ABCA4 Gene Mutations in Japanese Patients with Stargardt Disease and Retinitis Pigmentosa

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PURPOSE. To evaluate photoreceptor cell-specific adenosine triphosphate (ATP)–binding cassette transporter (ABCA4) gene mutations in Japanese patients with Stargardt disease (STGD) and the correlation of these mutations to clinical phenotypes.

METHODS. Serum was obtained from 10 unrelated Japanese patients with STGD and 96 unrelated Japanese patients with autosomal recessive retinitis pigmentosa (arRP). All 50 ABCA4 gene exons of the patients with STGD were screened for mutations by a combination of single-strand conformation polymorphism analysis and polymerase chain reaction (PCR) direct-sequencing techniques. By restriction enzyme digestion, primer extension analysis, and PCR direct sequencing techniques, the patients with arRP were screened for three segregated, presumably null ABCA4 gene mutations observed in Japanese patients with STGD.

RESULTS. Three novel, presumably null mutations of the ABCA4 gene, IVS7-45_952delinsTCTGACC, IVS12+2T→G, and 1894delA, were identified. The Arg2149stop mutation that had been found in a white patient with STGD in a prior study was also found in a Japanese patient. Two arRP-affected siblings and two unrelated patients with STGD were found to be homozygous for the same IVS12+2T→G mutation, and three other arRP-affected siblings were carriers of the IVS12+2T→G mutation and/or the IVS7-45_952delinsTCTGACC mutation. These three siblings with arRP showed only atrophic degeneration in the macula early after the onset of the disease, and STGD had been diagnosed.

CONCLUSIONS. Three novel ABCA4 gene mutations were identified in Japanese patients with STGD and arRP. Mutations in the ABCA4 gene can cause panretinal degeneration that changes its clinical appearance from STGD to arRP over time. (Invest Ophtalmol Vis Sci. 2002;43:2819–2824)

S targardt disease (STGD) is a frequent cause of autosomal recessive macular dystrophy. It is characterized by onset in the juvenile to young adult years, decreased central vision, progressive bilateral atrophy of the retinal pigment epithelium (RPE), and appearance of orange-yellow flecks in the macula and/or midperiphery of the retina.1,2 By a positional candidate approach, the causative gene for recessive STGD was identified as a retina-specific adenosine triphosphate (ATP)–binding cassette transporter (ABCR) renamed the ABCA4 gene.3,4 Subsequently, linkage analysis and direct sequencing demonstrated that mutation of the ABCA4 gene also causes autosomal recessive retinitis pigmentosa (arRP) and autosomal recessive cone-rod dystrophy (arCRD).5–13 Most patients with STGD are compound heterozygotes, with two missense mutations or one null mutation and one missense mutation.14–16 In contrast, patients with arRP, arCRD, or severe STGD are homozygous for null mutations.6–16 It has been suggested that there is an association between null mutations in the ABCA4 gene and panretinal degeneration.10–14,16

To our knowledge, there are no reports regarding mutations in the ABCA4 gene in Japanese patients with STGD. In this study, we screened Japanese patients with STGD for ABCA4 gene mutations and analyzed the association between the mutations and clinical phenotype.

METHODS

Ascertainment of Patients

Retina specialists in the Departments of Ophthalmology at Osaka University Graduate School of Medicine, Kyorin University School of Medicine, and Nagoya University Graduate School of Medicine identified 10 unrelated Japanese patients with STGD. Diagnosis of STGD was based on the following: a history of a recessive mode of inheritance; age less than 30 years at first reported symptoms; bilateral central vision loss with a beaten-bronze appearance and/or the presence of orange-yellow flecks in the retina from the posterior pole to the midperiphery; typical dark choroid observed by fluorescein angiography; and normal to subnormal electroretinograms (ERGs). We were unable to obtain informed consent for the ERGs from one patient. The remaining patients fit all criteria. Retina specialists in the Department of Ophthalmology at Osaka University Graduate School of Medicine identified 96 unrelated patients with arRP. Diagnosis of arRP was based on the following: a history of a recessive mode of inheritance; pigmentary retinal degeneration; and markedly reduced scotopic ERG. Informed consent for genetic analysis was obtained from each patient, in accordance with the Declaration of Helsinki.

Mutation Detection

All 50 exons of the ABCA4 gene were screened for mutations by a combination of single-strand conformation polymorphism (SSCP) analysis and polymerase chain reaction (PCR) direct-sequencing techniques.19,20 Genomic DNA was isolated from peripheral blood leukocytes by using a DNA extraction kit (DNA Micro Extraction Kit;
TABLE 1. ABCA4 Gene Sequence Variations

<table>
<thead>
<tr>
<th>Nucleotide change</th>
<th>Effect</th>
<th>STGD (20†)</th>
<th>Control (192†)</th>
<th>Reference</th>
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<tr>
<td>IVS7-45_952delinsTCTGACC*</td>
<td>Splice</td>
<td>0</td>
<td>0</td>
<td>This study</td>
</tr>
<tr>
<td>IVS12+2T→G*</td>
<td>Splice</td>
<td>5</td>
<td>0</td>
<td>This study</td>
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<td>0</td>
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<td>Ile604Ser</td>
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<td>Frameshift</td>
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<tr>
<td>G3366C</td>
<td>Glu1122Asp</td>
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<td>This study</td>
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<td>0</td>
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<td>1</td>
<td>0</td>
<td>This study</td>
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<td>Met1882Val</td>
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<td>0</td>
<td>This study</td>
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<td>32</td>
<td>12,13,14,16,22,23</td>
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<tr>
<td>A5814G</td>
<td>Leu1938Leu</td>
<td>4</td>
<td>32</td>
<td>12,14,23</td>
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<tr>
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<td>4</td>
<td>32</td>
<td>11,14,16,23</td>
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<tr>
<td>A5844G</td>
<td>Pro1948Pro</td>
<td>4</td>
<td>32</td>
<td>11,12,14,16,22,23,24,27</td>
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<tr>
<td>T6285C</td>
<td>Asp2095Asp</td>
<td>3</td>
<td>27</td>
<td>11,12,14,16,23</td>
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<tr>
<td>G6445T</td>
<td>Arg2149stop</td>
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<td>0</td>
<td>15</td>
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<tr>
<td>T6563C</td>
<td>Phe2188Ser</td>
<td>1</td>
<td>0</td>
<td>This study</td>
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</table>

* Mutations observed in patients with arRP.
† Number of alleles.

RESULTS

Seventeen sequence variations in the ABCA4 gene were detected (Table 1). The IVS7-45_952delinsTCTGACC, IVS12+2T→G, IVS12-50G→A, Ile604Ser, 1894delA, Glu1122Asp, Leu1583Pro, Gly1623Ser, IVS38-36delT, Met1882Val, Arg2149stop, and Phe2188Ser sequence variations were not found in the 192 normal control alleles. Two STGD-affected families, F9 and F22, and two arRP-affected families, F1 and F26, participated in segregation analysis, and correct segregation of the disease alleles was demonstrated in these families (Fig. 1). The IVS12+2T→G mutation was found in members of these STGD- and arRP-affected families, all of whom were homozygous or compound heterozygous. The IVS7-45_952delinsTCTGACC, IVS12+2T→G, and 1894delA mutations all segregated with the diseases (Fig. 2).

Table 2 shows the clinical characteristics of four patients with STGD from three families and five patients with arRP from two families, who were found to be homozygous or compound heterozygous for the presumably null mutations in the ABCA4 gene. All four patients with STGD had early onset of the disease, poor visual acuity, and a dark choroid (Fig. 3A). Patients F9-II-2 and F18-II-1, both homozygous for the IVS12+2T→G mutation, had RPE degeneration in the macula...
extending to the vascular arcades (Fig. 3B). ERGs obtained early in the course of the disease in these two patients were subnormal. At 11 years of age, patient F18-II-1 was found to have roughly one third of normal scotopic function and one half of normal photopic function (Fig. 4). Two siblings (F22-II-1 and F22-II-3), who were compound heterozygotes with the IVS12\[H11001\]^2T\[G and 1894delA mutations were observed to have posterior pole pigmentation. Three arRP siblings (F1-III-4, F1-III-6, and F1-III-8), all carriers of the IVS12\[H11001\]^2T\[G mutation and/or the IVS7-45_952delinsTCTGACC mutation were observed to have bone spicule-like pigmentation, severe RPE degeneration, and attenuated retinal vessels extending into the periphery with almost complete loss of both photopic and scotopic ERG amplitudes (Figs. 3E, 4). Two arRP siblings (F26-II-4 and F26-II-6), homozygotes for the IVS12\[H11001\]^2T\[G mutation, were found to have severe RPE atrophy, bone spicule-like pigmentation, and attenuated retinal vessels in all areas of the retina and atrophic optic disc, with almost complete loss of both photopic and scotopic ERG amplitudes (Figs. 3C, 4). The visual acuity of these patients with arRP was restricted to hand motions, with normal function remaining only in peripheral islands of the visual field.

### DISCUSSION

Although previous studies have demonstrated ABCA4 gene mutations in patients with STGD, to the best of our knowledge, mutations in the ABCA4 gene have not been reported in Japanese patients with STGD. In this study, we examined 10 Japanese patients with STGD and found 17 different sequence variations in the ABCA4 gene (Table 1). The IVS7-45_952delinsTCTGACC, IVS12\[2T\[G mutation, and Phe2188Ser sequence variations were not found in the 192 normal control alleles tested, and hence these

![Figure 2](http://tvst.arvojournals.org/) Three novel mutations in the ABCA4 gene were identified. The IVS7-45_952delinsTCTGACC mutation is a splice site mutation leading to an insertion that results in a TCTGACCTCCTG repeat sequence. The IVS12\[2T\[G mutation is a donor splice site mutation. The 1894delA mutation causes a frameshift.

### TABLE 2. Clinical Characteristics and Segregation of Null ABCA4 Gene Mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Phenotype</th>
<th>First Symptoms (y)</th>
<th>Present Age (y)</th>
<th>Visual Acuity</th>
<th>Dark Choroid</th>
<th>ERG</th>
<th>Allele 1</th>
<th>Allele 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1-III-4</td>
<td>arRP</td>
<td>8</td>
<td>28</td>
<td>OD 20/500</td>
<td>ND</td>
<td>Ext.</td>
<td>IVS12[H11001]^2T[G</td>
<td>IVS7-45_952delinsTCTGACC</td>
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<tr>
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<td>arRP</td>
<td>8</td>
<td>23</td>
<td>OD 20/500</td>
<td>ND</td>
<td>Ext.</td>
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<td>IVS7-45_952delinsTCTGACC</td>
</tr>
<tr>
<td>F1-III-8</td>
<td>arRP</td>
<td>8</td>
<td>28</td>
<td>OD 20/500</td>
<td>ND</td>
<td>Ext.</td>
<td>IVS12[H11001]^2T[G</td>
<td>IVS7-45_952delinsTCTGACC</td>
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<tr>
<td>F18-II-1</td>
<td>STGD</td>
<td>8</td>
<td>15</td>
<td>OD 20/250</td>
<td>ND</td>
<td>Ext.</td>
<td>IVS12[H11001]^2T[G</td>
<td>IVS12[H11001]^2T[G</td>
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<tr>
<td>F22-II-1</td>
<td>STGD</td>
<td>5</td>
<td>17</td>
<td>OD 20/250</td>
<td>ND</td>
<td>Ext.</td>
<td>IVS12[H11001]^2T[G</td>
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<td>F22-II-3</td>
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<td>6</td>
<td>12</td>
<td>OD 20/200</td>
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<td>Ext.</td>
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<td>41</td>
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<td>Ext.</td>
<td>IVS12[H11001]^2T[G</td>
<td>IVS12[H11001]^2T[G</td>
</tr>
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</table>

HM, hand motions; ND, not determined; Ext., extinguished; Sub., subnormal.

### Table 2. Clinical Characteristics and Segregation of Null ABCA4 Gene Mutations

- **Patient**: name of patient
- **Phenotype**: arRP or STGD
- **First Symptoms (y)**: age at first report of symptoms
- **Present Age (y)**: age at the time of the study
- **Visual Acuity**: dark choroid, scotopic, photopic
- **Dark Choroid**: OD 20/500, ND, Ext.
- **ERG**: OD 20/500, ND, Ext.
- **Allele 1**: IVS12\[H11001\]^2T\[G
- **Allele 2**: IVS7-45_952delinsTCTGACC

**Figure 2**: Three novel mutations in the ABCA4 gene were identified. The IVS7-45_952delinsTCTGACC mutation is a splice site mutation leading to an insertion that results in a TCTGACCTCCTG repeat sequence. The IVS12\[2T\[G mutation is a donor splice site mutation. The 1894delA mutation causes a frameshift.
are likely to be pathogenic mutations. The IVS7-45_delinsTCTGACC, IVS12+2T→G, and 1894delA mutations segregated with the diseases in this study, and the Arg2149stop mutation has been reported to segregate with STGD. The Arg2149stop mutation found in a white patient with STGD in that study was also found in a Japanese patient in the current study. The other mutations found in European and North American patients with STGD were not found in Japanese patients with STGD. Some major polymorphisms found in European and North American individuals (Leu1894Leu, Leu1938Leu, IVS41-11G→A, Pro1948Pro, and Asp2095Asp) were also found in the Japanese population. Two rare sequence variations (IVS12-50G→A and IVS38-36delT) were also detected. The effects of these variations are unknown. The P1948P polymorphism, which has been reported in a study on Japanese patients with AMD, was observed in Japanese patients with STGD. Other sequence variations found in Japanese patients with AMD were not found in Japanese patients with STGD. ABCA4 gene mutations that have been studied as possible markers for AMD in European and North American individuals were not found in the Japanese patients with STGD, and the patients with STGD had no siblings that had been diagnosed with AMD.

Previous studies have shown that most patients with STGD are compound heterozygous for two missense mutations or one null mutation and one missense mutation. In contrast, homozygotes with null mutations have arRP, arCRD, or severe STGD. The ABCA4 protein is present on the disc membrane of both cone and rod photoreceptor cells and has...
two ATP-binding domains, NB1D1 and NB2D2, which are important ATP sites.30–33 Mutations that cause arRP alter NB1D1 and/or NB2D2, resulting in ABCA4 proteins that are predicted to have no activity. Therefore, it is reasonable to assume that homozygotes or compound heterozygotes with null mutations in the ABCA4 gene would have reduced activity of the ABCA4 protein, leading to progressive degeneration of both cone and rod photoreceptors and resulting in the clinical features of arRP. In this study, all three families with STGD found to be homozygous or compound heterozygous for presumably null mutations had severe phenotypes (Table 2). These findings prompted us to screen 96 unrelated Japanese patients with arRP for the three segregated, presumably null mutations, IVS12+2T→G, 189delA, and Arg2149stop. Two arRP-affected siblings (F26-II-4 and F26-II-5) were homozygous for the IVS12+2T→G mutation and two arRP-affected siblings (F1-III-4 and F1-III-6) were heterozygous for the IVS12+2T→G mutation. Screening of family F1 was performed with PCR direct sequencing, and family members were found to be carriers of the IVS12+2T→G mutation and/or the IVS7–45_952delinsCTCTGACC mutation (Fig. 1).

As shown in Fig 3E, a patient from family F1 (F1-III-8) showed features of RP (night blindness, severe RPE atrophy, bone spicule-like pigmentation and attenuated retinal vessels in all areas of the retina) with an almost complete loss of scotopic ERG amplitude (Fig. 4). However, when this patient was first examined in another hospital at 8 years of age, she showed only macular degeneration, and STGD was diagnosed (Fig. 3D). These findings suggest that the clinical phenotype of this patient changed from STGD to arRP more than 20 years later. The clinical courses of patients F1-III-4 and F1-III-6 were similar to that of patient F1-III-8.

This study clearly showed that patient F26-II-6 (arRP, 41 years old) and patient F9-II-2 (STGD, 11 years old) shared the same ABCA4 gene mutation, IVS12+2T→G (Figs. 3B, 3C). Furthermore, when patient F26-II-6 was first examined in our hospital in the fifth decade of life, we were informed that her initial symptoms were decreased visual acuity and night blindness in the first decade of life. In another hospital, patient F26-II-6 was found to have macular degeneration during childhood and exhibited progressive central scotoma to the periphery. At present, her visual acuity is restricted to hand motions, with only peripheral islands remaining functional in her visual fields. The clinical history of patient F26-II-6 supports the idea that the fundus appearance in members of family F26 early after onset may have resembled that of STGD, because patients from family F26 with arRP were homozygous for the IVS12+2T→G mutation, similar to the two patients with STGD analyzed (F9-II-2 [Fig. 3B] and F18-II-1 [fundus photograph not shown]).

We speculate that null ABCA4 gene mutations can cause recessive panretinal degenerations such as central arRP, rather than typical arRP, in which retinal degeneration progresses from the periphery toward the macula. Furthermore, mutations in the ABCA4 gene can cause panretinal degeneration that changes its clinical appearance from STGD to arRP over time. Our study suggests that STGD and arRP, both resulting from ABCA4 gene mutations, may represent differing degrees of severity of the same disease. This idea is consistent with previous reports describing different phenotypic variations resulting from ABCA4 gene mutations and reports on the classification of STGD, in which patients (Gass’ classification Group III) showed atrophic maculopathy with late signs and symptoms of RP.5–13,35

In summary, we identified three novel ABCA4 gene mutations in Japanese patients with STGD and arRP. Mutations in the ABCA4 gene can cause panretinal degeneration that changes its clinical appearance from STGD to arRP over time.

Acknowledgments

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References