A New Leu253Arg Mutation in the RP2 Gene in a Japanese Family with X-Linked Retinitis Pigmentosa

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PURPOSE. To identify the clinical findings in a Japanese family with X-linked retinitis pigmentosa associated with mutation in codon 253 (Leu253Arg) in the RP2 gene.

METHODS. Case reports included clinical features and results of fluorescein angiography, electroretinogram, kinetic visual field testing, and DNA analysis. Two affected hemizygotes with retinitis pigmentosa associated with transversion mutations in codon 253 (Leu253Arg) of the RP2 gene and the obligate carriers were examined.

RESULTS. A novel Leu253Arg mutation of the RP2 gene was found to cosegregate with retinal degeneration in two affected males and two carriers in female heterozygote in a Japanese family. The ophthalmic findings in hemizygote showed severe retinal degeneration. In the obligate carrier, mild chorioretinal degeneration was observed in both eyes but a tapetal-like reflex of the fundus was not apparent.

CONCLUSIONS. The mutation at codon 253 of the RP2 gene is the first mutation reported in a Japanese family. It is concluded that the mutation of the RP2 gene also causes the X-linked retinitis pigmentosa in Japanese patients. (Invest Ophthalmol Vis Sci. 2000;41:290–293)

Retinitis pigmentosa (RP) is a progressive retinal degeneration with bone spicules and shows genetic heterogeneity, with autosomal dominant, recessive, and X-linked forms. The X-linked form of RP is the most severe, and patients show partial or total blindness by the third or fourth decade.1

In our population of patients with RP, X-linked RP (XlRP) comprises approximately 2% of patients with RP. The ratio of X-linked to non-X-linked RP in Japan is lower than that in other European countries.

In 1984, the X-linked form of RP (RP2) was mapped to Xp11.3,2 and another X-linked RP gene (RP3) was mapped to Xp21.3.

Positional cloning of the RP3 gene was performed in 1996 and found to encode a putative guanine nucleotide exchange factor.1 It was discovered that mutations in this gene are in less than 20% of the patients with XlRP. In 1998, RP2 gene, which showed homology with human cofactor C, which was protein involved in the ultimate step of β-tubulin folding, was positionally cloned, and six mutations in the RP2 gene were discovered in European patients with X-linked RP.5 Furthermore, five mutations in the RP2 gene were reported for the XlRP families in a North American cohort.6 We describe herein the ocular features associated with a newly identified RP2 gene mutation in a Japanese family with XlRP. The aim of this study was to assess the phenotypic manifestation associated with this mutation and the frequency of RP2 mutation in Japanese patients with XIRP.

The mutation gave rise to a thymine to guanine transversion in the second nucleotide at codon 253, resulting in a substitution of an arginine residue for a leucine at the codon. The phenotypic expression produced by this mutation is characterized by severely accelerated retinal degeneration. The present report is the first to describe the clinical features associated with an RP2 mutation.

METHODS

Subject and Mutation Analysis

We screened 59 genomic DNA samples isolated from 25 patients with XIRP and 14 carriers to search for mutations in the RP2 gene by means of nonradioisotopic single-strand conformation polymorphism (SSCP) with a modification previously described.7 We further screened 150 control X-chromosomes with this gene. Genomic DNA was isolated from leukocytes prepared from a sample of the patient’s blood (10–15 ml), using a protocol previously described.8

The sequence from exon1 to exon5 of the RP2 gene was amplified by polymerase chain reaction. Eight sets of oligonucleotide primer pairs were prepared to cover the application of these sequences. The polymerase chain reaction products were analyzed by SSCP analysis. After electrophoresis, DNA bands were visualized by silver staining. The mutation or polymorphism was detected by the presence of abnormal bands derived from a mutant allele.

One pedigree was the focus for this study (Fig. 1), which was identified as having a Leu253Arg mutation. None of 150...
control X-chromosomes had this mutation. Furthermore, we screened the RPGR gene to search for other mutations on our families, and no mutation was found in the RPGR gene. The tenets of the Declaration of Helsinki were followed, and informed consent was obtained from all subjects who participated in this study.

**Clinical Examination**

We examined the two affected patients and the carriers of this pedigree at Tohoku University Hospital (Sendai, Japan). The ophthalmic examinations included best corrected visual acuity, slit-lamp biomicroscopy, kinetic visual field examination, fundus examination, and electroretinograms (ERGs). Ophthalmoscopic findings were recorded by color fundus photography. Fluorescein angiography was performed for one affected patient. Kinetic visual field examination was performed with a Goldmann perimeter with V-4-e and III-4-e isopters. The ERG testings were obtained using a single flash or 30-Hz flicker stimulus of red light under light-adapted conditions for cone-isolated responses, a dim blue flash in the dark-adapted condition (30 minutes) for rod-isolated responses, and a bright white flash (20 J) in the dark-adapted state for recording maximal responses of both rods and cones. These tests were performed under controlled conditions that conform to the standard of the International Standardization Committee.9

**RESULTS**

**Ophthalmologic Examination**

The proband (III-4), a 29-year-old man, realized early in the first decade of life that he had impaired night vision and visual acuity. When he was 6 years old, he was diagnosed by a nearby ophthalmologist as having RP. The patient (III-1) was diagnosed as having RP in his early teens by the local ophthalmologist. He could not be referred to our hospital because he lived in a distant place and was restricted because of the progressive retinal degeneration. The proband’s visual acuity was corrected to 0.2 OD with a −6.5 diopter sphere and 0.06 OS with −9.00 to 1.00 × 180 refraction. Fundus examination showed bilateral pigmentary retinal degeneration and attenuation of retinal arteries. Fluorescein angiography disclosed hyperfluorescence from the posterior pole to the peripheral retina that corresponded to the mottled retina, suggesting atrophic changes in the retinal pigment epithelial layer (Fig. 2). Goldmann kinetic visual field testing showed severely constricted central visual field for V-4-e target and constricted oval visual field in the lower area (Fig. 3). The ERG testing, a bright white flash in the dark-adapted state, showed a nonrecordable response.

The two carriers had no visual complaints. The carrier’s (II-4) corrected visual acuity was 0.8 OD with −2.5 diopter sphere and 0.7 OS with −3.0 diopter sphere. Slit-lamp biomicroscopy disclosed a normal-appearing cornea, anterior chamber, iris, lens, and vitreous in each eye. A mild change of retinal pigment epithelium in the posterior pole was detected in the II-4 carrier, but no retinal change was seen in the other carrier (II-3; Fig. 2).

**DNA Analysis**

The results of nonradioisotopic SSCP analysis of exon2 showed that a mutant band was observed in the proband, and both the mutant band and the normal band were observed in his mother, which showed that she was the obligate carrier (Fig. 4). The subsequent nucleotide sequence disclosed that the proband had a transversion change of thymine to guanine in the second nucleotide at 253 and his mother had both normal and abnormal sequences. This alteration causes an amino acid substitution of arginine residue for leucine residue in codon 253 of the RP2 protein (Fig. 4). To confirm that this novel Leu253Arg mutation is a disease-causing mutation, we further screened the other affected patient, the carrier, and non affected family members with the RP2 gene by direct sequencing. The Leu253Arg mutation cosegregated with the disease in the pedigree and was not detected in other 24 XIRP patients and 150 control X-chromosomes.

**DISCUSSION**

No previous reports regarding phenotypes associated with RPGR gene or RP2 gene have been shown in Japanese patients with XIRP. In fact, only 12 mutations in the RP2 gene have been reported, and 1 of these mutations was a missense mutation and the rest were nonsense mutations.5,6 Furthermore, the clinical features of XIRP associated with the mutation in the RP2 gene have not been reported. In this report, we first described the ocular findings with the Leu253Arg mutation in the RP2 gene in a Japanese patient with XIRP. This mutation within exon2 occurred outside the region homologous to a cofactor C, where most of the reported mutations have been found. Although the precise effect of the Leu253Arg change on the protein product is still unclear, we can assume that a positive charge of arginine residue instead of a hydrophobic leucine residue can impede, to some extent, physiological structure and function of the RP2 protein.

The ocular finding in the affected male showed a severe form of RP; he had an impairment of night vision and

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**FIGURE 1.** A pedigree showing affected members with XIRP associated with Leu253Arg mutation in the RP2 gene. Open symbols indicate unaffected subjects; solid symbols, affected; X, individuals examined in this study; dot in the circle, carrier; symbols with slashes, deceased member; and arrow, proband.
a deterioration of central vision within the first two decades of life. These findings are similar to those in patients who had mutations in the RPGR gene\textsuperscript{10,11} and in the RP2 gene.\textsuperscript{5}

Recently it was reported that mutations in the RPGR gene and the RP2 gene were found in approximately 20% and 18%, respectively, of the patients with XIRP.\textsuperscript{5,12} In our study, we found mutations in approximately 4% of Japanese patients with XIRP, which implies that RP2 mutations are less frequent in Japanese patients than in European and American patients. One explanation of the low frequency of the mutation in the RP2 gene may be an ethnic difference.

This novel Leu253Arg mutation, which we first detected in a Japanese patient with XIRP, supports the theory that mutation in the RP2 gene causes XIRP in Japanese patients. In the present study, we did not detect nonsense mutation in RP2

![Fundus photograph from the proband with Leu253Arg mutation and the obligate carrier.](image1)

**Figure 2.** Fundus photograph from the proband with Leu253Arg mutation and the obligate carrier. The right eye of the proband (top left) and the right eye of the carrier (top right). Fluorescein angiography of the proband. Both eyes had hyperfluorescent spots corresponding to the atrophy of retinal pigment epithelium. The right eye (bottom left) and the left eye (bottom right).

![Results of Goldmann visual field testing of the proband.](image2)

**Figure 3.** Results of Goldmann visual field testing of the proband. L, left eye; R, right eye.
Gene, we could not examine the difference between the clinical features caused by the missense mutation and those by the nonsense mutation. Therefore, additional families with XRIP must be studied for mutations to ascertain the phenotype-genotype correlation in the RP2 gene.

From a clinical point of view, further correlations between specific mutations and their phenotypes are needed to augment our understanding not only of the molecular mechanism of diseases but also of diagnostic and prognostic values.

References