Autosomal Dominant Macular Degeneration Associated With 208delG Mutation in the FSCN2 Gene

Yuko Wada, MD; Toshiaki Abe, MD; Toshitaka Itabashi, MD; Hajime Sato, MD; Miyuki Kawamura, MD; Makoto Tamai, MD

Objective: To assess the clinical and genetic characteristics of 2 Japanese families with autosomal dominant macular degeneration (ADMD) associated with a 208delG mutation in the retinal fascin (FSCN2) gene.

Design: Case reports with clinical findings and results of fluorescein angiography, electroretinography, kinetic visual field testing, and DNA analysis.

Setting: University medical center.

Results: The 208delG mutation in the FSCN2 gene was identified in 14 members of 4 Japanese families with autosomal dominant retinitis pigmentosa and in 5 members of 2 Japanese families with ADMD. The characteristic features associated with this mutation led to 2 different phenotypes, autosomal dominant retinitis pigmentosa and ADMD.

Conclusions: The 208delG mutation in the FSCN2 gene produces not only autosomal dominant retinitis pigmentosa but also ADMD in the Japanese population. This mutation is relatively common in Japanese patients with autosomal dominant retinal degeneration and showed clinical variability.

Clinical Relevance: Autosomal dominant retinitis pigmentosa and ADMD can be caused by the same 208delG mutation. We suggest that mutations in the FSCN2 gene can lead to a spectrum of phenotypes.

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MACULAR DEGENERATION (MD) can have a dominant or recessive inheritance pattern and shows clinical and genetic heterogeneity. To date, 4 loci and 6 candidate genes for MD have been reported, and 10 loci and 28 candidate genes for retinitis pigmentosa (RP) have been identified. Among these candidate genes, the peripherin/RDS gene causes autosomal dominant retinitis pigmentosa (ADRP) and autosomal dominant macular degeneration (ADMD). Therefore, it was reported that the Arg172Trp, Tyr258Stop, and Gly167Asp mutations cause ADRP and that the Trp179Arg, Cys165Thr, Phe211Leu, and Asn244Lys mutations cause ADRP. These reports suggested that other candidate genes for RP caused other phenotypes, such as MD.

In 2001, it was first reported that the human retinal fascin gene (FSCN2) was one of the causative genes for Japanese patients with ADRP. This and other studies disclosed that about 3.0% of the patients with ADRP (14 patients from 4 unrelated families) have the identical 208delG mutation in the FSCN2 gene. FSCN2, a candidate gene for ADRP, is located on chromosome 17q25 and plays an important role in photoreceptor disc formation. The clinical characteristics of the 14 patients from 4 families with the 208delG mutation were those of typical RP.

However, 1 patient had an atrophic MD in addition to the pigmentary retinal degeneration. This finding suggested the possibility that mutations in the FSCN2 gene were related to not only ADRP but also ADMD. Thereafter, 148 patients from unrelated families with ADRP and 54 patients from unrelated families with cone-rod dystrophy or MD were screened to search for mutations in the FSCN2 gene. Fourteen patients from 4 unrelated families with ADRP and 5 patients from 2 unrelated families with MD had the identical 208delG mutation in the FSCN2 gene. We describe herein the ocular findings associated with the 208delG mutation in 2 of these Japanese families with ADMD.

From the Department of Ophthalmology, Tohoku University School of Medicine, Sendai, Japan. The authors have no relevant financial interest in this article.
METHODS

Genomic DNA samples isolated from 148 patients from unrelated families with ADRP and from 54 patients from unrelated families with cone-rod dystrophy or MD were screened to search for mutations in the FSCN2 gene. One hundred control chromosomes from 50 normal subjects were further screened for mutations of this gene. Informed consent was obtained from all patients before their entry into this study.

The sequences from exon 1 to exon 5 of the FSCN2 gene were amplified by polymerase chain reaction. Nine sets of oligonucleotide primer pairs were used to cover the extent of these sequences. Products of the polymerase chain reaction were directly sequenced in the forward and reverse directions on an ABI sequencer (model 3100; Applied Biosystems, Foster City, Calif). The clinical features of the 4 Japanese families with ADRP associated with the 208delG mutation have already been reported. We present herein our findings on the 5 patients from 2 Japanese families with ADMD. The heterozygous 208delG mutation in the FSCN2 gene was identified in all affected members from the 2 families (Figure 1).

Ophthalmologic examinations included best-corrected visual acuity, kinetic visual field examination, slitlamp biomicroscopy, fundus examination, fluorescein angiography, and electroretinography. Ophthalmoscopic findings were recorded by color fundus photography. Kinetic visual field examinations were performed on the Goldmann perimeter with the V-4-e, I-4-e, I-3-e, and I-2-e targets.

Electroretinograms (ERGs) were recorded under controlled conditions that conformed to the standards of the International Society of Clinical Electrophysiology of Vision. Briefly, photopic ERGs were elicited by a single flash or a 30-Hz flicker stimulus of red light under light-adapted conditions (cone-isolated responses); a dim blue flash under dark-adapted conditions (30 minutes) was used for the rod-isolated ERGs (rod-isolated responses); and a bright white flash (infrared range) was used to elicit the mixed cone-rod responses. The means ± SDs of the amplitudes of the ERGs of the normal subjects for the normal allele were: rod-isolated b-wave, 230.1 ± 51.2 µV; photopic a-wave, 57.2 ± 17.1 µV; photopic b-wave, 110.3 ± 22.6 µV; mixed a-wave, 376.3 ± 49.4 µV; mixed b-wave, 560.2 ± 72.9 µV; and 30-Hz flicker, 127.5 ± 24.1 µV.

RESULTS

The 5 affected members from the 2 families with ADMD (Figure 1) had an identical abnormal nucleotide sequence. There was a deletion of nucleotide G at the complementary DNA position 208, and this was designated as a 208delG mutation in the FSCN2 gene (Figure 2). The nonaffected members did not have this mutation, and this mutation cosegregated with the phenotype of MD (Figure 1). We further screened our patients for mutations in the peripherin/RDS, CRX, and GUCY2D genes, and no mutation was detected. We also confirmed that the 208delG mutation was not present in 100 normal control chromosomes.

REPORT OF CASES

FAMILY 1, PATIENT III:4

The proband, a 62-year-old man, had a gradual progression of visual impairment, photophobia, and constriction of the visual field during his 40s. In 1992, at age 52, he visited our clinic to have a detailed assessment of his eyes. His visual acuity was corrected to 0.70 OD with a 2.00 diopter (D) sphere and to 0.06 OS with a 1.00 D sphere.

Slitlamp biomicroscopic examination showed normal-appearing cornea, anterior chamber, iris, lens, and vitreous in both eyes. Fundus examination showed the mottled appearance of retinal pigment epithelium (RPE) in the posterior pole bilaterally (Figure 3A). Fluorescein angiography disclosed diffuse hyperfluorescence around the macula in the right eye and oval and round hyperfluorescent lesions in the posterior pole of the left eye (Figure 4A).

The rod-isolated, photopic, mixed cone-rod, and 30-Hz flicker ERGs were within the normal range (Figure 5). The patient could not identify the numbers on the Ishihara plates.

He experienced an accelerated deterioration of central visual acuity after his first visit in 1992 at age 52. In 2001, his visual acuity had decreased to counting fingers in both eyes. Fundus examination at this time showed bilateral atrophic MD, a mottled appearance of the RPE, which appeared to have progressed since his first visit,
and diffuse retinal mottling in the midperipheral areas (Figure 3B). Fluorescein angiography showed oval-shaped hyperfluorescence with hypofluorescence within the lesions (Figure 4B). Kinetic visual field testing showed only small residual islands of visual field remaining in the paracentral region bilaterally. Kinetic visual field testing had low reliability because of his age, poor visual acuity, and poor fixation. The ERG examinations in 2001 also showed normal rod-isolated, mixed cone-rod response, photopic, and 30-Hz flicker ERGs.

**FAMILY 1, PATIENT IV:2**

Patient IV:2, the 37-year-old son of patient III:4, had no subjective impairment in his central, peripheral, or night vision. His visual acuity was 1.0 OS with a −1.50 to −2.50 × 30° refraction and 1.2 OD with a −1.30 to 1.25 × 150° refraction. Slitlamp biomicroscopic examination showed normal-appearing cornea, anterior chamber, iris, and lens in both eyes. Fundus examination showed a mottled appearance of the RPE, yellowish deposits in the macula, and an irregular ring reflex bilaterally (Figure 6A). Fluorescein angiography disclosed granular hyperfluorescence from the posterior pole to the midperipheral areas in both eyes. The a- and b-wave amplitudes of the mixed cone-rod ERGs were normal, although the oscillatory potentials were reduced. The rod-isolated, photopic, and 30-Hz flicker ERGs were of normal amplitude (Figure 5). Goldmann kinetic visual field testing showed constricted visual fields for the I-3-e and I-2-e targets (Figure 7A). Color vision was normal on the panel D-15 test.

**FAMILY 2, PATIENT III:1**

A 26-year-old man first manifested a decrease of visual acuity when he was an elementary schoolchild. When he was 8 years old, he was diagnosed as having retinal degeneration. In 1990, he visited our clinic to have a detailed assessment of his eyes. His visual acuity was corrected to 0.08 OD with a −1.00 D sphere and to 0.80 OS with a −0.75 × 150° refraction. Slitlamp biomicroscopic examination showed normal-appearing cornea, anterior chamber, iris, lens, and vitreous in both eyes.
Fundus examination at age 13 showed mildly demarcated, atrophic MD associated with diffuse mottling of the retina in the midperipheral area in both eyes (Figure 3C). Fluorescein angiography demonstrated round-shaped hyperfluorescence associated with hypofluorescence within the lesions (Figure 4C). The hyperfluorescent lesions corresponded to the atrophy of the RPE, and the hypofluorescent lesions corresponded to the chorioretinal atrophy.

The rod-isolated response and photopic ERGs were within the normal range. The amplitudes of the mixed cone-rod b-waves were reduced to 75% of the normal amplitude in both eyes (percentage reduction below the normal mean, 560.2 µV), and the oscillatory potentials were also reduced. The 30-Hz flicker responses were mildly reduced (61.3 µV OD and 65.3 µV OS; controls, 127.5 ± 24.1 µV; Figure 5). Color vision testing showed a tritanopic pattern in the right eye and a normal pattern in the left eye on the panel D-15 test.

He experienced an accelerated deterioration of central visual acuity after his first visit in 1990 at age 13. In 2001, his visual acuity was corrected to 0.2 OD with a −2.5 D sphere in both eyes. Fundus examination at this time disclosed atrophic MD with pigmentation in both eyes that had progressed since 1990 (Figure 3D). Fluorescein angiography disclosed round hyperfluorescent lesions associated with an enlargement of the hypofluorescent areas in the posterior pole of both eyes (Figure 4D). Kinetic visual field testing showed absolute scotomas with relatively well preserved peripheral areas (Figure 7B). The ERG examinations in 1999 demonstrated no remarkable changes.

**FAMILY 2, PATIENT III:3**

The 15-year-old younger sister of patient III:1 had no impairment of visual acuity or night vision. Because her older brother (III:1) was diagnosed as having MD in our clinic, she also visited our clinic at age 8 years. Her best corrected visual acuity was 0.9 OD with a 1.0 to 0.5 × 150° refraction and 0.9 OS with a −0.5 D sphere. Slitlamp biomicroscopic findings in the cornea, anterior chamber, iris, and lens were normal in both eyes.
Fundus examination at age 8 showed a mottled appearance of the RPE in the posterior pole. The rod-isolated ERGs and 30-Hz flicker ERGs were within the normal range. The amplitude of the a-wave of the mixed cone-rod ERGs was reduced to 64% OD and to 79% OS, and the b-wave was reduced to 85% OD and was within the normal range in the left eye. The oscillatory potentials were also reduced (Figure 5).

In 2001, her visual acuity was corrected to 0.7 OD with a −1.5 D sphere and to 1.0 OS with a −1.5 D sphere. Fundus examination showed tortuous vessels, reddish optic discs, a mottled appearance of the RPE in both eyes, and pigmentation in the left macula (Figure 6B). The atrophic lesions had slightly enlarged since she first visited our clinic.

Goldmann kinetic visual field testing showed a slight peripheral constriction with the V-4-e and I-4-e targets, although she did not have a paracentral and central scotoma (Figure 7C).

FAMILY 2, PATIENT II:2

The 50-year-old mother of the family 2 patients had no visual complaints. Her visual acuity was corrected to 1.0 OD with a −1.5 D sphere and to 1.0 OS with a −1.5 D sphere. Slitlamp biomicroscopic examination showed normal-appearing cornea, anterior chamber, iris, lens, and vitreous in each eye. Fundus examination disclosed tortuosity of retinal vessels and mild atrophy of the RPE in the posterior pole and midperipheral retina, although the degenerative changes of the RPE and macula are not obvious in the fundus photograph (Figure 6C). Visual field testing showed a mild peripheral constriction with the V-4-e, I-4-e, and I-2-e targets (Figure 7D).

Macular degeneration is characterized by a reduction of visual acuity, abnormal color vision, and a central scotoma. The inheritance pattern for MD can be autosomal dominant or recessive. Recent molecular genetic analyses have shown that inherited retinal degeneration has allelic or nonallelic heterogeneity and that the phenotype depends on the type of mutation (eg, mutations of peripherin/RDS and ABCA4 genes can lead to MD and RP). Also, mutations in the rhodopsin and bPDE genes have been implicated in not only congenital stationary night blindness but also RP. On the other hand, an identical 1147delA mutation in the arrestin gene is the cause of not only Oguchi disease but also autosomal recessive RP. These findings indicate that some other candidate genes for RP will cause other phenotypes. In this study, we focused on the relationship between ADMD and mutation of the FSCN2 gene in the Japanese population.

Retinal fascin (FSCN2) is a photoreceptor-specific gene located on chromosome 17q25 and is a member of the fascin gene family. It encodes 516 amino acids and is responsible for the assembly of actin-based structures of the connecting cilium plasma membrane and is involved in photoreceptor disc formation. An earlier study showed that a 208delG mutation was the cause of ADRP in the Japanese population. Interestingly, some variation was found in the clinical features of patients with this mutation. In particular, the presence of a sharply demarcated MD in 1 patient with ADRP led to the suggestion that the FSCN2 gene might also be a candidate gene for MD.

Figure 5. Electroretinograms (ERGs) of 4 affected members. Patient IV:2 of family 1 and patients III:1 and III:3 of family 2 show reduced oscillatory potentials. Patient III:1 shows mildly reduced b-waves of the mixed cone-rod ERG and mildly reduced 30-Hz flicker ERGs. Patient III:3 shows mildly reduced a- and b-waves in the mixed flash ERG. Patients are described in detail in the “Report of Cases” section of the article. R indicates right eye; L, left eye.
for MD. These study findings were extended herein, and we found that 5 affected members from 2 unrelated families with MD had a mutation in the \textit{FSCN2} gene.

The clinical features produced by this mutation in the 5 affected members showed extreme variation in severity. The asymptomatic members had only slight atrophy of the RPE, or mildly constricted visual fields, and an irregular ring reflex in the macula. The symptomatic patients had decreased visual acuity, central scotoma, severely constricted visual field, atrophy of the RPE, and central retinal degeneration.

To examine the possibility of a digenic inheritance, we screened other candidate genes for macular dystrophy and cone dystrophy, such as peripherin/RDS, CRX, and \textit{GUCY2D} genes, to search for additional mutations. The results did not show any disease-causing mutation in these 3 genes; therefore, mutations in at least these genes are unlikely to be related to the pathogenesis of MD in our patients. However, further molecular genetic analysis is needed to eliminate other possibilities.

Taken together with previous results, we conclude that the 208delG mutation in the \textit{FSCN2} gene is responsible for not only ADRP but also ADMD. However, we cannot explain the variability of expressivity of the 208delG mutation in the \textit{FSCN2} gene and the 1147delA mutation in the ar-

\textbf{Figure 6.} Fundus photographs of asymptomatic patients. A, Patient IV:2 of family 1 at age 37 years. The retinal pigment epithelium (RPE) is mottled, and yellowish deposits in the macula and an irregular ring reflex can be seen. B, Patient III:3 of family 2 at age 15 years shows the mottled appearance of RPE, tortuous vessels, and a reddish-colored optic disc. C, Patient II:2 of family 2 at age 50 years. Tortuosity of the retinal vessels is observed.
restin gene have been found only in Japanese patients. Although the Pro23His and Pro347Leu mutations in the rhodopsin gene and the Arg677Ter mutation in the RP1 gene are representative mutations for ADRP, they are not found or are very rare in Japanese patients with ADRP. Moreover, we found 4 polymorphisms in exon 1 (Thr211Thr, Leu238Leu, Thr244Thr, and Pro246Pro) of the FSCN2 gene, 2 of which (Thr211Thr and Pro246Pro) were previously reported,14 and the others were found only in Japanese patients.

These findings suggest that the kind and frequency of mutations depend on the ethnic population. The phenotypic differences (ADMD and ADRP) induced by the 208delG mutation in the FSCN2 gene were probably due to the functional loss of 1 allele, resulting in haploinsufficiency. Further molecular biological analysis, such as transgenic experiments or gene expression studies, will augment our understanding of the mechanism of photoreceptor degeneration.

In conclusion, we have identified a heterozygous 208delG mutation in the FSCN2 gene among Japanese families with not only ADRP but also ADMD. We suggest that the 208delG mutation may be a relatively common cause of inherited retinal degeneration in Japanese patients. Our study provides evidence that a mutation in the FSCN2 gene is related, in part at least, to the pathogenesis of MD.

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Corresponding author and reprints: Yuko Wada, MD, Department of Ophthalmology, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-77, Japan (e-mail: YUKOW@oph.med.tohoku.ac.jp).

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