched population had mutation of ABCR. However, the methodology they used in that study was controversial.<sup>15</sup> Among 26 patients that had ABCR-associated alteration, 25 patients had dry AMD.

The purpose of the present study is to explore whether a mutation in the ABCR gene contributes to the prevalence of the dry form of age-related macular degeneration (dry AMD) in Japanese unrelated patients.

## Materials and Methods

We selected 25 unrelated Japanese patients with dry AMD (20 men and 5 women; mean age = 70.2 years) and 40 Japanese controls (25 men and 15 women; mean age = 40.5 years) from the ophthalmic

clinic of the Tohoku University School of Medicine, Sendai, Japan. We did not choose an elderly, AMD-free control group, because this would require the differentiation between the retinal changes involved in normal aging and early AMD. Routine ophthalmic examinations were performed in all patients on each parameter. Examination included slit-lamp examination, indirect ophthalmoscopy, macula examination with contact lens biomicroscopy, and fluorescein and indocyanine green (ICG) angiography with a scanning laser ophthalmoscope. The diagnosis of dry AMD includes one or more of the following: the presence of drusen in or under the retinal pigment epithelium (RPE), and irregularities in the pigmentation of the RPE (or geographic atrophy). Apparent choroidal neovascularization and vascularized pigment epithelial detachment were absent in all cases. Cases with other macular disease, for example, idiopathic central serous chorioretinopathy, were excluded from this study. All patients and controls were anamnesticly unrelated. All patients were given sufficient explanation of the purpose of this study, and informed consent was obtained.

Genomic DNA was extracted from leukocytes of peripheral blood and was purified using the Qiagen QIAmp Blood Kit (Qiagen, Chatsworth, CA, USA). The exons shown in Table 1 were amplified by polymerase chain reaction (PCR) using 0.5  $\mu$ M concen-

**Table 1.** Mutations Found in ABCR\* Gene in 26 Exons Examined in This Study

| Exon | AMD <sup>†</sup> | Stargardt's Disease               | Exon | AMD   | Stargardt's Disease            |
|------|------------------|-----------------------------------|------|---|--------------------------------|
| 11   | E471K            |                                   | 29   | T1428M  |                                |
| 15   |                  |                                   | 31   | R1517S  |                                |
| 16   |                  | G818E, G863A<br>(D847H)           | 33   | I1562T<br>G1578R                                |                                |
| 17   |                  |                                   | 34   |   | N1614FS                        |
| 18   |                  |                                   | 35   |   |                                |
| 19   |                  | V931M, 2884delC<br>N965M, (R943Q) | 36   | 5196+1G→A                                       | 5041deL15<br>5196+2T→C         |
| 20   |                  |                                   | 40   | R1898H  | R1898H                         |
| 21   |                  | A1028V                            | 42   | G1961E  | G1961E                         |
| 22   |                  | 3211insGT, V1072A<br>E1087K       | 43   | L1970F  | 6006+1G→T                      |
| 23   | R1129L           |                                   | 44   |   | L2027F, R2038W<br>(I2023I)     |
| 24   |                  |                                   | 45   |   | V2050L, R2077W<br>(I2083I)     |
| 25   |                  |                                   | 46   |   | R2106C<br>(V2094V)             |
| 27   |                  |                                   | 48   | 6519 $\Delta$ 11bp<br>D2177N<br>6568 $\Delta$ C | 6519 $\Delta$ 11bp<br>6709insG |

\*ABCR: ATP-binding cassette transporter.

<sup>†</sup>AMD: Age-related macular degeneration.

tration of 26 pairs of intronic primers<sup>13</sup> in an amplification mixture (50  $\mu$ L) containing 0.2 mM dNTPs and 1 U Ex *Taq* polymerase (Takara, Tokyo). Ten exons were reported to include AMD-associated mutations. The other 16 exons were the ones that Allikmets et al<sup>7</sup> had used for the mutation analyses of the ABCR gene in patients with Stargardt's disease. These exons include ATP-binding domain and in fact many identified mutations were included in these exons.

The PCR products were purified using the PCR Purification Kit: Nucleo Spin Extract kit (Macherey-Nagel) or GeneClean II (Funakoshi, Tokyo) after loading onto a 1.2% agarose gel. Purified fragments were directly sequenced using primers specific for the intron sequence and BigDye<sup>TM</sup> Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Norwalk, CT, USA) on an automated DNA sequencer (ABI Prism<sup>TM</sup> 310 Genetic Analyzer, Perkin-Elmer).

## Results

We screened for ABCR sequence mutations in unrelated dry AMD patients. Of the 50 exons so far identified with the ABCR gene,<sup>16</sup> 26 exons were screened for variations in each dry AMD patient. Among the chosen 26 exons, 10 exons (exon 11, 23, 29, 31, 33, 36, 40, 42, 43, and 48) contained AMD-associated alterations reported by Allikmets et al.<sup>14</sup> The other exons had been previously used for mutation analysis in Stargardt's disease patients.<sup>7</sup> After direct sequencing, a point mutation in exon 29 was found in one patient, a polymorphism in exon 45 was found in two other patients, and three sequence variations in exon 23 were detected in all patients. Table 2 shows the frequency of changes in nucleotides found in the patients and in normal controls.

The point mutation found in exon 29 was a heterozygous mutation (cytosine to thymine; C→T) at the second nucleotide in codon 1428, which changes the amino acid threonine to methionine. The mutation in exon 29 (4%) in AMD patients was less frequent than in the controls (5%). In exon 23, three sequence variations existed in conserved amino acid positions affecting the encoded protein sequence (P1116S, H1125L, Q1126L) in all AMD patients. Three alterations in exon 23 were also detected in all controls.

In exon 45, a polymorphism was found; there was heterozygous base change (C65249T) at the third nucleotide in codon 2083 in two AMD patients, but this base change did not cause amino acid change (12083I). It was reported that 12083I was detected in Stargardt's disease patients.<sup>7</sup> Screening of the ABCR coding sequence and intron-exon boundaries also led to the identification of two homozygous intronic base changes 4773+48C→T (8%) and four heterozygous intronic base changes 4773+48C→T (4%) in intron 33. Neither a deletion nor a splice donor/acceptor site change was found.

## Discussion

We performed a mutation screening of 26 exons of the ABCR gene in 25 unrelated Japanese dry AMD patients in this study. Of the 50 exons identified with the ABCR gene, 10 exons containing AMD-associated alterations<sup>14</sup> and 16 exons used for mutation analysis in Stargardt's disease<sup>7</sup> were studied. Allikmets et al<sup>14</sup> showed that 10 exons contained 13 AMD-associated alterations. Our study revealed a missense mutation and three sequence variations in conserved amino acid positions. The direct sequencing identified a heterozygous transitional change at

**Table 2.** Sequence Variations, Mutation, and Polymorphism in ABCR\* Gene Detected in This Study

| Alteration                      | Base Change               | Dry AMD <sup>†</sup><br>(n = 25) | Controls<br>(n = 40) |
|---------------------------------|---------------------------|----------------------------------|----------------------|
| P1116S                          | CCC→TCC (homozygote)      | 25 (100%)                        | 40 (100%)            |
| H1125L                          | CAC→CTC (homozygote)      | 25 (100%)                        | 40 (100%)            |
| Q1126L                          | CAA→CTT (homozygote)      | 25 (100%)                        | 40 (100%)            |
| T1428M                          | ACG→ATG (heterozygote)    | 1 (4%)                           | 2 (5%)               |
| Polymorphism<br>I2083I          | ATC→ATT (heterozygote)    | 2 (8%)                           | 0 (0%)               |
| Intron basechange<br>Intron 33: | 4773+48C→T (heterozygote) | 4 (16%)                          |                      |
|                                 | 4773+48C→T (homozygote)   | 2 (8%)                           |                      |

\*ABCR: ATP-binding cassette transporter.

<sup>†</sup>AMD: Age-related macular degeneration.

the second nucleotide in codon 1428 (ACG→ATG) resulting in an amino acid substitution of methionine (M) for threonine (T), which was the same alteration Allikmets et al reported as an AMD-associated mutation with a prevalence of 0.6% (1/167). Our results showed that the incidence of the T1428M mutation was 4% in Japanese dry AMD patients, but this mutation was found more frequently (5%) in the controls. The other 12 AMD-associated mutations were not detected in this study.

In exon 23, three sequence variations (P1116S, H1125L, Q1126L) were detected in all patients and controls. This may be explained by racial differences or sequence variations. Azarian and Travis reported the same sequences as our finding.<sup>12</sup> These sequence variations may not influence the ABCR function, because exon 23 lacks transmembrane and ATP-binding domains. A polymorphism (I2083I) detected in exon 45 had no genetic importance. Also, there is probably no causative meaning for a mutation in intron 33, because this position is 48 bases removed from the splice donor site.

We selected dry AMD patients for this study. It is true that there is difficulty in the diagnosis of dry AMD, but we excluded patients with apparent wet AMD and other maculopathy similar to AMD, using fluorescein and ICG angiography. It has been reported that 25 patients had dry AMD among 26 patients that had ABCR-associated alteration.<sup>14</sup> Therefore, we focused on dry AMD in the present study.

Stone et al<sup>18</sup> reported that allelic variation in ABCR is associated with Stargardt's disease but not with AMD. In Japanese patients, Kuroiwa et al<sup>19</sup> examined 10 exons in Japanese wet and dry AMD, and suggested the lack of association between ABCR genotypes and AMD. The hypothesis that the mutations of the gene that causes Stargardt's disease may enhance susceptibility to AMD in the heterozygous state is fascinating. But this survey revealed only one AMD-associated mutation and no missense mutation similar to that in Stargardt's disease. It was reported that four mutations found in AMD patients (R1898H, G1961E, 6519del11, and G863A) were also found in patients with Stargardt's disease.<sup>14</sup> In our study, only one polymorphism (I2083I) was found.

Although the transport function of ABCR is not yet well-defined, its location lies exclusively in the outer segments of rods and makes this gene an excellent candidate for involvement in diseases such as retinitis pigmentosa and cone-rod dystrophy. Subsequently it has been reported that a homozygous mutation in ABCR causes autosomal recessive retinitis pigmentosa,<sup>20,21</sup> and cone-rod dystrophy.<sup>21</sup> Re-

cently, Weng et al<sup>22</sup> characterized the ocular phenotype in abcr knockout mice. They suggested that ABCR-mediated retinal degeneration may result from a major lipofuscin fluorophore (A2E) in the RPE, with secondary photoreceptor degeneration due to loss of the RPE support role. Klevering et al<sup>23</sup> described mutations in the ABCR gene that can cause clinical characteristics resembling autosomal recessive retinitis pigmentosa and autosomal recessive cone-rod dystrophy. Further investigation of the structure and function of the ABCR protein would be helpful in understanding these retinal diseases.

Our present mutation study in 26 exons did not detect AMD-associated mutations in the ABCR gene. Though we cannot deny the possibility that AMD-associated mutations exist in other exons, we found no association between allelic variation in the ABCR gene and dry AMD in Japanese unrelated patients.

---

The authors thank Kurt Scheibner for editing the manuscript. This work was supported in part by Grants-in-Aid for Encouragement of Young Scientists (to NF) from the Ministry of Education, Science, Sports, and Culture of Japan.

---

## References

1. Klein R, Klein BEK, Linton KLP. Prevalence of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology* 1992;99:933–43.
2. Klein R, Klein BEK, Jensen SC, Meuer SM. The five-year incidence and progression of age-related maculopathy. *Ophthalmology* 1997;104:7–21.
3. Seddon JM, Willett WC, Speizer FE, Hankinson SE. A prospective study of cigarette smoking and age-related macular degeneration in women. *J Am Med Assoc* 1996;276:1141–6.
4. Seddon JM, Ajani UA, Mitchell BD. Familial aggregation of age-related maculopathy. *Am J Ophthalmol* 1997;123:199–206.
5. Heiba IM, Elston RC, Klein BEK, Klein R. Sibling correlations and segregation analysis of age-related maculopathy: the Beaver Dam Eye Study. *Genet Epidemiol* 1994;11:51–67.
6. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. *Surv Ophthalmol* 1995;39:367–74.
7. Allikmets R, Singh N, Sun H, et al. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nature Genet* 1997;15:236–45.
8. Kaplan J, Gerber S, Larget-Piet D, et al. A gene for Stargardt's disease (*fundus flavimaculatus*) maps to the short arm of chromosome 1. *Nature Genet* 1993;5:308–11.
9. Allikmets R, Gerrard B, Hutchinson A, Dean M. Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the expressed sequence tag database. *Hum Mol Genet* 1996;5:1649–55.
10. Illing M, Molday LL, Molday RS. The 220kDa rim protein of retinal rod outer segments is a member of the ABC transporter superfamily. *J Biol Chem* 1997;272:10303–10.

11. Sun H, Nathans J. Stargardt's ABCR is localized to the disc membrane of retinal rod outer segments. *Nature Genet* 1997;17:15-6.
12. Azarian SM, Travis GH. The photoreceptor rim protein is an ABC transporter encoded by the gene for recessive Stargardt's disease (ABCR). *FEBS Lett* 1997;409:247-52.
13. Stargardt K. Über familiäre, progressive Degeneration in der Maculagegend des Auges. *Albrecht von Graefes Arch Klin Exp Ophthalmol* 1909;71:534-50.
14. Allikmets R, Shroyer NF, Singh N, et al. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. *Science* 1997;277:1805-7.
15. Dryja TP, Briggs CE, Berson EL, et al. ABCR gene and age-related macular degeneration. *Science* 1998;279:1107.
16. Gerber S, Rozet JM, van der Pol TJ, et al. Complete exon-intron structure of the retina-specific ATP binding transporter gene (ABCR) allows the identification of novel mutations underlying Stargardt disease. *Genomics* 1998;48:139-42.
17. Nasonkin I, Illing M, Koehler MR, Schmid M, Molday RS, Weber BHF. Mapping of the rod photoreceptor ABC transporter (ABCR) to 1p21-p22.1 and identification of novel mutations in Stargardt's disease. *Hum Genet* 1998;102:21-6.
18. Stone EM, Webster AR, Vandenberg K, et al. Allelic variation in ABCR associated with Stargardt disease but not age-related macular degeneration. *Nature Genet* 1998;20:328-9.
19. Kuroiwa S, Kojima H, Kikuchi T, et al. ATP binding cassette transporter retina genotypes and age related macular degeneration: an analysis on exudative non-familial Japanese patients. *Br J Ophthalmol* 1999;83:613-5.
20. Martinez-Mir A, Paloma E, Allikmets R, et al. Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene ABCR. *Nature Genet* 1998;18:11-2.
21. Cremers FP, van de Pol DRJ, van Driel M, et al. Autosomal-recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. *Hum Mol Genet* 1998;7:355-62.
22. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. Insight into the function of rim protein in photoreceptors and etiology and Stargardt's disease from the phenotype in *abcr* knockout mice. *Cell* 1999;98:13-23.
23. Klevering BJ, van Driel M, van de Pol DRJ, Pinckers AJLG, Cremers FP, Hoyng CB. Phenotypic variations in a family with retinal dystrophy as result of different mutations in the ABCR gene. *Br J Ophthalmol* 1999;83:914-8.