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Multiple Skin Cancers in Adults with Mutations in the XP-E (*DDB2*) DNA Repair Gene

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TO THE EDITOR

Xeroderma pigmentosum complementation group E (XP-E) patients exhibit sunlight-induced lentiginous pigmentation without blistering on minimal sun exposure, yet they are prone to develop multiple skin cancers. Only eight XP-E patients have been reported (Bootsma *et al.*, 1970; De Weerd-Kastelein *et al.*, 1974; Kraemer *et al.*, 1975; Nichols *et al.*, 1996; Rapic *et al.*, 1998; Itoh *et al.*, 1999, 2000; Rapic-Otrin *et al.*, 2003) with mutations in the *DDB2* gene (Tang and Chu, 2002; Itoh, 2006), resulting in the loss of UV-damaged DNA-binding protein (UV-DDB) activity (Nichols *et al.*, 2000; Rapic-Otrin *et al.*, 2003) (Table 1). UV-DDB is a heterodimer of DDB1 (p127) and DDB2 (p48) (Keeney *et al.*, 1994; Kazantsev *et al.*, 1996) that binds with high affinity to DNA damaged by UV and is involved in initiation of global genome nucleotide excision repair (GG-NER) (Sugasawa, 2010).

We identified four adult XP-E patients from three kindreds with large numbers of skin cancers (Table 1). Patients' written, informed consent was obtained. The Declaration of Helsinki guidelines were followed and all necessary institutional approvals were obtained. Patient XP1GO, 45 years old, in family A from Germany never experienced a blistering sunburn (Figure 1a). Diagnosed with XP at age 22, he works as a train conductor. His first tumor was removed at age 12. He had >400 basal cell carcinomas (BCCs) and squamous cell carcinoma (SCCs) and 6 melanomas treated by age 30, and now he develops ~20 skin cancers per year. He has no neurological abnormalities. Patient XP37BE is a 45-year-old Caucasian female of Dutch ancestry in family B living in the western United States (Figure 1b). She denies ever having a blistering sunburn. She developed a keratoacanthoma on her face at 7 years and was diagnosed with XP. XP37BE has had >300 BCC and SCC skin cancers but no melanomas. She has no neurological abnormalities. Patient XP66BE is a 43-year-old brother of XP37BE. He was diagnosed with XP at age 4 at the same time his older sister was diagnosed and exhibits similar clinical symptoms, yet, milder because of improved sun protection. Patient XP408BE is a 53-year-old Caucasian female in family C from the eastern United States (Figure 1c). She had no sunburns and tanned easily, but did experience significant photophobia. At age 14, she was found to have multiple skin cancers (BCCs and SCCs) on her face and a diagnosis of XP was made. She has no XP neurological abnormalities.

CONFLICT OF INTEREST

The authors state no conflict of interest.

All cells were either established at the Human Genetic Mutant Cell Repository, the NCI Repository, or in the Department of Dermatology, Goettingen, Germany. Plasmid host cell reactivation assay was performed for cellular DNA repair capacity measurement (Emmert *et al.*, 2000). The cells were transfected with a UV-treated plasmid containing a reporter (luciferase) gene (pCMVLuc). Compared with normal and XP variant cells, XP1GO, XP37BE, XP66BE, and XP408BE/GM01389 cells had a reduced level of luciferase expression whereas severe XP-B control cells had an even lower level (data not shown). To determine the complementation group we co-transfected the UV-irradiated pCMVLuc with plasmids that carry cloned wild-type XP complementary DNA (cDNA). Only co-transfection of the *DDB2* cDNA resulted in markedly enhanced reporter gene activities (data not shown).

Human primary XP-E fibroblasts have been reported to show abnormally low or undetectable levels of p53 and its downstream-regulated proteins (Hwang *et al.*, 1999; Itoh *et al.*, 2003; Itoh, 2006). In agreement with this observation, the intensities of p53 and p21 bands were reduced ~60–80% and 40–60%, respectively, in untreated XP37BE, XP66BE, and XP408BE/GM01389 cells (ECL Western blotting; Amersham, Piscataway, NJ) (data not shown).

Sequence analysis (NC_000011.8 for genomic sequence, NM_000107.1 for cDNA, and NP_000098.1 for protein) revealed a, to our knowledge previously unreported, homozygous C-to-A transversion (c.914 C > A) in exon 7 in the *DDB2* gene of XP1GO. This missense mutation resulted in a p.Thr305Asn substitution (Table 1). His parents and brother were heterozygous for this mutation. The restriction enzyme *Btg*I cuts the normal but not the mutant sequence.

XP37BE and XP66BE showed homozygous G-to-A transitions in exon 6 of *DDB2*. This missense mutation (c.818 G > A) resulted in p.Arg273His and was also found in their mother and father but not their unaffected brother (Table 1). This mutation inactivates a *Hha*I restriction site. This mutation was previously reported in XP2RO and XP3RO cells from the Netherlands (Bootsma *et al.*, 1970; De Weerd-Kastelein *et al.*, 1974; Kraemer *et al.*, 1975; Nichols *et al.*, 1996).

The cells from patient XP408BE had compound heterozygous mutations in exon 8. One allele showed a T-to-C transversion (c.1049 T > C) resulting in p.Leu350Pro, and the other allele had a three-base deletion (c.1045_1047del) resulting in p.Asn349del (Table 1). These two mutations were identical to the mutations previously reported in cell line GM01389 (Nichols *et al.*, 2000). We measured 15 single-nucleotide polymorphisms (SNPs) in the *DDB2* gene to determine the relationship between these two cell lines (XP408BE and GM01389). All 15 SNPs were identical in both cells (data not shown). CODIS DNA fingerprinting of highly polymorphic short tandem repeats (STRs) was then performed (Azari *et al.*, 2007). All 13 CODIS core STR loci were detected and were identical in both cell lines (data not shown). Thus, the likelihood that the cells are not identical is approximately one in one billion. Indeed, the patient recalled having a skin biopsy for fibroblast culture when she was 21 years old.

Figure 1d shows the crystal structure of DDB2 stabilized by DDB1 and contacting the damaged DNA extensively (Chu and Yang, 2008; Scrima *et al.*, 2008). The heterozygous DDB2 mutations (Leu350Pro and Asn349del) in XP408BE impair DDB1 binding (DDB1–DDB2 interface mutations). In contrast, the Arg273His mutation in XP37BE and XP66BE directly interferes with DNA binding (DNA-binding mutation). The new mutation, p.Thr305Asn in XP1GO cells, is located in the WD domain near a known Asp307Tyr

mutation. This mutation has been reported to disrupt damage detection and complex formation with DDB1 (Rapic *et al.*, 1998; Rapic-Otrin *et al.*, 2003).

The diagnosis of XP-E can be considered in adults with freckle-like pigmentation without blistering on minimal sun exposure who have many skin cancers.

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Abbreviations

BCC	basal cell carcinoma
cDNA	complementary DNA
GG-NER	global genome nucleotide excision repair
SCC	squamous cell carcinoma
SNP	single-nucleotide polymorphism
STR	short tandem repeat
UV-DDB	UV-damaged DNA-binding protein
XP-E	xeroderma pigmentosum complementation group E

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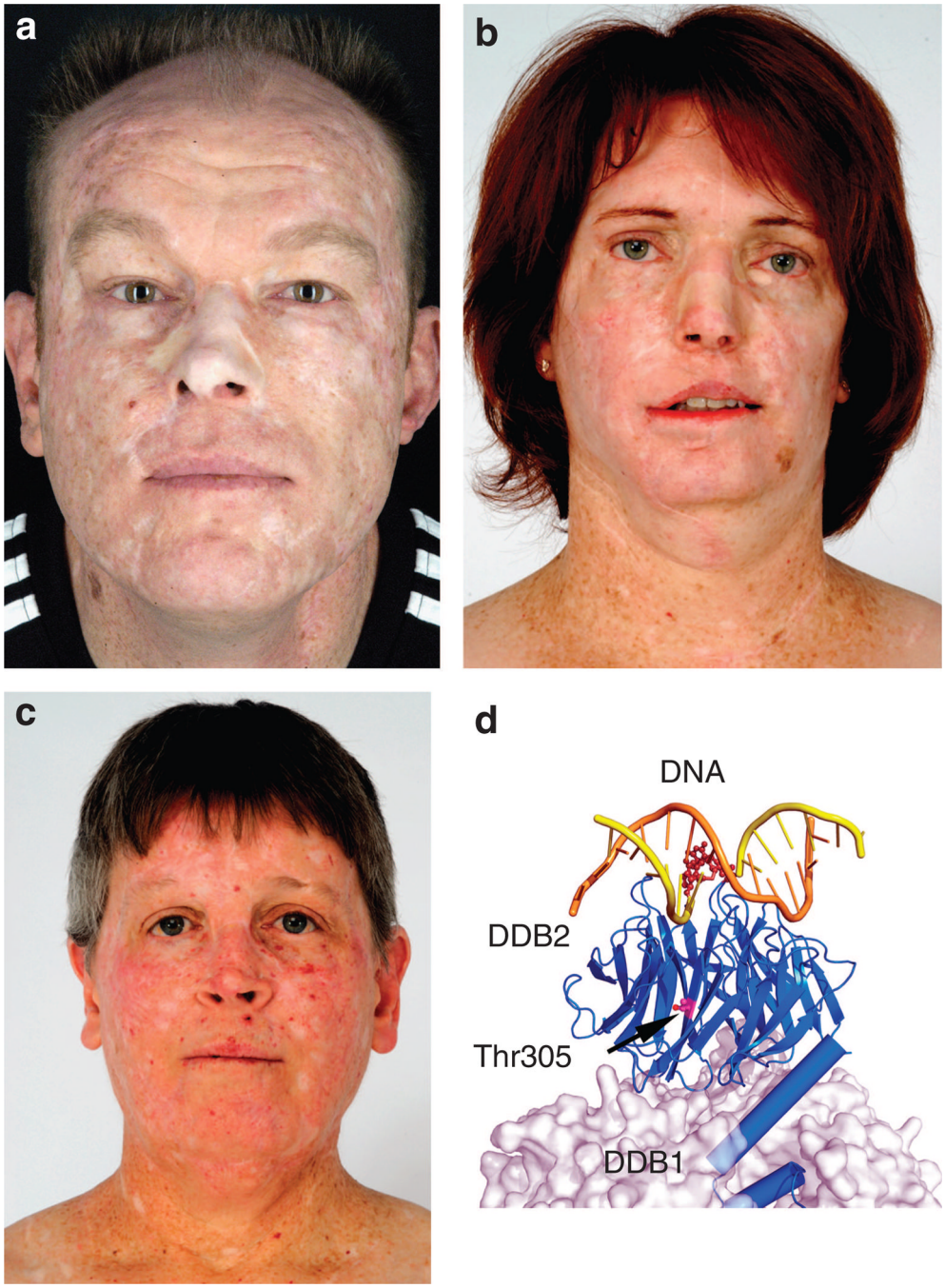

Figure 1. Clinical features and DDB2 crystal structure

(**a**) XP1GO in family A is a 45-year-old German train conductor with a history of >400 skin cancers including basal cell carcinomas (BCCs), squamous-cell carcinomas (SCCs), and melanomas. His face shows multiple surgical scars and grafts. (**b**) XP37BE in family B is a 45-year-old woman with a history of >300 non-melanoma skin cancers. She had removal of part of her jaw from SCC. Her face shows multiple surgical scars and grafts. Lentiginous hyperpigmentation is present on her neck. (**c**) XP408BE in family C is a 53-year-old woman with a history of >600 skin cancers including BCCs, SCCs, and melanomas. She has multiple surgical scars and telangiectasias in sun-exposed areas. (**d**) Crystal structure of

DDB1–DDB2 complexed with a 6–4 photoproduct. DDB2, shown as a blue ribbon diagram, is stabilized by DDB1, shown as a semitransparent molecular surface. The undamaged DNA strand is shown in brown, and the damaged strand in yellow, with the 6–4 photoproduct shown in dark brown. The p.Thr305 residue (magenta and red) (arrow) lies buried within the DDB2 protein and not near the DNA or DDB1 interfaces. (Image courtesy of Dr Wei Yang, modified from Chu and Yang, 2008.) Patients gave written permission for the use of their photographs.

Table 1

Clinical features and *DDB2* mutations

					***DDB2* mutations[1]**						
					Allele 1			**Allele 2**			
Family	**Cell line**	**Last reported age/sex**	**Location**	**Clinical features**	**cDNA**	**Amino acid**	**Size (aa)[2]**	**cDNA**	**Amino acid**	**Size (aa)[2]**	**References[6]**
A	XP1GO	45y/M	Germany	>400 BCCs and SCCs; 6 melanomas before age 30	c.914 C>A (exon 7)	pThr305Asn	427	Homozygous			This paper
B	XP37BE[3]	45y/F	USA/the Netherlands	>300 BCCs and SCCs, no melanomas	c.818 G>A (exon 6)	p.Arg273His	427	Homozygous			This paper
B	XP66BE[3]	43y/M	USA/the Netherlands	6 Melanomas before age 40	c.818 G>A (exon 6)	p.Arg273His	427	Homozygous			This paper
C	XP408BE[4]	53y/F	USA	>600 BCCs, SCCs, and 12 melanomas by age 50	c.1049 T>C (exon 8)	p.Leu350Pro	427	c.1045_1047del (exon 8)	p.Asn349del	426	This paper
C	GM01389[4]	21y/F	USA	Multiple skin cancers	c.1049 T>C (exon 8)	p.Leu350Pro	427	c.1045_1047del (exon 8)	p.Asn349del	426	A
D	XP2RO[5]	34y/F	The Netherlands	Skin cancer developed at age 14	c.818 G>A (exon 6)	p.Arg273His	427	Homozygous			B, C, E
D	XP3RO[5]	29y/F	The Netherlands	Skin cancer present	c.818 G>A (exon 6)	p.Arg273His	427	Homozygous			B, C, E
E	XP82TO	41y/F	Japan	No skin cancer	c.730 A>G (exon 6)	p.Lys244Glu	427	Homozygous			B
F	XP23PV	18y/M	Italy	7 BCCs from age 16 to 18	c.703_1023del (del. exon 6 and 7)	p.Leu235_Lys341del	320	Homozygous			A, D
G	XP25PV	29y/F	Italy	5 BCCs and 1 SCC from age 22 to 28	c.919 G>T (exon 7) c.918 G>A (exon 7)	p.Asp307Tyr No change	427 427	Homozygous Homozygous			A, D
H	XP27PV	35y/F	Italy	BCC, SCC, and melanoma	1. c.730_733del (exon 6) 2. c.703_880 (del. exon 6) 3. c.703_1023del (del. exon 6 and 7)	p.Lys244X p.Trp236Valfs*10 p.Leu235_Lys341del	243 244 320	Homozygous Homozygous Homozygous			A
I	Ops1	62y/F	Japan	14 BCCs and 5 melanomas on face, 2 SCC extremities	c.937 C>T (exon 7)	p.Arg313X	312	Homozygous			F

Abbreviations: BCC, basal cell carcinoma; cDNA, complementary DNA; DDB, DNA-binding protein; SCC, squamous cell carcinoma.

[1] GenBank reference sequence NC_000011.8 for genomic sequence, NM_000107.1 for cDNA, and NP_000098.1 for protein.

[2] Predicted size.

[3] Siblings.

[4] Same patient, cultures established at different ages (see text for details).

[5] Second cousins.

[6] References: A, Rapic-Otrin *et al.* (2003); B, Nichols *et al.* (2000); C, Bootsma *et al.* (1970); D, Rapic *et al.* (1998); E, Kraemer *et al.* (1975); F, Itoh *et al.* (1999).