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Identification of novel mutations and re-assignment of archival xeroderma pigmentosum group C cell strains from Japanese patients

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Xeroderma pigmentosum (XP) is an autosomal recessive disorder, which is characterized by cutaneous hypersensitivity to sunlight exposure and a predisposition to skin cancer. Eight genetic complementation groups have been identified for XP: seven groups (XP-A through G) are associated with defects in nucleotide excision repair (NER), while the remaining one, an XP variant (XP-V) form, is caused by impairment of translesion DNA synthesis, but not NER [1,2]. The complementation group C is one of the most common forms of XP in the world, so that a large number of pathogenic mutations have been identified for the responsible gene *XPC* (https://www.ncbi.nlm.nih.gov/clinvar/?term=XPC[gene]). Although the overall frequency of XP in Japan is much higher than that in the whole world, XP-C is a relatively rare form. Only a few XP-C cases have been reported, for most of which the *XPC* gene mutation was not identified. Here we describe genetic analyses of skin fibroblasts from three Japanese XP patients (XP3KA, XP4KA, and XP40OS), which were assigned to XP-C (Clinical information of the patients is provided in Supporting information).

Mutations of the *XPC* gene were analyzed by sequencing of genomic PCR and RT-PCR products and the results are summarized in TABLE 1 (detailed results of sequencing are provided in Supporting information, FIGURE S1). We identified compound heterozygous *XPC* mutations in XP3KA cells: one allele harbored a well-documented, 2-bp (TG) deletion in exon 9 [3], and the other lacked the entire exons 12 and 13, both leading to a frameshift. Breakpoints of the novel large (~2.3 kbp) deletion were identified within introns 11 and 13. On the other hand, XP4KA cells were homozygous for a novel 1-bp insertion in exon 2, also leading to a frameshift. As expected, the *XPC* mRNA levels were significantly reduced in both cell strains most likely due to the nonsense-mediated mRNA decay, while the expression of XPC protein was not detected by immunoblot analysis (see Supporting information, FIGURE S2). Therefore, these results indicate that both patients showed typical null-like phenotypes, which are common to most of the XP-C cases so far reported. Intriguingly, both XP3KA and XP4KA cells were homozygous for Ala499Val polymorphism (see Supporting information, FIGURE S3), which could be implicated in the development of melanoma in both patients [4].

Surprisingly, we failed to identify any pathogenic *XPC* mutation in XP40OS cells. Therefore, complementation with the known XP-related genes was re-examined, which revealed that the *ERCC2* (*XPD)* gene, but not *XPC*, could restore UV-induced unscheduled DNA synthesis in XP40OS cells (see

Supporting information, FIGURE S4). Sequencing of the *ERCC2* genomic locus revealed the presence of compound heterozygous mutations (TABLE 1). One allele harbored a base substitution at the second nucleotide in exon 22, resulting in an amino acid substitution (Arg683Gln) that has been frequently found in Japanese XP-D cases [5]. The other had a novel deletion encompassing last 23 nucleotides of exon 21. From RT-PCR analysis, we identified at least two types of transcripts expressed from this second allele, both of which causes a frameshift and potentially encodes mutant XPD proteins of larger size than the wild-type protein. However, immunoblot analyses detected only the XPD protein of normal size (see Supporting information, FIGURE S2), indicating that the unrelated C-terminal amino acid sequences destabilize the mutant XPD proteins in cells. Therefore, it is likely that only the XPD Arg683Gln protein is relevant to the phenotypes of XP40OS. Based on these results, we conclude that XP40OS should be re-assigned as XP-D. Indeed, this patient exhibited exaggerated sunburn 2 months after birth [6], which is characteristically observed symptoms in XP-D, but not in XP-C.

In this study, we identified three novel pathogenic mutations of XP-related genes (two in *XPC*, and one in *ERCC2*), which could be specific for Japanese XP cases. Recently next generation sequencing has become a powerful approach for diagnosis of various hereditary diseases, especially for detection of known mutations in responsible genes. It is important for future diagnosis to accumulate data of pathogenic mutations from analysis of individual cases.

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CONFLICTS OF INTEREST

None declared.

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TABLE 1 Summary of mutations identified in this study.

cell line	mutated gene	mutation identified			predicted protein change
XP3KA	*XPC*	allele 1	c.1643_1644delTG	2-bp deletion in exon 9 (ref. 3)	XPC, p.Val548Alafs*25
		allele 2	c.2116_2420del (g.33142_35462del)	deletion of exons 12 and 13	XPC, p.Met706Aspfs*2
XP4KA	*XPC*	allele 1/2	c.218_219insT	1-bp insertion in exon 2	XPC, p.Lys73Asnfs*9
XP40OS	*ERCC2*	allele 1	c.2048G>A	single base substitution in exon 22 (ref. 5)	XPD, p.Arg683Gln
		allele 2	c.2024_2046del	23-bp deletion in exon 21	XPD, p.Gly675Alafs*91 XPD, p.Leu676Alafs*141