A Novel XPA Gene Mutation and its Functional Analysis in a Japanese Patient with Xeroderma Pigmentosum Group A

Miki Tanioka,*+ Arief Budiyant,* Takahiro Ueda,* Tohru Nagano,* Masamitsu Ichihashi,* Yoshiki Miyachi,+ and Chikako Nishigori*

*Department of Dermatology, Kobe University Graduate School of Medicine, Kobe, Japan; †Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan

Most Japanese patients with xeroderma pigmentosum group A (XPA) have the homozygous intron 3 splicing mutations (AlwNI mutation). Here, we report a Japanese XPA patient, XP79KO, a compound heterozygote with a newly identified T to G transversion at splice donor site in intron 1 in one allele, and with the AlwNI mutation in another allele in the XPA gene. The mutation in intron 1 creates two new abnormal splice sites that resulted in two types of aberrant mRNA. These abnormal splicings cause frameshifts that make stop codons downstream. No XPA protein was detected in XP79KO fibroblasts.

Key words: group A xeroderma pigmentosum/Japanese/intron 1/splicing mutation analysis

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Xeroderma pigmentosum (XP) is an autosomal recessively inherited disease that is classified into seven genetic complementation groups, A–G, and a variant type (Kreamer et al., 1987). Patients with group A XP (XPA, OMIM 278700) usually show the most severe clinical symptoms among these groups, and about 50% of the XP patients in Japan belong to complementation group A (Takebe et al., 1987). Among Japanese patients with XPA, 80% of them have the homozygous AlwNI mutation in the XPA gene (Genbank accession number MN000380) (Tanaka et al., 1990; Satokata et al., 1992; Nishigori, 1994; Sato et al., 1996).

Case Report

XP79KO, a 6-y-old boy, had presented with severe photosensitivity since 6 mo old and was tentatively diagnosed as XP at the age of 1 y. He developed freckles on his face at the age of 2 y. He has never shown neurological symptoms. No skin cancer had developed under strict sun avoidance. There was no consanguinity in his parents. His photo irradiation test revealed that MED was 200 J per m² and the peak time causing erythema delayed up to 72 h after ultraviolet (UV)B exposure. He and his family gave their consent for investigating inherited variation of XP genes. The Medical Ethics Committee of Kobe University approved this investigation, which was conducted in accordance with the Declaration of Helsinki principles.

Mutations in the XPA Gene in XP79KO

Genomic DNA was extracted from normal and XP79KO-cultured fibroblasts according to a standard phenol/chloroform protocol. All exons and exon–intron boundaries of the XPA gene were PCR amplified. At first, we examined the frequently mutated sites in exons 3, 4, 6 in Japanese patients with XPA that can be recognized by restriction enzymes AlwNI, MseI, and HphI, respectively, as described (Nishigori et al., 1993). The PCR-RFLP analysis showed that XP79KO is heterozygous for the AlwNI mutation (data not shown). Direct sequence analysis showed that the other undetermined mutation was T to G transversion at the second nucleotide in intron 1 (Fig 1a). This mutation was not found in genomic DNA from 30 non-affected unrelated Japanese individuals or the SNP database network in Japan (http://snpnet.jst.go.jp/top_e.html). The father of the patient is heterozygous for the AlwNI site and the mother is heterozygous for this intron 1 mutation.

Aberrant Splicing of the XPA Pre-mRNA in XP79KO Cells

Since this intron 1 mutation disrupts the normal 5′ splice donor site of intron 1, we examined the abnormal splicing in XP79KO cells by RT-PCR. Total RNA extracted from XP79KO and normal cells was reverse transcribed and amplified using primers (RT-E1F: 5′-GAGCTAGGTCCCTCGGAGTG-3′, RT-E6R: 5′-TGAAAAAGGACCAATCTAAATTTCC-3′) encompassing the entire coding region of XPA mRNA (Fig 1b). These DNA fragments were cloned and sequenced. Band I had the normal XPA cDNA sequence. Band II for XP79KO contained three PCR prod-

Abbreviations: UDS, unscheduled DNA synthesis; UV, ultraviolet; XPA, xeroderma pigmentosum group A

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products. One was identified as a DNA fragment lacking exon 3, which was designated as def I I (Satokata et al., 1992), and the other two were found to be lacking the latter parts of exon 1, which were named def E1A and def E1B (Fig 1c).

The sizes of the RT-PCR products from def I I, def E1A, and def E1B were 811, 816, and 822 bp, respectively, and these products consisted of band II (Fig 1d). Both def E1A and def E1B caused frameshifts that result in a stop codon at the fifth triplet of exon 2 (Fig 1c).

No XPA Protein was Detected in XP79KO Cells

Cell extract was prepared from normal, XP79KO, and XP101KO cells, which were confirmed to have the homozygous AlwNI mutation, and separated by SDS-PAGE (Maniatis et al., 1989). Western blot analysis using two kinds of anti-XPA protein antibodies showed that two bands of 40 and 38 kDa were detected in normal cells (Fig 2, arrows). The 38 kDa protein is tentatively considered to be a degradation product of the 40 kDa protein (Miura et al., 1991). No XPA protein, however, was detected in XP79KO and XP101KO cells (Fig 2).

Cellular UV Sensitivity and UV-Induced Unscheduled DNA Synthesis (UDS)

The ability to repair the UV-induced DNA damage was determined by measuring cellular sensitivity to UV by means of the colony formation assay and the UDS as described (Nishigori et al., 1994). D0 value of both XP79KO and XP101KO cells was 0.8 J per m² whereas that of normal cells was 5.0 J per m². UV-induced UDS in XP79KO cells was 4.75% of the normal level. Both the post-UV colony-forming ability and UV-induced UDS of the cells from XP79KO’s mother showed a normal level.
mozygous logical manifestations compared with those with the homozygous AlwNI mutation (Sato et al, 1997; Miura et al, 1991). At first, we thought that the other undetermined mutation of XP79KO should be located near the 3' end of the XPA gene that would result in mild clinical manifestations because he had shown no neurological symptoms at the age of 6 y. This patient, however, has the novel intron 1 mutation that results in aberrant mRNA splicing and no XPA protein production. These in vitro data coincide with cellular repair data such as UV sensitivity test and UV-induced UDS that were similar to that of XPA patients with the homozygous AlwNI mutation.

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Address correspondence to: Chikako Nishigori, Division of Dermatology, Clinical Molecular Medicine, Graduate School of Medicine, Kobe University, 7-5-1, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. Email: chikako@med.kobe-u.ac.jp

References


Discussion

Eighteen mutation sites in the XPA gene in the world have been identified so far (Tanaka et al, 1993; States et al, 1998). We found the nineteenth mutation that is the first mutation around exon 1 of the XPA gene.

The severity of neurological abnormalities in Japanese patients with XPA correlates with the locations of the mutations. XPA patients with the homozygous AlwNI mutation (skipping the zinc-finger motif in exon 3) manifest the hearing impairment, which is one of the earliest neurological symptoms, around the age of 5 y. Around the age of 10 y, they show weak tendon reflexes and gait disturbance. An XPA patient with the homozygous HphI mutation (codon 228 nonsense mutation in exon 6) had less severe neurological manifestations compared with those with the homozygous AlwNI mutation. This difference can be explained by the fact that the mutated XPA protein produced from the homozygous HphI mutation remains zinc-finger domain (exon 3) intact and is more stable than that from the homozygous AlwNI mutation (Sato et al, 1997; Miura et al, 1991).