SHORT COMMUNICATION

A Japanese trichothiodystrophy patient with *XPD* mutations

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Trichothiodystrophy (TTD) is a rare autosomal recessive disorder characterized by sulfur-deficient brittle hair complicated with ichthyosis, physical and mental retardation, and proneness to infections. Approximately half of TTD patients exhibit cutaneous photosensitivity because of the defect of nucleotide excision repair. Three genes, *XPB*, *XPD* and *TTDA*, have been identified as causative genes of photosensitive TTD. These three genes are components of basal transcription factor IIH. Most TTD cases have been reported in Europe and North America. We report a severely affected Japanese TTD patient with *XPD* mutations. Interestingly, his father has ichthyotic skin. The alteration in the paternal allele was a nucleotide substitution leading to Arg-722 to Trp (R722W), as previously reported in TTD patients. The other alteration in the maternal allele was a novel 3-bp deletion at nucleotides 67–69, resulting in the deletion of Ser-23, which is located upstream of helicase motif I and is the closest to the N-terminal end of *XPD* in reported mutations. The expression study showed that the two alterations were causative mutations for TTD. In Asia, it is likely that there are TTD patients who have not been diagnosed.

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INTRODUCTION

Trichothiodystrophy (TTD) is a rare autosomal recessive disorder characterized by sulfur-deficient brittle hair with an alternative dark and light banding appearance referred to as a 'tiger-tail' pattern under polarizing microscopy. TTD patients show a wide variety of clinical symptoms including intellectual impairment, growth retardation, ichthyosis, nail abnormalities and proneness to infections. Approximately half of the patients with TTD exhibit photosensitivity due to a defect of nucleotide excision repair. Three genes, *XPB*, *XPD* and *TTDA*, have been identified as causative genes of photosensitive TTD. The three genes encode distinct subunits of the basal transcription factor IIH that consists of 10 subunits and have an important role in nucleotide excision repair as well as in basal transcription.^{1,2} Mutations in the *XPB* and *XPD* genes also result in two related photosensitive disorders, xeroderma pigmentosum and xeroderma pigmentosum–Cockayne syndrome complex.³

A recent systemic literature review identified 112 TTD patients containing 47 photosensitive cases. Most cases have been reported in Europe and North America, whereas only one Indian patient has been reported in all of Southern and Eastern Asia.⁴ In this report, we describe a Japanese photosensitive TTD patient with *XPD* mutations.

MATERIALS AND METHODS

Case report

The male patient was born at 33 weeks' gestation with the typical features of a collodion baby, and was the second child of non-consanguineous Japanese parents. His father had ichthyosis diagnosed by histopathological examination, whereas he did not have any other physical impairments. The patient's sister was healthy, and exhibited no hair, skin or developmental abnormalities. His birth weight was 1380 g (below the 10th percentile), length was 41 cm (10–25th percentile) and head circumference was 28.5 cm (10–25th percentile). He developed ichthyosis on the face, trunk and extremities after collodion improved (Figure 1a). His nails were dysplastic and his hair was thin, brittle and sparse (Figures 1b and c). Using polarizing microscopy, all of his hairs that were examined demonstrated a 'tiger-tail' pattern (Figure 1d). Moreover, his hair showed a variety of hair shaft abnormalities, such as trichoschisis, trichorrhexis nodosa-like fracture and ribboning, with scanning electron microscopy (Figures 1e and f). At the age of 4 months, he developed a red skin rash on his face and extremities with fever, which did not occur on the day he was bathed in sunshine for a few minutes, but occurred 1 or 2 days later. He had repeated solar dermatitis with delayed erythema after short sun exposure. He frequently suffered from bacterial infections (mainly bronchitis and enteritis) and was diagnosed with chronic neutropenia at 14 months of age. Magnetic resonance imaging of the brain at 18 months showed dysmyelination. He showed no ocular and bone abnormalities. His developmental delay was gradually prominent. He smiled and controlled his head at 4 months, sat alone at 1 year, pulled himself to standing at 2 years and said his first

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Figure 1 Clinical features of the patient. He has (a) ichthyotic skin, (b) nail dysplasia, (c) thin, brittle, and sparse hair, (d) an alternative dark and light 'tiger-tail' banding pattern of the hair under polarizing microscopy, (e) trichorrhexis nodosa-like fracture and (f) ribbing of hair as shown by scanning electron microscopy.

word at 22 months. Currently, at 3 years, he has taken small steps and has a vocabulary of around 10 words. His height (79.0 cm) and weight (7.8 kg) are below the 3rd percentile and his head circumference (47.0 cm) is below the 10th percentile. He is currently being treated with sulfamethoxazole–trimethoprim for infection prophylaxis and with sunscreen for protection from sun exposure.

Mutational and functional analyses of *XPD*

Mutations in the *XPD* gene were analyzed using procedures as described in our previous studies.^{5,6} Genomic DNA and complementary DNA extracted from white blood cells were amplified by PCR using *XPD*-specific primers. The amplified fragments were purified and sequenced either directly or after subcloning into pBluescript SK(–). To construct mutant *XPD* expression plasmids, *in vitro* mutagenesis reactions were carried out on *XPD* complementary DNA by a PCR-based method as described in our previous studies with modifications.^{5,7} XP6BE, which is an xeroderma pigmentosum group D cell strain, was transfected with each of the wild- or mutant-type *XPD* expression plasmids. Stably transfected cell lines were established by selection with neomycin. Exponentially growing cells were plated in Petri dishes and exposed to ultraviolet (UV) irradiation at various dosages. Colonies were counted after the cells were cultured for 8–12 days.

RESULTS

Analyses of the *XPD* gene revealed two alterations in our patient. One mutation was a heterozygous C-to-T transition at nucleotide position 2164 (Figure 2a), resulting in an Arg-to-Trp substitution at amino acid position 722 (R722W), which has been previously reported in TTD patients.¹ Another alteration was a novel heterozygous deletion of TCC (Figure 2b) at nucleotide 67–69, which encodes serine at amino acid position 23 (Ser23del). Analyses of the *XPD* gene in the parents of the patient showed that the father was heterozygous for R722W and the mother was heterozygous for Ser23del. The parents did not have any other alterations of the *XPD* gene (data not shown). To examine whether the alterations would affect *XPD* function, we measured UV sensitivity of the XP6BE cells that were transfected with the expression plasmid of the wild-type (pCAGGS-XPDW) or mutant-type *XPD* complementary DNA containing each mutation. Both pCAGGS-XPDSer23del and pCAGGS-XPDR722W failed to restore the UV

Figure 2 (a) The nucleotide sequence of *XPD* complementary DNA from the patient was aligned with a normal counterpart. The arrow indicates the position of a C-to-T substitution at nucleotide 2164. (b) Nucleotide sequence of subcloned wild-type and mutant allele PCR products of exon 2. The box indicates the position of the 3 bp deletion of nucleotide 67–69.

sensitivity of XP6BE cells compared with pCAGGS-XPDW (Figure 3). This finding indicates that these two mutations affect the repair activity of *XPD*. On the other hand, the transfectants with

Figure 3 Ultraviolet (UV) sensitivity measured by colony-forming ability. ● pCAGGS-XPDW-transfected XP6BE; ▲ pCAGGS-XPDR722W-transfected XP6BE; ■ pCAGGS-XPDser23del-transfected XP6BE; ◆ pCAGGS-transfected XP6BE. The points are average values for duplicate dishes.

pCAGGS-XPDser23del were less UV resistant than the pCAGGS-XPDR722W-transfected cells.

DISCUSSION

Our patient exhibited typical TTD phenotypes. Very recently, Moslehi *et al.*⁸ reported that the incidence of gestational complications, such as small for gestational age, preeclampsia, preterm delivery and decreased fetal movement, was significantly higher in TTD-affected pregnancies compared with unaffected pregnancies of the same mothers. They hypothesized that TTD genes are important for normal placental development. The finding that our patient was born preterm and was small for gestational age is compatible with the findings described by Moslehi *et al.*⁸

We identified two *XPD* mutations, R722W and Ser23del, in our patient. The R722W substitution has been reported in nine TTD patients; one was homozygous and the others were compound heterozygous for the mutation.^{9–14} Patients with R722W normally exhibit severe physical and mental impairments. However, one patient (TTD24PV) has been found with a mutation at the 3' end of intron 10 that results in production of a small amount of normal *XPD* transcripts, and exhibits a relatively mild TTD phenotype.¹⁴ Our patient showed a severely affected phenotype compatible with typical TTD with R722W. The allele containing R722W was inherited from his father. The finding that the father has ichthyosis suggests that the mutation might have a dominant-negative effect against another *XPD* allele. However, transfected *rad15*, which is the *Schizosaccharomyces pombe* ortholog of human *XPD* with a mutation corresponding to R722W in humans, did not affect viability of the wild-type *S. pombe* cells, demonstrating that R722W does not have a dominant-negative effect on viability.¹⁵ Therefore, it is not clear whether the substitution is related to ichthyosis observed in the father of the patient.

The other alteration found in our patient, Ser23del, has not previously been reported. UV survival of the XP6BE cells transfected

with *XPD* containing Ser23del was much more reduced than that of cells transfected with wild-type *XPD*, suggesting that the deletion is a causative mutation for TTD. Ser-23 resides upstream of helicase motif I,^{16,17} and is the closest to the N-terminal end compared with other *XPD* mutations previously reported.¹ The role of the N-terminal region of *XPD* remains unclear. Considering that *XPD* interacts with other proteins and forms the transcription factor IIH complex,^{2,18} the serine deletion might result in an insufficient interaction and instability of the protein complex.

Cellular studies in TTD patients have revealed that 32 cases have defects in *XPD*, two have defects in *XPB*, and two have defects in *TTDA*.⁴ In all, 24 types of mutations in the *XPD* gene have been reported in photosensitive TTD patients.¹ Recently, Kleijer *et al.*¹⁹ reported that in Western Europe the incidence of photosensitive TTD is ~1.2 per million live births and the incidence of the disorder in immigrants is higher than that in autochthonic people. There might be TTD patients in Asia that have not yet been diagnosed.

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