Young Monozygotic Twin Sisters With Fundus Albipunctatus and Cone Dystrophy

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Objective: To describe young monozygotic twin sisters with fundus albipunctatus (a type of autosomal recessive stationary night blindness caused by mutations of the 11-cis retinol dehydrogenase gene [RDH5]) associated with cone dystrophy, previously reported in elderly men.

Methods: Ophthalmologic examinations were performed, and the RDH5 gene was analyzed by direct genomic sequencing.

Results: Twin 23-year-old sisters with high myopic refractive errors of approximately −13 diopters were diagnosed as having fundus albipunctatus. Their photopic electrophysiologic responses were markedly reduced, and cone dystrophy was diagnosed. One twin had macular degeneration with reduced best-corrected visual acuity, while the other twin had normal maculae with good visual acuity. A compound heterozygous mutation, Val132Met and Arg280His, in the RDH5 gene was found in both sisters.

Conclusions: Cone dystrophy can be present in patients with fundus albipunctatus, not only elderly men but also young women. The clinical severity differed between monozygotic twins with fundus albipunctatus and cone dystrophy.

Clinical Relevance: The patient’s sex is not critical for the presence of cone dystrophy in patients with fundus albipunctatus. The discordant findings in the twins indicate that factors other than genetics influenced the phenotype.

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tion of the purpose and procedures. The sisters were examined in the Department of Ophthalmology, Nagoya University, Nagoya, Japan. A complete ophthalmologic examination was performed, including best-corrected visual acuity, slit-lamp and fundus examinations, fundus photography, fluorescein angiography, and ERG.

Genomic DNA was extracted from leukocytes of peripheral blood, and exons 2, 3, 4, and 5 of the RDH5 gene were amplified by polymerase chain reaction. The polymerase chain reaction conditions and the procedures for direct sequencing have been described in detail elsewhere. To search for polymorphisms, 100 alleles from normal, unrelated Japanese individuals were also directly sequenced.

Standardized full-field ERGs, elicited by Ganzfeld stimulation, were recorded after pupillary dilation with 0.5% tropicamide and 0.5% phenylephrine hydrochloride. The rod (scotopic) ERGs were recorded with a blue stimulus of a luminance of $5.2 \times 10^{-3}$ candela-seconds per square meter ($cd-s/m^2$). The mixed rod-cone, single-flash (bright white) ERGs were elicited by a white stimulus of $44.2 cd-s/m^2$. They were recorded after 20 minutes and also after 3 hours of dark adaptation. The photopic (cone) single-flash ERGs and 30-Hz flicker ERGs were elicited by white stimuli at an intensity of 4 cd/s/m$^2$ and 0.9 cd/s/m$^2$, respectively, on a white background of 68 cd/m$^2$.

![Figure 1](image1.png)

**Figure 1.** Pedigree of a family with fundus albipunctatus showing affected (solid symbols) and unaffected (open symbols) members. Individuals whose DNA was tested are indicated by X's. Squares indicate men; circles, women; and slash through symbol, deceased. The parents of the affected twin sisters are consanguineous.

![Figure 2](image2.png)

**Figure 2.** Fundus photographs (A, B) and fluorescein angiograms (C, D) of the patients with a mutation of the 11-cis retinol dehydrogenase gene. A, Left eye of patient IV:3 showing multiple yellow-white lesions excluding the macula, as well as high myopic changes and retinal degeneration in the macula. B, Left eye of patient IV:2 showing multiple yellow-white punctate lesions and high myopic changes. No degenerative changes are seen in the macular area. C, Fluorescein angiogram of the left eye of patient IV:3 showing hyperfluorescence in the macula. D, Fluorescein angiogram of the left eye of patient IV:2 showing no abnormality in the macular area.
The 23-year-old twin sisters were referred to our hospital for a diagnosis for their visual difficulties. They were considered to be monozygotic because they bore a close resemblance to each other. Both patients had noticed night blindness from childhood, while only twin 1 (IV:3, Figure 1) had noticed a gradual reduction of vision during the previous 5 years. Their paternal grandmother and maternal great-grandfather were siblings (Figure 1). The corrected visual acuity of twin 1 was 1.2 OD and 0.2 OS with refractive errors of $-12.50 \times 15^\circ$ OD and $-13.00 \times 175^\circ$ OS, and that of twin 2 (IV:2, Figure 1) was 1.2 OU with refractive errors of $-12.00 \times 175^\circ$ OD and $-13.00 \times 3.00^\circ$ OS. They stated that their healthy mother was also highly myopic.

In both patients, numerous small, discrete yellow-white dots were observed at the level of retinal pigment epithelium with scarring of the macula in both eyes (Figure 2A and B). Both maculae of twin 1 (IV:3) demonstrated macular degeneration (Figure 2A), and fluorescein angiography showed hyperfluorescence in the corresponding areas (Figure 2C). Twin 2 (IV:2) did not show any abnormality in the macula by either indirect ophthalmoscopy (Figure 2B) or fluorescein angiography (Figure 2D) in both eyes. The intraocular pressures and anterior segments were normal. Neither nystagmus nor strabismus was found in either patient.

The full-field rod ERGs elicited by Ganzfeld stimulation were significantly reduced after 20 minutes of dark adaptation, and they improved but remained subnormal after prolonged dark adaptation in both patients (Figure 3). The amplitudes of the a and b waves of the bright-flash, mixed rod-cone ERG were reduced in both sisters (Figure 3). Although a bright-flash negative-type ERG (b wave < a wave) after 20 to 30 minutes of dark adaptation is a distinct characteristic in FA, it was not possible to use this diagnostic feature because of blink artifacts (Figure 3).

The photopic a and b waves and 30-Hz flicker ERGs were significantly reduced in both patients, indicating the presence of CD (Figure 3).

Molecular genetic examination disclosed a compound heterozygous mutation of G to A at nucleotide 394 (Val132Met) and G to A at nucleotide 539 (Arg280His) in the RDH5 gene (Figure 4). Their healthy mother was
heterozygous, with a mutation at nucleotide 394 but normal findings at nucleotide 539. The same mutations in the gene have been detected in other Japanese patients with FA. No such base substitutions were found in 100 alleles from healthy individuals. In addition to the genotype of the RDH5 gene, the monozygosity of the twin was determined by totally concordant results of the polymerase chain reaction with fluorescent-labeled primers for 22 highly polymorphic loci including D1S249, D2S337, D3S1267, D4S402, D5S630, D6S257, D7S636, D8S550, D9S286, D10S1686, D11S4175, D12S58, D13S159, D14S68, D15S205, D16S520, D17S1852, D18S61, D19S220, D20S186, D21S1914, and D22S283.

Figure 4. Nucleotide sequences of the 11-cis retinol dehydrogenase gene determined with sense primers in the patients. A compound heterozygous mutation of G to A at nucleotide 394 (Val132Met) and G to A at nucleotide 539 (Arg280His) in the gene was identified in the patients. Bars indicate the positions of the mutations.

We have examined the RDH5 gene in 6 cases of FA associated with CD and in 8 cases without CD. A homozygous or compound heterozygous mutation in the gene was detected in all of the patients. Because the CD was seen more frequently in older patients, and because some of those with CD had progressive macular degeneration with gradual decline of visual functions during a long follow-up period, we concluded that mutations of the RDH5 gene led to a progressive CD resulting in a severe loss of visual function with increasing age in some patients with FA. All of these patients with CD were older than 50 years, and they began to notice a decrease in their vision after 40 years of age. Only 1 young patient (a 9-year-old) with FA has been reported to have reduced visual acuity and macular dystrophy. This boy had compound heterozygous mutations of Tyr281His and Leu310GluVal in the RDH5 gene and had symmetric atrophic lesions in the macula of each eye. The amplitudes of his focal cone macular ERGs were significantly reduced, indicating that he had macular dystrophy. However, he was not considered to have CD because his full-field photopic ERGs were normal. Thus, to date, no young person with FA associated with CD has been described to our knowledge.

The present twin sisters were diagnosed as having CD despite their young age (23 years) because their full-field photopic ERGs were significantly reduced. These results indicated that CD can be present not only in elderly patients but also in young patients with FA. The mutations found in these sisters have already been found in other Japanese patients, including a 53-year-old woman who had the same combination of compound heterozygous mutation of Val132Met and Arg280His. She maintained her good visual acuity of 1.2 in both eyes without macular degeneration, and her cone ERG responses were within normal limits. These observations lead us to suggest that other genetic or environmental factors might have induced the CD in the twins.

One possibility may be that high myopia played a role in the development of the cone dystrophy in the twins. However, no other case with FA has been reported to be associated with high myopia. In this case, it was presumed that some other factors in addition to myopia or genetics had an influence on the progress of cone dystrophy in the twins because one twin had bilateral macular degeneration with unilateral reduction of best-corrected visual acuity, whereas the other twin had good vision without macular degeneration in both eyes.

We previously suggested that the sex of the patient would have some effect on the presence of CD because all of the patients with FA associated with CD were men. However, the patients in the present report were women, indicating that the sex is not a critical condition for the presence of CD.

The clinical phenotype of patients with FA is heterogeneous. Recently, we examined the RDH5 gene in a number of patients with FA with or without macular dystrophy; however, we observed no clear correlation between genotype and phenotype. The details of the factors affecting the progress of CD are still unknown, and additional data must be gathered to help make this clear.

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